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Ligand-Binding Proteins: Their Potential for Application in Systems for Controlled Delivery and Uptake of Ligands

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***Abstract*——Unstable or harmful agents, such as drugs, vitamins, flavors, pheromones, and catalysts, for use in pharmaceutics, personal care, functional foods, crop protection, laboratories, offices, and industrial processes, require stabilization against oxidation and degradation or shielding from sensitive environments. Therefore, binding them to carriers with high affinity and selectivity for targeting to the right environment and subsequent controlled release is beneficial, especially if this allows improved control of (stimulus-induced) release. Proteins often possess one or more of these properties, whereas modern biotechnology and bioinformatics provide an increasing number of tools to engineer and adapt these properties. Carrier systems are now developed that incorporate proteins as the central ligand-binding component, e.g., lectins for glucose-triggered release of glycosylated insulin and bispecific antibodies for brain targeting of drugs, but ligand-binding proteins can potentially be used in many other applications. Collectively, the proteins available in nature bind an impressive variety of ligands and non-natural analogs. In this light, various ligand-binding protein classes are surveyed, including biotin-, lipid-, immunosuppressant-, insect pheromone-, phosphate-, and sulfate-binding proteins, as well as bacterial periplasmic proteins, lectins, serum albumins, immunoglobulins, and inactivated enzymes. Disadvantages, such as enzymatic degradation or immunogenicity, associated with the pharmaceutical use of certain proteins can be avoided by incorporating these proteins in more complex carrier and targeting systems. In other applications, this may not be necessary. The enclosure of high-affinity (potentially stimulus-sensitive) binding proteins within an envelope that acts as a diffusion barrier for the ligand may provide excellent slow release. Many possibilities seem to be as yet unexplored.**

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I. Introduction: The Concept of Ligand-Selective Carrier Proteins

Many active agents used in pharmaceutics, food, agriculture, and chemical processes require temporal stabilization and protection against degradation or oxidation (Hattori et al., 1995; Iametti et al., 1995). Alternatively and/or additionally, a sustained or triggered release may be a prerequisite. Finally, the efficacy of such agents may be improved by increasing their solubility or by masking unwanted properties, such as toxicity or bad taste, at least before the target environment is reached (Poznansky and Juliano, 1984; Tomlinson, 1987; Jain, 1989; Vingerhoeds et al., 1994; Pothakamury and Barbosa-Cánovas, 1995; Risch and Reineccius, 1995). In many cases, a combination of requirements may apply (Fig. 1A). For example, the oral administration of an unstable, insoluble, and bitter-tasting drug would call for 1) the prevention of early drug degradation, 2) an improvement of the solubility or dispersability of the drug in water, 3) a masking of its bitter taste, 4) a more efficient routing to its target environment (leading to a reduction of generic toxicity), and 5) a controlled release once the target environment has been reached. To meet (part of) such demands, carrier systems have been developed (Fig. 1B), including particulate systems like nano- and microcapsules, nano- and microspheres, liposomes (Felgner, 1990; Kreuter, 1992; Karsa and Stephenson, 1993, 1996; Risch and Reineccius, 1995), and even resealed erythrocytes (Dale, 1980). Whereas liposomes consist of (phospho)lipids, the other particulate systems normally consist of natural or synthetic biocompatible polymers. These can be proteins, such as gelatin, albumin, casein, or fibrin (Thies and Bissery, 1984; Chen et al., 1987; Wolkoff, 1987; Gupta and Hung, 1989; Senderoff et al., 1991; Banga, 1995; Narayani and Rao, 1996; Yu et al., 1996), but more often other polymers are used (Hsieh et al., 1981; Kost et al., 1989; Davis et al., 1991; Leung et al., 1991; Sanders, 1991; Hirabayashi et al., 1996; Mi et al., 1997) usually because they are more stable or less immunogenic. Several systems are used for the specific targeting (homing) of the entrapped agents (Poznansky and Juliano, 1984; Tomlinson, 1987; Felgner, 1990; Vingerhoeds et al., 1994; Hirabayashi et al., 1996), whereas most systems are designed to control the kinetics of release of the active agent in a site- and time-dependent manner. Where targeting rather than controlled release is the sole or prime goal, covalent attachment of the active agent to the carrier system is often used (Poznansky and Cleland, 1980; Trouet et al., 1982; Fiume et al., 1986; Franssen et al., 1994; Molema and Meijer, 1994; Narayani and Rao, 1996; Pirrung and Huang, 1996; Dosio et al., 1997; Lebbe et al., 1997). Alternatively, encapsulation of free or noncovalently bound drugs in liposomes and attachment of antibodies to the liposome is applied in drug targeting (Crommelin et al., 1992; Vingerhoeds et al., 1993, 1994; Storm et al., 1995). Many controlled release systems are designed to create a sustained release (Bagshawe et al., 1988; Merlin, 1991; Karsa and Stephenson, 1993; Vingerhoeds et al., 1993; Risch and Reineccius, 1995). However, release of the active compounds can also be designed to be burst-like, as triggered by the local conditions in the target environment (Brownlee and Cerami, 1979; Hsieh et al., 1981; Jeong et al., 1985; Albin et al., 1987; Fischel-Ghodsian et al., 1988; Kost et al., 1989; Merlin, 1991; Pinnaduwage and Huang, 1992; Kim et al., 1994) or by externally applied triggers (Jain, 1989; Kreuter, 1992; Karsa and Stephenson, 1993, 1996; Vingerhoeds et al., 1994; Pothakamury and Barbosa-Cánovas, 1995).

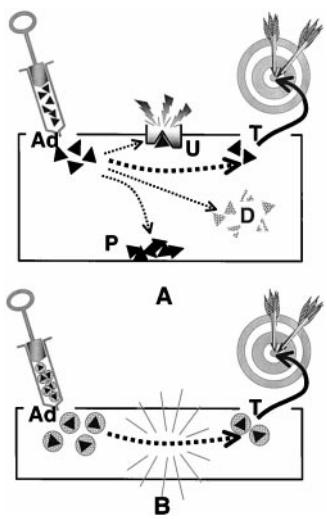

FIG. 1. A, schematic representation of the possible fates of active agents in view of the various requirements for their functional application. The active agent is represented by black triangles. Ad, site of administration; U, unwanted interaction (for example, bad taste resulting from interaction with taste receptors, toxicity resulting from interaction with hormone receptors at unintended sites, etc.); P, (undesirable) precipitation; D, (undesirable) degradation and/or oxidation; T, target site. B, attachment of the active agent to a carrier system (a single protein, a polymer capsule, or a more complex system, schematically represented by gray-speckled balls) may prevent unwanted side effects and loss of the agent. Furthermore, it may result in improved delivery and in controlled release of the active agent at the target site (T). (Again, the agent is represented by black triangles.).

The field of controlled release, which is of prime importance in drug delivery, especially in cancer therapy and the treatment of endocrinological disorders (e.g., Karsa and Stephenson, 1993), finds increasing application also in cosmetics and household materials (e.g., Schaeffer and Brooks, 1992; Joshi, 1996; Withenshaw et al., 1996), food (Yolles, 1973; Risch and Reineccius, 1995), agrochemicals/crop protectants (e.g., Allan and Neogi, 1971; Kuderna and Saliman, 1974; Cohen et al., 1977; Knight et al., 1995; Scher, 1999), fertilizers (e.g., Fersch and Stearns, 1976; Knight et al., 1995), industrial and other reactants

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(Mestetsky, 1973; Segalman and Wallace, 1995), and office supplies, e.g., "carbonless carbon paper" (see Karsa and Stephenson, 1993). In crop protection, triggers like rainfall, heat, photolysis, natural plant-associated molecules, or infestation-induced generation of by-products can be used. In cosmetics, as in pharmaceutical applications, carrier systems consisting of polymers and proteins with a low immunogenicity are advantageous, e.g., for the slow or stimulated release of fragrances, radical scavengers, etc. In food-oriented applications, food-grade protein-, polysaccharide-, or lipid-mediated carrier systems are particularly appropriate (e.g., lactoglobulin, albumin, starch, lecithin). Food-associated compounds that can profitably be protected and targeted by binding to carrier proteins include flavoring agents, colorants, vitamins, antioxidants, or preservatives.

In most traditional systems, the control of release and the stabilization of the agent is based on encapsulation (Brownlee and Cerami, 1979; Hsieh et al., 1981; Fischel-Ghodsian et al., 1988; Kost et al., 1989; Karsa and Stephenson, 1993; Kim et al., 1994; Vingerhoeds et al., 1994; Pothakamury and Barbosa-Cánovas, 1995; Risch and Reineccius, 1995) and/or on nonspecific reversible interactions between the carrier and the active agent (e.g., Chen et al., 1987; Wolkoff, 1987; Gupta and Hung, 1989; Senderoff et al., 1991; Chuo et al., 1996; Lubbe et al., 1996). However, the application of carrier components that *selectively* and reversibly bind the active agent with high affinity offers additional possibilities to stabilize the agent and improve the control of its release (Cohen et al., 1977, 1979; Mitchell, 1986; Schaeffer and Brooks, 1992; Hattori et al., 1995), reducing unwanted side effects of the active agent (Fig. 2). This is especially the case when the binding site and binding mechanism is well characterized and accessible to engineering. Thus, agent-specific ligand-binding (bio)polymers can be considered as a special class of carrier components either to be used as such or, alternatively, as agent-selective parts of more complicated (multicomponent) particulate carrier systems. Particularly, ligand-binding proteins could be potentially promising as such agent-specific binding components. The ligand-binding sites of a great number of proteins are well characterized, with known crystal structures, and well accessible to engineering via modern genetic techniques (site-directed mutagenesis, directed evolution, and/or screening of large numbers of more or less random mutants). In natural systems, the occurrence of high selectivity and binding affinity for a specific small ligand is almost invariably attributable to the presence of proteins. Examples are the binding of biotin by (strept)avidin, the binding of antigens by specific antibodies, and the binding of small molecules to receptors. In fact, the binding of a molecule to a protein is the basic step in most biological processes and functions. The first step in biological catalytic conversions is the binding of the target substrate by an enzyme. Cell signaling, DNA replication, gene expression, and the sorting and trafficking of any cellular component to the various compartments of the cell all depend on the specific binding of smaller and larger molecules to proteins and proteinaceous receptors. The cascade of events in the immune response is triggered by the recognition of an antigen molecule by an antibody. Carrier proteins bind small molecules to transport them through the intra- and extracellular compartments.

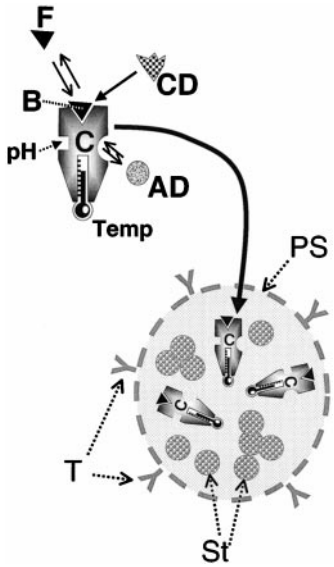

FIG. 2. Use of carrier molecules (C) that selectively bind the active agent with high affinity. Ligand- (i.e., agent-) binding proteins are especially suitable for this purpose. They can be selected or genetically engineered to be sensitive to, for example, pH, temperature changes, and other molecules that displace the ligand by competitive or allosteric binding. Such selective high-affinity carrier molecules (proteins) can in turn be incorporated in more complex carrier systems that also include targeting devices like antibodies, stabilizing agents, and a selectively permeable capsule (schematically represented here by an interrupted black line). B, bound agent (ligand); F, free (unbound) agent; CD, competitive displacer; AD, allosteric displacer; Temp, temperature sensitivity; T, targeting device (e.g., antibody); St, stabilizing molecule.

Although agent-carrying controlled release systems may comprise several additional carrier components (see Fig. 2), the central function of the ligand-binding protein, with its high affinity and selectivity for the active agent and its ligand-stabilizing power (e.g., Hattori et al., 1995), would be to provide control over the binding, stabilization, and release of the active agent. Such application has already been proposed by others for some specific proteins and specific applications (e.g., Geisow, 1992; He and Carter, 1992; Kost and Langer, 1992; Batt et al., 1994; Stayton et al., 1995; Verhoeyen et al., 1995). We would like to consider this idea as a more general concept, potentially applicable in pharmacology and cosmetics but also in food technology, crop protection, industrial chemical processes, and various household purposes.

II. Survey of Ligand-Binding Protein Classes

One of the aims of this review is to give a general overview of different classes of ligand-binding proteins

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in view of their possible application in protein-mediated transport and controlled release of small molecules. Because many excellent reviews have already been devoted to the structural and ligand-binding properties of individual proteins and protein classes, we will give an overview of *various* classes and key proteins in terms of their ligand-binding properties and possibilities for modification. We concentrate on protein groups that can be used to bind small, chemically attractive, and commercially important molecules such as pharmaceuticals, flavors, pesticides, and/or insect pheromones that are used to control insect plagues. Excluded are proteins that bind metals, single-element ligands, or very large biopolymeric ligands such as nucleic acids and proteins. Thus, we will discuss 1) biotin-binding proteins, 2) lipid-binding proteins (LBPs)[2]/transporters of hydrophobic molecules, 3) bacterial periplasmic binding proteins, 4) lectins, 5) serum albumins, 6) immunoglobulins, 7) inactivated enzymes, 8) odorant-binding proteins (OBPs), 9) immunosuppressant-binding proteins, and 10) phosphate- and sulfate-binding proteins (PiBPs and SBPs). The treatment of each group could not possibly be exhaustive but merely serves to give an overview of the basic properties that may provide new ideas and may be relevant to the development of protein-mediated controlled release systems.

A. Biotin-Binding Proteins

Of all ligand-binding proteins, the biotin-binding proteins avidin and streptavidin are perhaps most thoroughly studied. Their exceptionally high affinity for biotin ($K_d = 6 \times 10^{-16}$ M for avidin and 4×10^{-14} M for streptavidin) has lead to many biotechnological applications (Wilchek and Bayer, 1990). The novel approach of Stayton et al. (1995) in conjugating the protein to a stimuli-responsive polymer is likely to find applications in biotechnological and medical areas. The coupling of the protein to a temperature-sensitive polymer enables reversible, environmentally-triggered binding and release of the ligand. Normal binding of biotin is possible at temperatures below 32°C, but at temperatures above this, the coupled polymer collapses and blocks the binding site (Fig. 3). Another possibility is to engineer the loop involved in locking in the bound ligand or the monomer-monomer interfaces to render the protein susceptible to specific protease activity, temperature, pH, etc., thus making the release of ligand triggerable by these stimuli. In conjunction with the detailed molecular dynamics simulations performed using the biotin-binding system (Leckband et al., 1994; Moy et al., 1994; Vajda et al., 1994; Berendsen, 1996; Grubmüller et al., 1996), such studies bring these proteins closer to being ready for application in the area of ligand transport and release. It may, however, be difficult to engineer the binding site itself to obtain radical changes of ligand specificity (when molecules unrelated to biotin should be bound) because the molecular shapes and interacting groups of the binding site and its natural ligand are tightly complementary. The versatility of the (strept)avidin system is thus very limited. In this respect, the group of LBPs, which is discussed in the next section, offers much broader possibilities for adaptation.

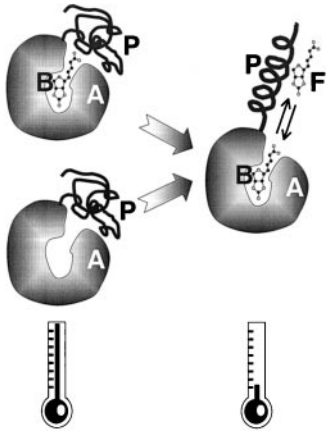

FIG. 3. Avidin modified with a temperature-sensitive polymer that blocks the binding site for biotin at high temperature, as described by Stayton et al. (1995). Left, high temperature. Right, low temperature. This figure was not taken from Stayton et al. (1995) but was newly designed to illustrate the principle. A, avidin; B, bound biotin; F, free avidin; P, polymer (indicated schematically with a black line).

The highly stable avidin-biotin complex (Green, 1990) has found actual application in drug targeting rather than in controlled release (Yoshikawa and Pardridge, 1992; Bickel et al., 1993; Kang and Pardridge, 1994; Shin et al., 1997; Schechter et al., 1999). It is an alternative to covalent attachment of drug to a carrier protein, which often requires harsh methods that can lead to denaturation, unwanted modification, and loss of functionality of the carrier and/or drug. Normally, a combination of biotinylated drug and an avidin-containing targeting system is used. The biotinylation of some peptide drugs can be obtained under relatively mild conditions, for example, by disulfide linkage (e.g., Yoshikawa and Pardridge, 1992; Bickel et al., 1993). Although the biotin-(strept)avidin complex is highly stable, the biotinylated drug can still be released at the target site. The release is easier in cases where coupling of avidin to a targeting device leads to a decreased affinity for biotin (Shin et al., 1997). The biotinyl group is small enough to allow in many cases a proper functioning of the drug at the target site. For targeting, the avidin has been chemically conjugated to antibodies or to cationized albumin (Bickel et al., 1993; Kang and

[2] Abbreviations: LBP, lipid-binding protein; OBP, odorant-binding protein; BsAb, bispecific antibody; DPP, dipeptide-binding protein; HBP, histidine-binding protein; HSA, human serum albumin; I-FABP, intestinal fatty acid-binding protein; eLBP, extracellular LBP; iLBP, intracellular LBP; ALBP, adipocyte LBP; LAOBP, lysine/arginine/ornithine-binding protein; MBP, maltodextrin-binding protein; GOBP, general OBP; OPP, oligopeptide-binding protein; PBP, pheromone-binding protein; PiBP, phosphate-binding protein; RBP, retinol-binding protein; pRBP, plasma (serum) RBP; SBP, sulfate-binding protein.

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Pardridge, 1994). Alternatively, antibodies and avidin have been fused to a single protein via genetic engineering (Shin et al., 1997), avoiding the need for chemical modification of the carrier (Fig. 4). The clearance of the fusion protein was more than 10 to 20 times slower than the clearance of free avidin (Shin et al., 1997). Probably due to its cationic nature and to glycosylation, free avidin has a very short plasma half-life (approximately 1 min) and is rapidly taken up by the kidney and liver. Removal of the glycosyl group of avidin and chemical modification may result in a prolonged half-life (Kang et al., 1995). Chemically modified avidin has also been used as a targeting device itself, negating the need for antibodies. Thus, trinitrophenylated streptavidin was used to effectively target 5-fluorouridine to the liver in mice (Schechter et al., 1999). The immunogenic nature of (strept)avidin is a point of concern. However, because most people have been exposed to egg avidin and because oral antigens are known to be tolerogenic, a certain degree of tolerance to avidin can be expected (Weiner, 1994). Antibody-avidin, cationized albumin-avidin fusion proteins (obtained by genetic engineering or chemical modification), and more complicated avidin-containing systems have been used for the successful delivery of biotin, biotinylated bioactive peptide, biotinylated nucleic acid, and other molecules to the brain as well as to cancer cells (e.g., Bickel et al., 1993; Kang and Pardridge, 1994; Shin et al., 1997; Penichet et al., 1999; Vinogradov et al., 1999). Because of the so-called blood-brain barrier, brain delivery is notoriously difficult (Halmos et al., 1997).

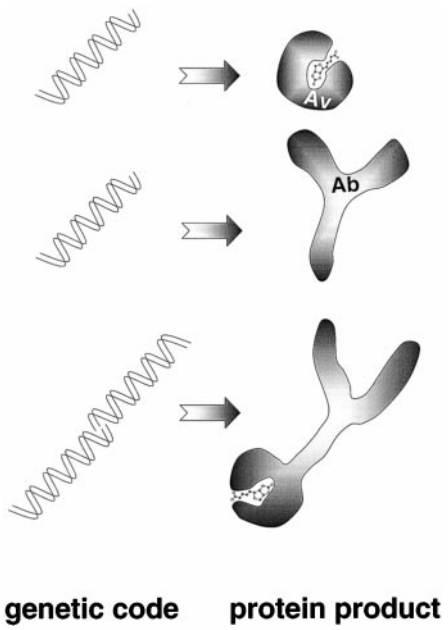

FIG. 4. Avidin and antibody genes can be fused to encode a single fusion protein molecule with bispecific function (biotin binding and antigen recognition/targeting). Ab, antibody; Av, avidin. A similar principle can be applied to combinations of other ligand- (i.e., agent-) binding proteins.

The function of biotin-binding proteins in nature still remains a puzzle because, despite the apparent exquisite design, it is still unknown whether biotin is the sole or even the primary ligand for either protein (Linvah et al., 1993). Avidin is a basic tetrameric glycoprotein isolated from egg white (Fig. 5). Each monomer of 128 amino acids is capable of binding a single biotin molecule, giving a 62.4-kDa protein capable of binding four molecules of vitamin H (Pugliese et al., 1994). Streptavidin, secreted by *Streptomyces avidinii,* is slightly larger with 159 residues per subunit. However, such full-length molecules are rarely detected under the conditions used to culture the bacteria (Sano et al., 1995). The terminal regions of the protein are particularly susceptible to proteolysis, and truncated core streptavidins are more commonly formed, considerably improving solubility properties. Natural core streptavidin consists of 127 residues, forming a monomer of 13.3 kDa and a biotin-binding tetramer of ~53 kDa. Streptavidin lacks the carbohydrate chain present in avidin and has a lower isoelectric point. The resultant decrease of nonspecific binding has lead to increased application of the bacterial protein. Another difference between the two proteins is their sulfur content; avidin contains a disulfide bridge and two methionine residues per subunit, whereas streptavidin is free of sulfur-containing amino acids (Sano and Cantor, 1990). With a view to possible modification, it is essential for the protein to be expressed in a suitable vector. Both avidin and streptavidin have been successfully expressed in *Escherichia coli* (Sano and Cantor, 1990; Thompson and Weber, 1993; Airenne et al., 1994). Although the streptavidin gene is extremely lethal to the host cells, it can be expressed efficiently using T7 RNA polymerase/T7 promoter expression systems. As generally observed in *E. coli* overexpression systems, most of the streptavidin is insoluble in the cell, forming inclusion bodies.

The primary structures of the two proteins are similar (Linvah et al., 1993), and major structural motifs are conserved as well as the significant residues in the binding site. The proteins are constructed of eight antiparallel β-strands forming the classic β-barrel. The biotin-binding site is positioned toward one end of the barrel,

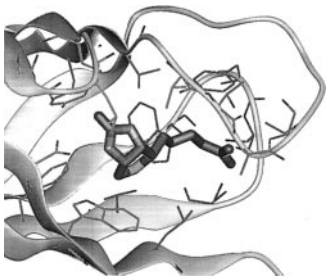

FIG. 5. Detail of a crystal structure of an avidin molecule complexed with biotin. The structure was obtained from the Brookhaven protein database (PDB), as deposited by O. Livnah and J. Sussman (see Livnah et al.,1993). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc. (San Diego, CA).

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where a number of aromatic and polar amino acids are involved in ligand binding being positioned to provide a precise fit for biotin. It has been reported (Linvah et al., 1993) that in the unoccupied binding site, the structure of the bound solvent molecules resembles that of the substrate. During the course of binding, solvent molecules are replaced by biotin and an exposed loop, residues 36 through 44 in avidin, orientates itself to lock the substrate in the binding site. Within the binding site, a number of aromatic amino acids form a "hydrophobic box" surrounding the biotin molecule. The two biotin-binding proteins differ somewhat in this area; in avidin, a phenylalanine, Phe^{79}, is involved in the binding, but this is replaced by a tryptophan, Trp^{92}, in streptavidin. Also, an additional aromatic amino acid, Phe^{72}, is involved that has no equivalent in streptavidin. The importance of the regions of monomer-monomer interaction is also emphasized in the binding site; for instance, the residues in the binding site of avidin comprise Trp^{70}, Phe^{72}, Phe^{79}, and Trp^{97} from one monomer and Trp^{110} from another. Similarly, the binding site of streptavidin contains only four aromatic residues, but these are again derived from adjacent monomers with Trp^{79}, Trp^{92}, and Trp^{108} from one monomer and Trp^{120} from the adjoining one. Several crucial hydrogen bonds are formed between biotin and the side chains of polar amino acid residues. Interestingly, the biotin carboxyl group is involved in five hydrogen bond interactions in avidin but only two in streptavidin. Together with the additional aromatic residue in the avidin-binding site, this has been used (Linvah et al., 1993) to explain the 100-fold tighter binding of biotin to avidin compared with streptavidin. Another contributing factor may be the length of the loop that becomes ordered on binding and locks the ligand in the binding site. The longer loop in avidin contains nine residues, compared with six residues in streptavidin, and provides tighter closure.

The importance of the tryptophans in the binding site of streptavidin has been investigated (Chilkoti et al., 1995b) using site-directed mutants. This work confirmed the hypothesis that van der Waals and hydrophobic interactions, which are crucial to the binding process, are largely mediated by the aromatic side chains of the tryptophan residues. Similarly, the role played by the tryptophan residue Trp^{120} of one subunit in binding a biotin molecule held by an adjoining subunit has been investigated (Sano and Cantor, 1995). Mutation of this residue to a phenylalanine had no effect on the biotin-induced formation of streptavidin tetramers or on the amount of binding sites per tetramer but resulted in a drastic drop in biotin-binding affinity, verifying the significance of the role played by Trp^{120} in the tight binding of biotin. It has been proposed that the mutation of Trp^{120} to Phe may have introduced additional structural transformations in the protein at or near the binding site, considerably lowering the affinity for biotin (Sano and Cantor, 1995).

The question of whether binding of biotin to the streptavidin tetramer is cooperative has been addressed (Jones and Kurzban, 1995), and it was found that despite the large binding energy involved, biotin slowly equilibrates between tetramers, and the equilibrium distribution indicates that binding is not cooperative. The degeneracy of the streptavidin tetramer can be abolished by the construction of chimeric tetramers composed of unmodified wild-type subunits and genetically engineered subunits (Chilkoti et al., 1995a). The chimeric streptavidin tetramers are produced by mixing wild-type streptavidin and site-directed mutants, followed by denaturation and slow renaturation. This approach allows tetramers to be produced in which one or more monomers retain the high affinity for biotin and one or more monomers possess a novel property such as reduced affinity due to a greater off-rate, enabling enhanced recovery in applications where streptavidin is used as a capture molecule. In applications where streptavidin is conjugated to functional moieties such as antibodies, a mutant subunit can be incorporated that spatially directs the assembly of the tetramer to encourage conjugation to a particular face of the protein, leaving another face free for biotin binding.

The widespread interest in avidin and streptavidin largely results from their high specificity for biotin. However, other molecules are known to bind to these proteins, albeit with much lower affinity. A number of peptide ligands have been reported that contain the consensus tripeptide sequence HPQ (Giebel et al., 1995; Katz, 1995). The amino acids flanking this sequence play important roles, but the interactions with the HPQ portion of the peptide are all very similar, involving common hydrogen bonds and van der Waals interactions. Some disulfide-bonded cyclic peptides bind with several hundred-fold greater affinity than their linear counterparts, and binding is seen to be greater at neutral pH than at pH 5 (Weber et al., 1992). The relationship between 1) the structural differences of biotin and the peptides studied so far and 2) their different binding characteristics is not clear at this moment. The stereochemical features of biotin that lead to the high binding affinity are not easily derived from the corresponding bound peptide structures. The binding of other ligands, such as synthetic azobenzenes, has also been reported (Weber et al., 1994). Biotin accounts for half the avidin-binding material in human serum with the remaining 50% comprising biotin metabolites (Mock et al., 1995).

B. Lipid-Binding Proteins

The effective transport of hydrophobic molecules within a cell system is mediated in part by LBPs. These low-molecular weight proteins, which bind, among other compounds, fatty acids and retinol analogs (Newcomer, 1995), are present in a diverse range of cell types. Like many other binding proteins, their exact role is unclear, but there is presently little to suggest a function other

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than solubilization and transport of hydrophobic ligands.

The large and expanding group of LBPs includes commonly known members such as the milk whey protein β-lactoglobulin as well as lesser known proteins such as α_1-microglobulin (Åkerström and Lögdberg, 1990), plasma or tear transthyretin (formerly called prealbumin) (Redl et al., 1992; Garibotti et al., 1995; Monaco et al., 1995), proteins secreted by the mammalian von Ebner's salivary glands of the tongue, soluble OBPs secreted by nasal glands, and mammalian aphrodisin (Kruhoffer et al., 1997; Magert et al., 1999). The OBPs appear to function as cofactors in olfaction and taste sensing (Pevsner et al., 1990; Korsching, 1991; Kock et al., 1994; Garibotti et al., 1995). Distantly related members of the group may have less than 20% amino acid sequence identity, but many have highly similar tertiary structures as will be discussed below. The LBPs (also called "calycins" because of the shape of the binding pocket) can be divided into two groups: the intracellular LBPs (iLBPs) and the extracellular LBPs (eLBPs; also referred to as "lipocalins"). A number of LBPs has been studied crystallographically (for example, see Table 1) and/or by NMR (e.g., Lassen et al., 1995). Many LBPs still await characterization.

A detailed description of the structural features of individual proteins is unnecessary here because this has been the subject of an excellent review by Banaszak et al. (1994). However, because the family members share many structural motifs, a brief overview will be useful to anyone interested in using or modifying these proteins. The eLBPs are composed of approximately 175 amino acid residues, whereas the iLBPs are slightly smaller with about 130 residues. The principal feature of both groups is an antiparallel β-barrel with a repeated +1 topology, composed of 10 strands in the iLBPs and 8 in the lipocalins. The iLBP barrel is more elliptical than that of the eLBPs and is not continuously hydrogen bonded. The binding site has been found to exist almost exclusively within this β-barrel, and moreover, the stoichiometry of binding is generally one hydrophobic molecule per molecule of protein. Comparison of the group structures shows that the initial three and final two strands of the iLBP barrel correspond well to the initial three and final two strands of the eLBPs (Flower et al., 1993). The strands of both families are mostly connected by tight turns, except the first two strands of the iLBPs, which are connected by a helix-turn-helix motif that is absent in the corresponding region of the eLBPs. Also, a "gap region" exists in the iLBP structure between strands 4 and 5 that is missing in the eLBP structure (Banaszak et al., 1994).

The conformational similarity between members of the LBP group is remarkably high; the β-barrel, the link regions, and the binding cavity are virtually superimposable. Despite the high degree of structural homology, the sequence identity is generally low, particularly in the eLBPs where it is often less than 20%. However, there are regions where primary structure conservation is greater. Within the eLBPs, it has been reported (Katakura et al., 1994) that the only completely conserved residue is Trp^{19} and that this residue, although not vital for ligand binding, was critical for maintaining the environment surrounding the bound ligand for correct positioning of the ligand and for stabilizing the overall protein structure. Compared with the eLBPs, the iLBPs show a greater range of intragroup homology from 20 to over 80%, the average being 20 to 30%. Even though the number of homologous residues is low, a number of conserved amino acids do occur, many of these being involved in the formation of a structural backbone. The conserved residues appear to be either internal hydrophobic residues or neighboring hydrophilic amino acids that help to stabilize the backbone structure. Some of the hydrophobic residues within this backbone also form part of the wall of the binding site (Banaszak et al., 1994).

As a group, the LBPs are able to bind a diverse range of ligands, but also the individual members of the group bind a wide range of ligands (Richieri et al., 1994). Different LBPs can have ligand binding spectra that overlap to various degrees as summarized by Banaszak

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TABLE 1
Some lipid-binding proteins with known crystal structure

Lipid-Binding Protein	Abbreviation	Size	Ligand	Resolution	Ref.[a]
		kDa		*Å*	
β-Lactoglobulin	β-LG	18	retinol, fatty acids	2.8	1
Serum retinol-binding protein	pRBP	21	retinol	1.7	2–4
Urinary α_2-globulin	A2U	18	pheromones	2.8	5
Major urinary protein	MUP	18	pheromones	2.4	5
Intestinal fatty acid-binding protein	IFABP	15	fatty acids	1.2	6
Cellular retinol-binding protein	CRBP	15.5	retinol	2.1	7
Cellular retinol-binding protein II	CRBPII	15.5	retinol	1.9	8
Cellular retinoic acid-binding protein	CRABP	15.7	all trans retinoic acid	2.7	9
Adipocyte lipid-binding protein	ALBP	15.1	fatty acids, retinoic acid	1.6	10
Human muscle fatty acid-binding protein	M-FABP	14.9	fatty acids	2.1	11
Schistocerca gregaria muscle fatty acid-binding protein	sM-FABP	14	fatty acids	2.2	12

[a] References cited: 1, Papiz et al. (1986); 2, Zanotti et al. (1993a); 3, Zanotti et al. (1993b); 4, Zanotti et al. (1994); 5, Böcskei et al. (1992); 6, Scapin et al. (1992); 7, Cowan et al. (1993); 8, Winter et al. (1993); 9, Thompson et al. (1995); 10, Xu et al. (1993); 11, Zanotti et al. (1992); 12, Haunerland et al. (1994).

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et al. (Banaszak et al., 1994). The affinities of various proteins for fatty acid ligands are similar (Wootan et al., 1990), ranging from approximately 2.5×10^5 to 5×10^6 M^{-1} and showing a general trend of decreasing affinity for shorter hydrophobic chain lengths (Banaszak et al., 1994).

Unlike the biotin-binding proteins, the unoccupied binding site in the hydrophobic transporter molecules does not reflect the shape of the fatty acid ligand. The volume of the binding cavity is considerably larger than any prospective ligand with perhaps only one-third of the cavity volume being occupied by the bound fatty acid or retinoid. This leaves space for a number of ordered water molecules, with 16 being identified in holo-adipocyte LBP (ALBP) (Xu et al., 1993). In the absence of ligand, there is space for 40 disordered solvent molecules (Banaszak et al., 1994). There are more than 35 amino acids lining the binding cavity, of which perhaps only half are hydrophobic; the remainder are polar and/or ionizable. Generally, the polar head of a fatty acid ligand lies at the bottom of the cavity, and binding of ligand results in little conformational change in the protein.

By far, the most studied of the hydrophobic transporter molecules is the 18-kDa globular milk whey protein, β-lactoglobulin. The exact role of this protein is unknown. It is believed to function as a transporter of retinol (vitamin A) to the neonate in mammals, although its absence in the milk of many mammals, including humans, indicates that this role is not essential. Apart from retinol, it binds retinoic acid and other retinol analogs (Cho et al., 1994a), β-carotene, β-ionone compounds, saturated (Fig. 6) and unsaturated fatty acids (O'Neill and Kinsella, 1987), aliphatic hydrocarbons such as heptane and pentane, and carbonyl-based compounds such as 2-octanone (O'Neill and Kinsella, 1987). The affinities for the different compounds are widely different. For example, the K_a values of heptane and 2-heptanone are 4.8×10^5 and 1.5×10^2 M^{-1}, respectively, whereas that of retinol is 5×10^7 M^{-1} (O'Neill and Kinsella, 1987). It has been previously proposed that β-lactoglobulin could be used as a versatile carrier of small hydrophobic molecules in controlled delivery applications (Batt et al., 1994). This application is likely to be facilitated by expression of β-lactoglobulin in the food-grade yeast *Kluyveromyces lactis* (Rocha et al., 1996) and by the recently obtained high-yield expression in the methylotrophic yeast *Pichia pastoris* (Kim et al., 1997; Denton et al., 1998, de Wolf et al., 1998), which is food-grade according to the FDA and, although not officially approved, also seems to be a safe species for humans (Scrimshaw and Murray, 1995). The crystal structure of bovine β-lactoglobulin, exhibiting the characteristic β-barrel structure of eLBPs, has been known for some time (Papiz et al., 1986), and differences in the structure between the naturally occurring genetic variants have been studied (Dong et al., 1996). The variants A and B differ in just two positions with no significant effect on the binding affinity or crystal structure. In contrast to the other eLBPs, β-lactoglobulin contains two disulfide bonds and a free thiol group. For some time, there has been uncertainty over the location of the retinol-binding site. The initial observations based on its sequence and structural similarity to retinol-binding protein (RBP) indicated the retinol to be bound within the β-barrel. This hypothesis was questioned when crystallographic evidence seemed to indicate the retinol to be bound in a surface groove (Monaco et al., 1987). However, further studies, using selective amino acid modifications, confirmed the retinol-binding site to be within the central calyx (Cho et al., 1994a). After binding to β-lactoglobulin, retinol and other hydrophobic molecules are protected from (oxidative) degradation (Hattori et al., 1995; Iametti et al., 1995). Site-directed mutagenesis studies have been facilitated by the expression of β-lactoglobulin in *E. coli* (Batt et al., 1990; Chatel et al., 1996) and in the yeast *K. lactis* (Rocha et al., 1996; Kim et al., 1997; Denton et al., 1998). These highlighted the importance of individual residues for retinol binding (Zanotti et al., 1993b) and protein stability (Katakura et al., 1994). Much work has been devoted to the denaturation (Cho et al., 1994b; Dumay et al., 1994; Hong et al., 1994; Jeyarajah and Allen, 1994; Macleod et al., 1995; Qi et al., 1995; Boye et al., 1996) and renaturation (Dufour et al., 1994) behavior of β-lactoglobulin. Hattori et al. (1993) used β-lactoglobulin as a model protein to show that, following denaturation, specific moieties within a protein cannot return to the native conformation from a denatured state. The results were based on the inability of monoclonal antibodies to recognize renatured β-lactoglobulin. However, the incomplete refolding of the protein did not affect its biological properties. β-Lactoglobulin also exhibits the ability to form heat-induced aggregates and gels (Hong et al., 1994; Matsuura and Manning, 1994) both separately and in combination with other macromolecules such as polysaccharides (Ndi et al., 1996a,b), a property which could be valuable in the development of protein-mediated transport systems.

Another important eLBP is the plasma RBP (pRBP) found in the circulatory system bound to transthyretin

FIG. 6. Crystal structure of bovine β-lactoglobulin with palmitic acid (C16:0) in the binding pocket. The structure was obtained from the Brookhaven protein database (PDB), as deposited by S.-Y. Wu and L. Sawyer (see Wu SY et al., 1999). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc.

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(Monaco et al., 1995). This specific retinol carrier has been isolated from several vertebrates, including mammals, birds, and fish, although most work has been done on bovine and human pRBP (Zanotti et al., 1993a,b, 1994; Sivaprasadarao and Findlay, 1994). Recently, an *E. coli* expression system for pRBP has also been described (Müller and Skerra, 1993) that should make future mutagenesis possible. Bovine and human pRBP are found to be quite similar, exhibiting 92% sequence similarity and practically identical three-dimensional structure (Zanotti et al., 1993a). They exhibit the typical eLBP β-barrel structure with the retinol molecule completely encapsulated within the β-barrel with its β-ionone ring in the center, its tail pointing outward, parallel to the barrel axis, and the end of its tail close to the protein surface. In accordance, it was found that alteration of the cyclohexene ring of retinol may result in a lowering of binding affinity, whereas substantial modifications of the hydroxyl end do not preclude high-affinity binding to pRBP (Zanotti et al., 1993b). The binding of retinol to pRBP is thus stabilized mainly by hydrophobic interactions with the ring moiety playing the dominant role.

Interestingly, the orientation of the ligand in eLBPs is opposed to that in iLBPs, where the polar end is buried inside the cavity. Accordingly, the binding appears to be particularly sensitive to modifications of the polar end, e.g., the hydroxyl of retinol in the case of intracellular RBP possibly in addition to changes of the isoprene chain (Malpeli et al., 1995). A member of the iLBP family that binds exclusively fatty acids (no retinoids) is the intestinal fatty acid-binding protein (I-FABP). The 15-kDa protein is synthesized in the enterocytes of the small intestine. Each molecule binds a single saturated, monounsaturated, or polyunsaturated fatty acid. The apo and holo forms of I-FABP from the rat have been characterized to high resolution (1.2 and 2.0 Å, respectively) (Scapin et al., 1992). The protein has the common structural features of the iLBP family, the carboxylate of the fatty acid being buried in the β-barrel of the binding cavity. A comparison of apo and holo forms of the rat I-FABP shows that ligand binding does not induce clear conformational changes. The ligand-binding cavity of I-FABP is different from that of other proteins in the family in that the amino acid side chains that constitute the binding cavity are predominantly hydrophobic, and those that are in close contact with the ligand are quite different from those of related proteins of known structure. The protein has been successfully expressed in *E. coli* with no loss of function and an absence of co- or post-translational modifications. Studies based on such systems have yielded detailed information about the nature of the interaction between protein and fatty acid ligand (Sacchettini and Gordon, 1993) that deal comprehensively with the combination of X-ray crystallography and mutagenesis to analyze the binding forces. They propose that fatty acid binding results from a series of weak forces, including a complex range of electrostatic and dipolar forces between the fatty acid carboxylate, polar, and ionizable groups of the protein and solvent molecules, as well as a range of van der Waals contacts involving residues from both the β-sheets and the helices. The importance of a number of individual residues has been implicated in playing distinct roles in various aspects of the binding interaction (Scapin et al., 1992; Zanotti et al., 1992; Sacchettini and Gordon, 1993; Banaszak et al., 1994).

The ALBP is a 14.6-kDa protein found in adipose cells. It is known to bind long-chain fatty acids and retinol (LaLonde et al., 1994a,b), and its function is believed to be the transport and solubilization of fatty acids. It has been well characterized and shown to possess the typical LBP properties such as the β-barrel binding cavity and the retention of conformation between apo and holo forms. The specificity is generated as the result of interaction of the fatty acid carboxylate with two arginines and a tyrosine. In some adipocytes, the protein is phosphorylated in response to insulin (Xu et al., 1991). The effects of tyrosyl phosphorylation on the cellular function of ALBP is unknown, but its in vitro effect is the modulation of ligand binding, with the phosphorylated protein showing much reduced affinity for immobilized long-chain fatty acids (Xu et al., 1993). Murine ALBP has been successfully cloned and expressed in *E. coli* (Xu et al., 1991) and characterized crystallographically to 1.6 Å. The recombinant protein has been used for site-directed mutagenesis studies of the binding site (Sha et al., 1993). Increased insight into structure-function relationships of fatty acid-binding proteins is now coming from molecular modeling and dynamics studies (Woolf, 1998). Compared with the (strept)avidin system, the induction of triggered release or uptake of ligands is probably much more difficult to elicit in LBPs, even after modification of the protein structure by genetic means. However, the LBP system can be used with a broader range of (apolar or amphiphilic) ligands and allows easier switching between (such) ligands.

C. Periplasmic Binding Proteins

These proteins, found in the periplasmic space of Gram-negative bacteria, serve as initial high-affinity receptors in the active uptake of specific nutrients. After binding their specific substrate molecule, they interact with a membrane bound complex, triggering a series of events that results in translocation of the substrate. The mechanism of the transport system has been reviewed by Ames (1986), and although the number of binding proteins reported has increased since then, this work still provides a valuable basis for the understanding of bacterial periplasmic transport. More recently, Tam and Saier (1993) reviewed the mutual relationships between extracellular solute-binding receptors of bacteria, providing a clear overview of the various protein "clusters". The family of approximately 50 periplasmic binding pro-

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teins is valuable with respect to development of a system of protein-mediated delivery in that they collectively bind a wide range of ligands. This provides versatility (viz., when use can be made of several members of the group) without sacrificing ligand specificity, which is conferred by the individual proteins. The periplasmic binding proteins exhibit specificity for carbohydrates, amino acids, peptides, metals, or vitamins. Some binding proteins are highly ligand-specific, whereas others are known to bind several related ligands, each with comparable affinity. In general, the largest proteins bind the largest ligands, the protein molecular masses ranging from 20 to 58 kDa, with the average being approximately 33 kDa. The sequence similarity between members of the group is generally low. Nevertheless, the different proteins tend to exhibit a similar affinity for their respective ligands, the K_d being of the order of 5.0×10^{-7} M (Spurlino et al., 1991). The crystal structures of a growing number of periplasmic binding proteins have been elucidated, and, despite the low sequence homology, the overall three-dimensional structures are similar. The structural features responsible for different functions (binding of ligands, binding to membrane receptors) are located in structurally distinct regions in each protein. All the proteins are composed of two lobes, joined by two or three peptide strands, which function as hinges. The ligand-binding site is located within a cleft between the two lobes (Fig. 7). The bound ligand is typically buried within this cleft, where it is almost inaccessible to bulk solvent. This form of the protein is designated the "closed" form. The "open" form is obtained by rotation of the lobes relative to one another by means of the peptide hinge region (see Fig. 7). This allows the access of the solvent to the binding cleft as well as the entry or exit of the ligand. The lobes themselves do not change their conformation significantly during the rotatory movement. On closure, residues from both lobes are involved in the formation of a network of interactions with the bound ligand, interactions that involve polar and nonpolar side chains as well as the protein backbone and charged side chains. Thus, the presence of the bound ligand shifts the relative energies of the two conformations (i.e., their probabilities of occurrence) in favor of the closed form, which is stabilized by the ligand. There is still some doubt whether the empty proteins continually alternate between the open and closed form or whether the conformational change occurs only once on binding of the ligand. For example, the equilibrium conformational free energies of the two forms could be very different, implying that the chance of occurrence of the closed form would be low (or virtually zero) in the empty protein. Alternatively, the activation energy could be relatively large in the absence of ligand, preventing the empty protein from closing. Although not unambiguously proven, it is generally thought that a (net) reversion of the conformation from the closed to the open state and a subsequent (directional) release of ligand are specifically induced by the binding of the protein-ligand complex to a corresponding membrane protein (Ames, 1986; Dean et al., 1992; Mowbray, 1992; Shilton and Mowbray, 1995). The membrane protein translocates the ligand to the cytoplasm (Ames, 1986; Shilton and Mowbray, 1995). It specifically recognizes the closed conformation (top of the two lobes) of the periplasmic binding protein with its complexed ligand (Spurlino et al., 1991; Sharff et al., 1992). Interestingly, modifications of the hinge region have been shown to affect the ability of the periplasmic proteins to bind their ligands. For example, in the case of maltose-binding proteins, the bound ligand was found to directly interact with at least one hinge-region residue, viz., Glu^{111} (Spurlino et al., 1991; Sharff et al., 1995). In this light, the "hinge" region represents an interesting target area for protein engineering studies exploring the possibility of making periplasmic binding proteins stimulus- (e.g., protease-, pH-) responsive. Such developments could well find application in stimuli-responsive ligand release systems (pest control, hygiene, technical applications, and special therapeutic applications). However, it is not known whether the hinge region can still influence the binding after completion of the transition from the open to the closed conformation because the closed conformation is stabilized by interactions between the two lobes and the ligand. Such an influence could occur if the ligand-containing protein were subject to frequent alternation between the closed (major) and the open (minor) conformations, which would then be in equilibrium, in a manner similar to what has been proposed in relation to the empty protein. Obviously, this is an interesting subject that requires further research.

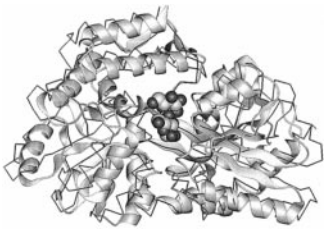

FIG. 7. Crystal structure of *E. coli* periplasmic maltodextrin-binding protein. The molecule represented as a ribbon was complexed with maltodextrin; the molecule represented as a thread is the empty protein without ligand. The structures were obtained from the Brookhaven protein database (PDB), as deposited by J. C. Spurlino and F. A. Quiocho for the complex (see Quiocho et al., 1997) and by A. J. Scharff and F. A. Quiocho for the unliganded protein (see Sharff et al., 1992). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc.

Examples of periplasmic binding proteins include those with specificities for maltodextrin [maltodextrin-binding protein (MBP), Fig. 7] (Spurlino et al., 1991; Dean et al., 1992; Sharff et al., 1992, 1995), histidine [histidine-binding protein (HBP)] (Oh et al., 1994b; Wolf et al., 1994, 1995), lysine/arginine/ornithine [lysine/arginine/ornithine-binding protein (LAOBP)] (Kang et al., 1991; Oh et al., 1993, 1994a), leucine/isoleucine/valine

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(leucine/isoleucine/valine-binding protein) (Ohla et al., 1993), glutamine (glutamine-binding protein) (Hing et al., 1994), dipeptides [dipeptide-binding protein (DPP)] (Dunten and Mowbray, 1995; Nickitenko et al., 1995), oligo-peptides [oligopeptide-binding protein (OPP)] (Hiles et al., 1987; Kashiwagi et al., 1990), arabinose (arabinose-binding protein) (Kehres and Hogg, 1992), galactose/glucose (galactose/glucose-binding protein) (Mowbray, 1992), sulfate (SBP) (Wang et al., 1994), phosphate (PiBP) (Wang et al., 1994), and ribose (ribose-binding protein) (Binnie et al., 1992; Björkman et al., 1994). The binding proteins with known tertiary structure have little in common with respect to size, amino acid composition, and specificity for the primary ligands, with the exception of the HBP and LAOBP pair, which show sequence identities of 70%. HBP and LAOBP are probably the most extensively studied of the periplasmic binding proteins (Oh et al., 1994a; Wolf et al., 1995). In addition to a high degree of sequence similarity, they contain an identical number of residues and have closely resembling domains that interact with common membrane-bound components of the transport system. Comparison of HBP and LAOBP is interesting because LAOBP also binds histidine, albeit with reduced affinity. The conformations of ligand-bound forms of LAOBP are essentially the same for lysine, arginine, and ornithine, with minor variations restricted to the area directly around the binding site. All of the residues surrounding the ligand in HBP are identical with those in LAOBP with the exception of residue 52, where a leucine in HBP is replaced with a phenylalanine in LAOBP. Furthermore, two other residues interact in a different way with the ligand in the two proteins (Oh et al., 1994b), reflecting the difference in shape and length of the ligands. Ser^{69} forms a hydrogen bond from its carbonyl oxygen in HBP, but in LAOBP, a hydrogen bond originates from its hydroxyl group. Asp^{11} in LAOBP makes an ionic and hydrogen bonding contribution, but in HBP, it is not close enough to the bound ligand to make a contribution.

Comparison of the residues lining the binding cleft in HBP and LAOBP show that only five amino acids differ between the two proteins, and it has been proposed that the differences in substrate affinities may be due to one or more of these residues. As mentioned above, only one of these five residues, residue 52, interacts with the ligand, although it is still possible that one or more of the other residues directly or indirectly contributes to the binding interaction. However, it is not apparent why a leucine at position 52 in HBP should result in this protein having a higher affinity for histidine than LAOBP, which has a phenylalanine in the corresponding position. Oh et al. (1994b) proposed that the presence of the bulkier phenylalanine prevented the formation of an essential ionic interaction. Hydrogen bonds play an important role in the recognition of the respective ligands, and their directional nature helps confer specificity on the binding site. In these particular binding proteins, the binding pocket seems to be large enough to accommodate the maximum common volume of the four ligands plus three water molecules. Residues within the binding pocket undergo small conformational changes to achieve geometric fit and most favorable interactions with the ligand. To achieve optimal fit, protein-bound water molecules can be displaced by the ligand.

To further understand the functions of individual amino acid residues in the periplasmic binding proteins, Wolf et al. (1995) carried out a series of studies on mutant proteins. They found that in many cases, the substitution of a single amino acid had quite pronounced effects, generally leading to the inability of the protein to assume the closed conformation of the liganded form. A total of 12 residues are involved in binding the histidine molecule, six of which have hydroxyl functions. In total, the histidine molecule is held in place by 10 hydrogen bonds, 2 salt links, and more than 60 van der Waals contacts (Yao et al., 1994). Tyr^{14} is the singly most involved protein residue, interacting with seven different histidine atoms.

Of the four sugar-binding proteins, the broadest specificity (or highest versatility) can be attributed to the MBP. It is able to bind linear maltodextrins of two to seven α(1–4)-linked glucosyl units as well as cyclodextrins such as cyclomaltohexose and cyclomaltoheptose. The other three well characterized sugar-binding proteins are able to bind only monopyranosides, although their specificity is not limited to an individual sugar. Arabinose-binding protein binds L-arabinose, D-galactose, and D-fucose; the galactose/glucose-binding protein binds D-galactose and D-glucose; and the ribose-binding protein binds D-ribose. Of these, MBP is the most extensively studied. As with the amino acid-binding proteins, its binding site is located at the base of a groove between the two domains, almost completely shielded from solvent and bound by a combination of hydrogen bonds and van der Waals interactions. Every polar and nonpolar atom of the bound sugar molecule forms extensive hydrogen bond and van der Waals interactions, respectively, within the binding site. Thus, the number of van der Waals contacts is unusually high, e.g., 65 in MBP. The sugar hydroxyl groups act as simultaneous hydrogen bond donors and acceptors. MBP has a relatively high number of aromatic residues, many of which are located in or near the binding groove and take part in stacking interactions with the carbohydrate pyranose ring. Together with the deep binding, which shields more than 96% of the sugar molecule from the bulk solvent, these interactions make the complexes between the binding proteins and carbohydrates some of the tightest.

Sharff et al. (1992, 1995) have studied MBP in detail both crystallographically and by the use of mutations of the *malE* gene that encodes MBP. They observed the classic rigid body "hinge-bending" between the two do-

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mains to reveal the sugar-binding site, which is characteristic of the periplasmic binding proteins, and proposed that the hinge-mediated closing is triggered by the ligand-induced exclusion of water from the binding site, which is in agreement with the lower number of ordered water molecules found in the closed, maltose-containing site. Using mutant proteins, it was shown that, apart from the binding site, the hinge region of the protein is particularly sensitive to minor changes, whereas several other regions were tolerant to substantial modifications. In one example, a helix was deleted with little effect on sugar binding, binding to the membrane, and general structure of the protein.

Other periplasmic binding proteins are less well studied. DPP and OPP are two of the largest members of the periplasmic binding protein family. DPP from *E. coli* shows 24% sequence homology with the slightly larger OPP of *Salmonella typhimurium*. Certain structural features of the oligopeptides seem to be essential for their binding to OPP. These include a protonated primary amino group, L-stereochemistry, and a modified terminal carboxyl (Nickitenko et al., 1995). The nature of the side chains appears to make little contribution to ligand binding. These proteins are distinguished from the previous periplasmic binding proteins by their broad specificity. DPP binds dipeptides and some tripeptides as diverse as glycine-glycine, lysine-lysine, and phenylalanine-phenylalanine with similar affinity. OPP binds peptides that vary in length from two to five amino acids with little consideration to their side chains; it will not bind single amino acids. In both proteins, specific interactions seem to be restricted to the peptide backbone, and there are few specific interactions, if any, between the binding protein and the side chains of the peptide. Off the binding site, pockets are present that accept these side chains and that are large enough to accommodate any of the naturally occurring side chains. Unlike other periplasmic binders, these proteins are composed of three domains. Domains I and III are analogous to the domains in the other family members in that they form the two lobes of the "Venus flytrap", which close to entrap the ligand. Domain II has no counterpart in the bilobate binding proteins. Its function is not clear, but it appears to contribute residues to line the pocket that accepts the side chain of the initial peptide residue. This domain contains two β-hairpins and consists of approximately 120 residues in OPP and approximately 150 residues in DPP; it makes few contacts with the ligand.

In contrast to the peptide transporters, the oxyanion transport molecules show extreme specificity (Wang et al., 1994). The structurally similar tetrahedral oxyanions, phosphate and sulfate, are bound, respectively, by the PiBP and the SBP. The high specificity for each oxyanion is vital in that one ligand cannot become an inhibitor for the transport of the other. The specificity is derived from the presence or complete absence of protons on the substrate molecule. The binding site of PiBP is designed to recognize the protons of weakly acidic mono- and dibasic phosphate, whereas the binding site of the SBP is constructed to receive only the fully ionized sulfate. Once bound, the phosphate is held in place by hydrogen bonds that involve 12 polar amino acid residues. The carboxyl function of one of these, Asp^{56}, serves as a charged hydrogen bond acceptor but disallows, by charge repulsion, the binding of a sulfate group. Site-directed mutagenesis studies by Wang et al. (1994) have shown that the specificity of PiBP can be even further enhanced. For instance, by replacement of Thr^{141} with an Asp residue, the binding can be limited to the monobasic form of phosphate ($H_2PO_4^-$), excluding the binding of the dibasic form (HPO_4^{2-}). Such exquisite modifications of the binding specificity provide information that can be applied in the tailoring of these proteins to carry other ligands.

D. Lectins

Lectins are proteins that reversibly bind carbohydrates with high affinity and specificity. The binding involves hydrophobic interactions as well as hydrogen bonds. The lectins are able to induce cell agglutination and are thus believed to play a key role in plant defense mechanisms. These ubiquitous proteins are found in microorganisms, animals, and plants (Lis and Sharon, 1986; Brossmer et al., 1992; Sharon, 1993; Jordan and Goldstein, 1994; Iobst et al., 1994; Kennedy et al., 1995; Peumans and Van Damme, 1996). Plant lectins have been purified from leaves, fruits, roots, and tubers but primarily from seeds. There has been much discussion about the definition of a lectin; the current definition for plant lectins is related to function rather than structural criteria. All plant proteins possessing at least one noncatalytic domain that binds reversibly to a mono- or oligosaccharide are considered to be lectins. If this definition were extended to all carbohydrate-binding proteins, the aforementioned periplasmic binding protein MBP would also fulfill the criteria of a functional lectin. Lectins have been divided into three classes. The merolectins, found in certain microorganisms (*Actinomyces*, *Myxococcus*, and *Mycoplasma* among others) consist of a single carbohydrate-binding domain. These small single-chain proteins are incapable of agglutinating cells. The hololectins behave as true agglutinins. They are similarly composed exclusively of carbohydrate-binding domains but contain at least two such domains. The chimerolectins are fusion proteins composed of at least one sugar-binding domain and an unrelated domain with a separate biological function, e.g., the membrane-anchoring domain of vertebrate lectins. The majority of currently known plant (holo)lectins can be further classified into four subgroups of related proteins. The legume lectins occur exclusively within the legume family. The monocot mannose-binding lectins occur in at least five different families and all have similar molecular structure and binding specificity. The chitin-binding lec-

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tins, found in unrelated taxonomic families, are composed of similar structural domains and exhibit comparable specificity. The type 2 ribosome-inactivating proteins are chimeric proteins composed of a catalytically active A chain covalently linked to a carbohydrate-binding B chain.

The lectins of leguminous origin are perhaps best characterized; the three-dimensional structures of a number of them are known (Einspahr et al., 1986; Shaanan et al., 1991). The primary structures are highly homologous, resulting in similar three-dimensional conformations. β-Sheets dominate, whereas α-helices are virtually absent. Another common feature is the presence of two metal ions, Ca^{2+} and Mn^{2+}, that appear to be essential for sugar binding. The amino acids involved in metal binding are highly conserved throughout the legume lectin subgroup. The lectins from pea (*Pisum sativum*), sweet pea (*Lathyrus ochrus*), lentil (*Lens culinaris*), and vetch (*Vicia faba*) of the legume tribe *Vicieae* (a subgroup of the subfamily *Papilionatae*) show identical binding specificity for D-mannose- and D-glucose-type residues (Van Eijsden et al., 1992). Lectins from pea, vetch, and sweet pea have almost identical three-dimensional structures. Not only are the amino acids involved in sugar binding highly conserved, but they occupy comparable molecular positions in a surface cleft adjacent to the metal-binding site.

The thoroughly characterized pea lectin is representative of the *Vicieae* group in that it is a dimeric β-barrel protein with post-translational modification of the monomers to form the small α-chain and larger β-chain and with overall $\alpha_2\beta_2$-configuration. Hoedemaeker et al. (1993) have succeeded in modifying the properties of this lectin by specific amino acid mutations. Substitution of a valine with an alanine, at a position within a loop believed to play a crucial role in carbohydrate binding, had a large effect on the stability of the protein: although the mutant protein still bound sugar at ambient temperature, the binding activity was lost at 37°C. Such a property could be exploited to provide carrier systems that release their ligand on entry into the body, suppressing the notorious allergenicity (resulting from the affinity for carbohydrates) at body temperature. Another study (Van Eijsden et al., 1992) highlighted the importance of the involvement of residue Asn^{125} in Ca^{2+} binding and directly interacting with the bound sugar molecule. Replacement of this asparagine with an aspartate destroyed the carbohydrate-binding activity. The highly conserved nature of this residue indicates that this effect is common to all legume lectins.

The binding sites of the lectins are generally shallow depressions on the protein surface (Fig. 8) with an adjustable topology that allows them to optimally align the specific ligands with the arrangement of residues involved in hydrogen bond formation and hydrophobic interactions. However, there are many factors that govern specificity, including the conformation of the carbohydrate ligand itself. Jordan and Goldstein (1995) highlighted particular residues responsible for ligand recognition in the lima bean lectin, whereas Nagahora et al. (1995) studied the pH-dependent role of individual aromatic residues in the sugar binding of wheat germ agglutinin by using site-specific mutagenesis.

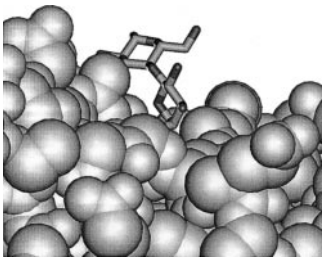

FIG. 8. Crystal structure of human lectin L-14-II complexed with lactose. The structure was obtained from the Brookhaven protein database (PDB), as deposited by Y. D. Lobsanov, M. A. Gitt, H. Leffler, S. Barondes, and J. M. Rini (see Lobsanov et al., 1993). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc.

The animal lectins consist of two major groups: the calcium-dependent C-type and the calcium-independent S-type lectins. The S-type lectins are soluble, β-galactoside-specific binding proteins found in all the vertebrate species studied thus far. They can be divided into three main classes according to their molecular masses: 14, ~30, and 67 kDa. Interestingly, the larger proteins that have been sequenced all contain a domain with sequence homology to the 14-kDa proteins. The C-type lectins fall into two groups: those that bind ligands containing galactose or *N*-acetylgalactosamine and those that bind mannose and/or *N*-acetylglucosamine. The specificity is linked to the orientation of the 3- and 4-hydroxyl groups.

A simple and elegant idea for the pharmacological application of lectins was the use of encapsulated (i.e., immunologically shielded) lectins for the delivery of (glycosylated) insulin, with glucose displacing the insulin in a concentration-dependent manner (Brownlee and Cerami, 1979; Jeong et al., 1985; see Fig. 9. Lectins, such as concanavalin A, have also been applied in the past to target drugs or to retain them at the site of administration rather than release them in a controlled manner. In all these applications, the drug or active compound was covalently attached (conjugated) to the lectin carrier. Examples of conjugated active agents are asparaginase and daunomycin for tumor suppression, dextranase against plaque formation, and trypsin for application sites where unwanted proteins should be degraded (Shier, 1979). In view of their imunogenicity, lectins could be of use especially in nonpharmacological and nonfood applications like agricultural pest control.

E. Serum Albumins

In addition to being the most abundant protein in the circulatory system and maintaining blood pH, the most outstanding property of serum albumin is its ability to reversibly bind a wide range of ligands. In a recent

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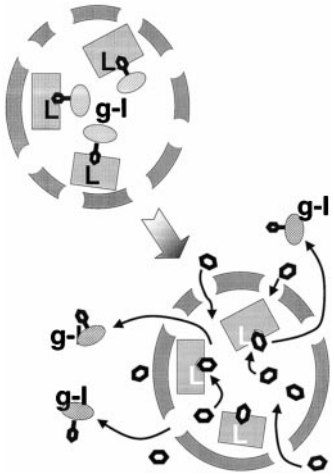

FIG. 9. Schematic representation of a complex system for the controlled, glucose-induced release of (glycosylated) insulin, as described by Brownlee and Cerami (1979). The insulin, bound to lectin, is encapsulated. On appearance of glucose in the environment (indicated schematically by the hexagons), the insulin is displaced from the lectin by the glucose, and the insulin is released from the capsule, whereas the lectin is retained by the capsule and remains shielded from the immune system. g-I, glycosylated insulin; L, lectin.

review, Carter and Ho (1994) listed more than 70 ligands for serum albumin for which binding constants were known. These ligands include fatty acids (Fig. 10), amino acids, drugs (irrespective of charge), and a number of inorganic ions (Kohita et al., 1994; Hage et al., 1995; Jakoby et al., 1995). Serum albumin is the principal carrier of fatty acids, which are hydrophobic and normally insoluble. In addition, it inactivates toxic lipophilic metabolites such as bilirubin. Albumin belongs to a multigene protein family that includes vitamin D-binding protein and α-fetoprotein, although unlike the other group members, serum albumin is not glycosylated. The sequence homologies for the albumins thus far determined are generally high, of the order of 75%. Human serum albumin (HSA), perhaps the most thoroughly studied serum albumin, consists of 585 residues. The 65-kDa protein contains a total of 17 disulfide bridges arranged in a characteristic repeating series of nine loop-link-loop structures centered around eight sequential disulfides. This highlights a further characteristic of serum albumin: the high percentage of cysteine and charged amino acid residues and the low abundance of tryptophan, glycine, and methionine. Unusual among extracellular proteins but analogous to β-lactoglobulin, albumin contains a single free sulfhydryl, Cys^{34}. The structure of HSA has been solved to a resolution of 2.8 Å (Geisow, 1992; He and Carter, 1992). The shape is noticeably asymmetric, created by three structurally homologous domains (I, II, and III), each of which is in turn formed by two smaller subdomains, A and B. Each domain contains 10 helices, and in total, two-thirds of HSA is helical. Rare in protein structures and possibly explaining the unusual stability of albumin, the disulfide pairings are located almost exclusively between helical segments. The blocking of the free sulfhydryl, Cys^{34}, blocks the formation of albumin dimers and also prevents the formation of mixed disulfides. A useful property of albumin is its high solubility, a quality probably related to its high negative charge at neutral pH, although there are distinct areas with neutral charge that may be important in the interaction with long-chain fatty acids.

FIG. 10. Crystal structure of HSA complexed with fatty acid. The structure was obtained from the Brookhaven protein database (PDB), as deposited by S. Curry, H. Mandelkow, P. Brick, and N. Franks (see Curry et al., 1998). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc.

Initially, the ability of albumin to bind such a diverse range of ligands was interpreted as nonspecific binding. However, it is now generally accepted that binding occurs at a number of distinct locations on the protein (each specific for a certain ligand or set of ligands), although controversy remains over the exact number. The consensus seems to be that there are two principal binding areas for small heterocyclic or aromatic carboxylic acids (often referred to as sites I and II), at least two sites for the binding of long-chain fatty acids (III, IV), and two metal-binding sites (V, VI).

The majority of the small organic ligands are bound in specialized cavities of subdomains IIA and IIIA (sites I and II, respectively). The range of molecules bound by sites I and II include aspirin, warfarin, ibuprofen, triiodobenzoic acid, and tryptophan. There is an absence of a corresponding site on subdomain IA. Site I shows a preference for large heterocyclic, negatively charged compounds with site II binding smaller aromatic carboxylic acids. The importance of Tyr^{411} in site II has been highlighted not only in ligand binding but also in the weak esterase activity of albumin. HSA is the most reactive of the albumins in terms of esterase activity, whereas equine serum albumin shows little or no reactivity.

The fatty acid-binding sites III and IV, although equally well studied, are perhaps less well defined. The total fatty acid capacity varies with fatty acid chain length, the average being six per albumin molecule. Under normal physiological conditions, albumin carries one or two fatty acids. The general consensus seems to be for two high-affinity long-chain fatty acid-binding sites and for four lower affinity sites. However, there are

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reports suggesting that no major sites dominate and that fatty acid binding is spread evenly over as many as nine sites (see Carter and Ho, 1994 and references cited therein). The exact binding location of fatty acids on the albumin molecule is unknown, but the following residues have been identified as playing important roles: Cys^{34}, Lys^{116}, His^{145}, Lys^{199}, Lys^{220}, Lys^{281}, His^{336}, Lys^{349}, Lys^{412}, Lys^{439}, Lys^{473}, and Lys^{525} (see Carter and Ho, 1994). Carter and coworkers (Carter and Ho, 1994) found that on binding of medium- to long-chain fatty acids such as laurate and palmitate, significant conformational changes take place in the protein. The binding of three or more fatty acids produces a slight opening of the interface between the two halves of the molecule and a rotation of domain I. To some extent, the binding of fatty acid molecules is also dependent on pH and ionic strength. Pedersen et al. (1995) found that for medium-length fatty acids such as laurate, the binding of the first few molecules was weakened with increasing ionic strength. Subsequent molecules seemed to react independently of ionic strength. Binding of myristate seemed to be totally independent of ionic strength. As could be expected, the effect of pH on binding was more complex.

A large effort has been devoted to the study of the metal-binding sites of albumin, but a detailed discussion of this particular binding property of the albumins is beyond the scope of this review. It suffices to say that albumin displays high affinity for Cu(II), Ni(II), Hg(II), Au(I), and Ag(II) with weaker affinities for Ca(II) and Zn(II). Of particular importance for the binding of metals is Cys^{34} (site V) and the N terminus of the protein (site VI).

HSA has been successfully produced by secretion from yeast cells (Carter and Ho, 1994) both in *S. cerevisiae* (Katoh and Watanabe, 1995) and *K. lactis* (Blondeau et al., 1994). The crystallographic structure of the recombinant protein (rHSA) is virtually identical with that of the natural protein (Carter and Ho, 1994), and there is no apparent loss of binding properties. These observations justify further mutagenesis studies to check the role and importance of the residues proposed above in the binding process.

Serum albumin microspheres have been successfully used for the delivery of cytostatic agents such as doxorubicin and 5-fluorouracil to tumors in the liver, breast, and lungs, rendering the albumin-bound drugs more effective than free drug (Chen et al., 1987; Gupta and Hung, 1989; Doughty et al., 1995), although albumin seemed to be inferior to casein in one study (Chen et al., 1987). Free serum albumin appeared to be a useful noncovalently bound vehicle for the highly insoluble and toxic zinc phtalocyanine, a second-generation photosensitizer for the photodynamic therapy of cancer (Larroque et al., 1996). This is a simpler alternative for liposome-incorporated zinc phtalocyanine. The albumin-borne agent appeared to effectively control tumor growth in a human colon carcinoma, T380, implanted in nude mice, whereas the dark toxicity and hepatic toxicity associated with zinc phtalocyanine appeared to be absent. The agent readily redistributed over the serum high-density lipoprotein fraction. Serum albumin was also involved in a strategy to optimize the pharmacokinetics of insulin and especially the glucose disposal curve elicited by insulin. The hormone was covalently coupled to fatty acids, which bind to serum albumin in vivo. This appeared to result in favorable pharmacokinetics but especially in an improved glucose disposal curve (Markussen et al., 1996; Hoffman and Ziv, 1997; Kurtzhals et al., 1997; Hamilton-Wessler et al., 1999). The big advantage of albumin is the compatibility with human blood, plasma, and body components. Albumin is probably also quite suitable for the delivery of drugs to the liver (Wolkoff, 1987; Meijer and van der Sluijs, 1989). Cationized albumin appeared to be useful for the delivery of active agents across the blood-brain barrier to the brain (Kumagai et al., 1987; Kang and Pardridge, 1994). Brain targeting and passage of the blood-brain barrier is notoriously difficult (Halmos et al., 1997). The active agents were either attached covalently (Kumagai et al., 1987) or bound to a cationized albumin (neutral)-avidin conjugate (Kang and Pardridge, 1994). Heterologous cationized albumin has been shown to be immunogenic (Muckerheide et al., 1987), whereas homologous proteins seem not to elicit a strong immunogenic response as deduced from a study in which rat protein was given to rats (Pardridge et al., 1990).

F. Immunoglobulins

Like enzymes, immunoglobulins occupy a special place in the field of ligand-binding proteins because of their ability to collectively recognize an almost infinite number of ligand molecules. In the context of the present review we can only briefly outline some general aspects relating to the potential application of these proteins in carrier and controlled release systems.

Antibody molecules are capable of both incredible diversity and high specificity. Individually, they may bind one or only a few compounds with high affinity, but collectively they are able to recognize virtually any molecule. As such, they offer an almost unlimited versatility. In addition, Ig-based systems are easily adapted to new types of ligands (Rees et al., 1994; Stanfield and Wilson, 1994; Chester and Hawkins, 1995). Despite this versatility, antibody molecules have common structural and functional features. The IgG molecule consists of a tetramer of two identical 25-kDa ("light") polypeptides and two identical 50-kDa ("heavy") polypeptides. The antibody-combining sites, which bind the antigen molecules, are formed by the juxtaposition of six hypervariable loops, three from the variable region of the light chain and three from that of the heavy chain. As the crystal structures of more antibody-antigen complexes become available, the nature of the interaction between

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these proteins and their ligands is starting to be understood (Davies and Padlan, 1990). From a practical point of view, the widely used technique involving hybridomas for the production of monoclonal antibodies is a readily available method for fast adaptation and production of highly specific carrier molecules. However, the high binding affinity is likely to cause problems with respect to the efficient release of the ligand in the target environment. Although this could possibly be remedied by using genetically modified systems that are, for example, pH-, temperature-, or protease-sensitive, the development of stable systems with the desired properties and ligand specificity will probably not be straightforward. In addition, the relatively large molecular weight of the immunoglobulins negatively influences the binding capacity in terms of weight of ligand per unit weight of protein.

Apart from their possible role in (highly specific) binding and carrying of active agents, it is obvious that antibodies can play an important role in the specific targeting of carrier complexes toward the precise environment where the active agent should be delivered and released. Thus, antibodies have been coupled to liposomes (Crommelin et al., 1992; Pinnaduwage and Huang, 1992; Vingerhoeds et al., 1993, 1994) and other drug carriers (Poznansky and Juliano, 1984; Tomlinson, 1987; Senter et al., 1988; Crommelin et al., 1992; Bickel et al., 1993; Shin et al., 1997; Penichet et al., 1999).

Antibodies have actually been applied as carriers of small ligands in a variety of different cases and with highly variable aims both in vivo and in vitro. We will discuss a few examples. In a way, the first example, which is only in the stage of development, is the inverse of the subject of this review, viz., the uptake of active agent at sites where the latter should not be active. We would like to point out that this principle is not restricted to antibodies but applies to any ligand-binding carrier molecule and is intrinsically related to the concept of ligand-binding proteins as carriers of small molecules. Methotrexate is an inhibitor of dihydrofolate reductase, an essential enzyme involved in the synthesis of the DNA precursor deoxythymidilate. As such, it is very valuable for chemotherapy of rapidly growing tumors such as leukemia and choriocarcinoma, but like all cytostatics, it is highly toxic. Anti-methotrexate antibodies, administered i.v., may be used to mask systemic toxicity of methotrexate, when methotrexate is locally injected to localized ovarian carcinoma and other localized tumors (Balthasar and Fung, 1995, 1996).

A second example concerns an unintentional effect that is most probably due to antibodies as carriers of peptide hormones. Contrary to expectation, anti-hormone antibodies can enhance hormone activity (Aston et al., 1989). The enhancement has already been observed in vivo and could thus be seen as highly promising. However, the predictability of the effect (e.g., in different individuals) is still low at this moment because a large number of partly unknown parameters appears to be involved. The enhancement can be due to one or more of the following six effects: 1) binding of the antibody induces conformational changes (allosteric effects) in the hormone; 2) the bifunctional nature of antibodies brings together, and simultaneously presents, two hormone molecules; 3) the antibody prevents unwanted conformational and chemical changes of the hormone and stabilizes its structure and activity; 4) the antibody prevents recognition of the hormone by the clearance machinery; 5) the antibody protects the hormone against protease; and 6) the antibody enhances the response by slowly (sustainedly) releasing the hormone. This is illustrated in Fig. 11. Obviously, prerequisites for antibody-enhanced hormone activity are 1) that the antibodies must either bind to epitopes that are different from those that bind to the hormone's receptors and 2) that they must not sterically hinder receptor binding. (If the antibodies would bind to the same epitope, the binding affinity and capacity of the receptor would then have to clearly outweigh that of the antibody.) In healthy individuals, the plasma half-life of exogenous insulin is only a few minutes, but in diabetes patients, the half-life can become as long as several (more than 10) hours. Probably, this is the result of insulin binding to endogenous antibodies. It demonstrates a principle that could be exploited in var-

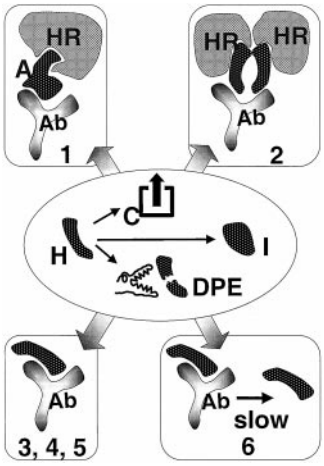

FIG. 11. Anti-hormone antibodies can enhance hormone activity due to one or more of the following six effects. 1, binding of the antibody induces conformational changes (allosteric effects) in the hormone, which promote the binding of the hormone to its receptor. 2, the bifunctional nature of antibodies brings together and simultaneously presents two hormone molecules, thus enhancing the response that results from binding of the receptor. 3, the antibody prevents unwanted conformational and chemical changes (I) of the hormone and stabilizes its structure and activity. 4, the antibody prevents recognition of the hormone by the clearance machinery (schematically represented by the exit symbol). 5, the antibody protects the hormone against proteolytic enzymes (DPE, schematically represented by the open jaws). 6, the antibody enhances the response by slowly (sustainedly) releasing the hormone. Ab, antibody; A, active conformation of the hormone induced by binding to the antibody; HR, hormone receptor molecule; C, clearance machinery; I, inactive conformation of the hormone resulting from spontaneous conformational or chemical changes of the free (unbound) hormone molecule; DPE, degradation of the hormone by proteolytic enzymes; the numbers correspond to the six possibilities mentioned in this legend.

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ious applications where controlled release systems could be based on carrier antibodies.

Probably, the most straightforward examples of antibodies as carriers of active agents are various kinds of bispecific antibodies (BsAbs). In a broader sense, fusion proteins or covalent conjugates containing (part of) an antibody as well as (part of) another protein such as avidin (Shin et al., 1997; Penichet et al., 1999) or another biopolymer such as DNA (Taylor and Ferguson, 1995; Taylor et al., 1997; Makrides, 1998) can be considered as BsAbs. Antibody-avidin fusion proteins have been discussed above in the section on biotin-binding proteins. In a stricter sense, BsAbs, carrying a variety of cytostatic agents such as interferon, vinca alkaloids, etc., are chimeric proteins consisting of (parts of) two or three different antibodies with different antigen (ligand) specificity (Berg et al., 1991; Fanger et al., 1992; Makrides, 1998; see Fig. 12. Such molecules can be constructed to include a targeting function (recognizing target antigens) as well as a carrier function recognizing and binding an active component. The active component can vary from relatively small compounds and drugs to complete killer-type T cells. Using BsAbs against the CD3 part of the antigen receptor on cytotoxic T cells and the CD4 T-cell receptor for HIV, T cells of irrelevant specificity were induced to effectively lyse HIV-infected cells (Berg et al., 1991). By binding to the CR1 receptor of erythrocytes, the clearance of antibody-antigen complexes and (opsonized) microbes can be enhanced because the erythrocyte-bound material is rapidly fagocytosed by macrophages, without lysis of erythrocytes, and subsequently cleared in the liver and spleen (Nelson, 1953, 1955; Ahearn and Fearon, 1989). Along these lines, anti-CR1 antibodies cross-linked to antibodies with specificity for targeted antigens were used to bind the targeted antigens to erythrocytes for enhanced clearance from the blood. After injection of the sensitized erythrocytes in monkeys, the antigens were rapidly cleared from the circulation, apparently without any harm to the erythrocytes (Taylor et al., 1992). The principle could be applied for the removal of pathogens from the blood and treatment of infections and infectious diseases (Taylor et al., 1991; Reist et al., 1993; Makrides, 1998). The excellent review by Fanger et al. (1992) describes a variety of other applications of BsAbs. For example, in clinical studies, saporin was successfully used to control non-Hodgkin's lymphoma by attaching the saporin to the malign lymphocytes with the help of anti-saporin/anti-CD22 $F(ab')_2$. BsAbs carrying a variety of cytostatic agents, such as interferon, vinca alkaloids, etc., were used to control various tumors and cancer cells. The BsAbs have further been used to control blood clotting and enhance fibrinolysis (anti-fibrin/anti-plasminogen activator combinations) and other applications. They may be used for the circumvention of the atherogenic process, virus removal, etc. The economical large-scale production of BsAbs is essential for the further development and clinical application of BsAbs (Fanger et al., 1992), and in this respect, the recently developed route to bispecific human IgG (Merchant et al., 1998) is worth mentioning.

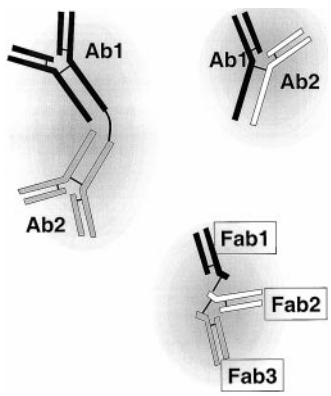

FIG. 12. Various types of BsAbs. Top left, two antibodies can be linked by chemical cross-linking or by gene fusion and recombinant production. Top right, a chimeric antibody can be produced by hybrid hybridomas. Bottom right, BsAbs and/or trispecific antibodies can consist merely of Fab fragments cross-linked to each other. Other BsAb forms are also possible (for example, see Fanger et al., 1992).

G. (Inactivated) Enzymes

The field of enzymology encompasses a vast number of proteins, each of which interacts in a highly specific manner with a particular ligand or with a group of structurally closely related ligands, generally resulting in a change of the covalent structure and/or redox state of the ligand (Creighton, 1993). The ability of an enzyme to catalyze such a specific reaction is a consequence of its specificity for the substrate molecule (Bennett and Steitz, 1980; Creighton, 1993; Kuzin et al., 1995; Lewis and Lake, 1995). In many enzymes, separate domains are involved in binding and in catalysis (e.g., Fothergill and Fersht, 1991; Creighton, 1993; Endrizzi et al., 1994; Lewis and Lake, 1995). A selective destruction of the catalytic activity with preservation of the binding properties by genetic engineering (Gerlt, 1987; Fersht, 1987; Wagner and Benkovic, 1990; Wilkin et al., 1994; Murray et al., 1995), allosteric inhibition, or other chemical means such as taking out cofactors, heme groups, etc. (Stryer, 1988; Creighton, 1993, Wilkin et al., 1994; de Ropp et al., 1995) would enable enzymes to function as specific carriers for their substrates that are no longer able to chemically modify these substrates (Fig. 13). For example, the binding and catalytic properties of chloramphenicol acyltransferase have been dissected (Murray et al., 1995) in structural and genetic engineering studies. A $T^{299}V$ mutant of *Streptomyces* R61 DD-peptidase was shown to have largely reduced catalytic and β-lactamase activity (especially for cephalosporin C and cefuroxime), whereas binding properties with respect to the substrate and β-lactam inhibitors were only marginally affected under certain conditions (Wilkin et al., 1994). For the purpose of controlled release, the larger

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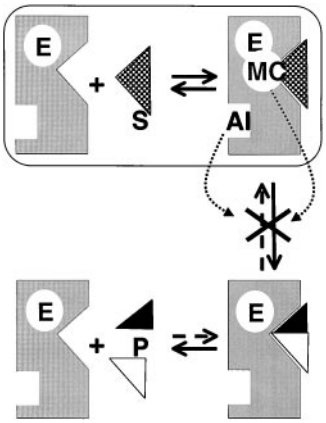

FIG. 13. Inactivated enzymes (E) can be used as specific agent-carrying molecules (either as such or in more complex delivery systems). The agent is then the natural substrate (S) of the enzyme or a substrate analog (S). However, it should not be converted to a different product (P), due to the catalytic activity of the enzyme. This can be obtained if 1) the agent (ligand) that is binding to the enzyme is a nonconvertible analog of the natural substrate, 2) the catalytic activity of the enzyme is blocked by allosteric inhibitors (this is unsafe because there is a serious risk of release of the inhibitor), or 3) the catalytic activity is blocked irreversibly by chemically or genetically modifying the catalytic site (MC), essentially without affecting its binding properties. E, enzyme; S, the agent (ligand), which is a substrate of the enzyme or a substrate analog, irreversibly blocking the catalytic site by chemical or genetic modification; AI, allosteric inhibition of the catalytic activity (but not of substrate binding); P, (set of) products generated by the catalytically active enzyme (undesirable if the protein is used merely as a carrier of its substrate (ligand).

proteins are less suitable (unless the potency of the ligand is very high) because a large protein weight will only be able to carry a small amount of ligand. In view of ease of handling, soluble enzymes are preferable to membrane- (lipid-) bound enzymes. Unlike the immunoglobulins, the enzymes lack the potential for fast adaptation to any type of ligand. Thus, suitable carriers should be selected from an essentially limited repertoire of pre-existing enzymes. Numerous enzymes have been identified, ranging in size from approximately 10 kDa, e.g., 13.7 and 14.4 kDa for bovine pancreatic ribonuclease A and chicken lysozyme, respectively (Canfield, 1963; Fedorov et al., 1996), to more than 100 to 200 kDa, e.g., the tetrameric prolyl-4-hydroxylase (Gong et al., 1988; Helaakoski et al., 1989), other multisubunit enzymes, and, if these are considered as enzymes, several membrane-incorporated transporters (Endicott et al., 1991). Many crystal structures are now available. For almost any chosen ligand (or group of closely related structural analogs), an enzyme will probably exist that is capable to bind it. Similarly, a number of allosteric enzyme inhibitors (of various size and nature) is known that could be used to suppress the unwanted enzyme-mediated modification of the ligand. Because such inhibitors may be easily lost, may be unstable, and/or toxic, it will be preferable in most cases to destroy the catalytic activity of the (recombinant) enzyme by removal of cofactors or by genetic means. Generally, there is little structural similarity between unrelated enzymes. Generalizations about binding sites or protein properties are therefore not possible and not relevant in this context. However, one aspect of enzyme action should be borne in mind when considering these proteins as possible carriers of small molecules: the postulated induced-fit mode of action, according to which the substrate can induce a considerable conformational change in the protein on binding (e.g., DelaFuente et al., 1970; Bennett and Steitz, 1980), may have some bearing on the modeling studies of the (native or engineered) binding site. Similarly, it should be borne in mind that enzymes can possess more than one binding site. Finally, cofactors or prosthetic groups may be necessary to facilitate ligand binding and/or execution of catalytic steps. In the former case, such cofactors or groups would have to be included in the carrier system, at least at certain stages of the carrying process. Conversely, in the latter case, they would have to be eliminated.

H. Other Protein Groups

The proteins discussed above have been well studied in terms of their binding characteristics and generally form large and expanding families. The proteins discussed below are, at present, less well studied. In many cases, their importance as binding or transport proteins is only just being revealed. In view of the interesting ligands bound by some of these proteins, it is nevertheless felt that they are worth being included in the list. As more becomes known about their structure and binding properties, they could well find interesting application in the field of engineered ligand transport.

1. Insect Pheromone-Binding Proteins and Odorant-Binding Proteins. This group of small, water-soluble proteins incorporates the insect pheromone-binding proteins (PBPs) and general OBPs (GOBPs) of insects. They bind volatile, hydrophobic odorants and are believed to mediate the delivery of lipophilic sex pheromones to specific receptor proteins (Prestwich, 1993; Du and Prestwich, 1994, 1995; Ozaki et al., 1995; Steinbrecht, 1996). The deduced protein sequences for several PBPs and GOBPs have been described (Du and Prestwich, 1995; Ozaki et al., 1995; Campanacci et al., 1999; Danty et al., 1999; Willet and Harrison, 1999; Rothemund et al., 1999). The insect PBPs and GOBPs differ completely in primary, secondary, and tertiary structure from the mammalian OBPs. However, the PBPs and insect GOBPs share highly conserved regions, including six conserved cysteine residues (Du et al., 1994) that form intramolecular disulfide bridges. Yet another type of protein seems to be the lipophilic stimulant carrier commonly found in insect taste as well as olfactory systems (Ozaki et al., 1995). The insect GOBPs, expressed in both the male and female antennae, show over 95% sequence conservation among the lepidopterous species (Du et al., 1994). The vertebrate OBPs are essentially constructed from 10 antiparallel β-strands and just two short α-helical stretches, whereas the insect binding proteins contain over 45% α-helix (Prestwich, 1993;

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Campanacci et al., 1999; Rothemund et al., 1999). There is as yet no direct evidence regarding the location of the pheromone-binding site, but photoaffinity labeling of pheromone components (Du et al., 1994) has indicated the involvement of certain amino acids that are apparently located in or around the binding site. Ligands bound by these proteins are based on long-chain unsaturated hydrocarbon acetyl esters. Each of the insect-derived OBPs shows affinity for a specific chemical group or structure, and it has been proposed that each pheromone component could have its own unique high-affinity PBP. The vertebrate OBPs, however, appear to bind a large number of structurally diverse odorants. The high-yield expression and purification of a recombinant PBP have been described (Prestwich, 1993), viz., for the protein Apo-3, first isolated from the antennae of the adult male *Antheraea polyphemus* moth. This 14-kDa, 142-residue recombinant PBP, which was overexpressed in *E. coli*, was indistinguishable from the native insect-derived protein. As yet, there is no crystallographic data relating to this group of proteins, and investigations of the binding sites are still in the early stages. However, because the first small diffraction crystals have been recently obtained (Campanacci et al., 1999), this information will soon emerge. In fact, the first NMR studies on α-helical insect PBP have already been performed (Rothemund et al., 1999). The availability of the recombinant protein (Prestwich, 1993; Campanacci et al., 1999; Danty et al., 1999) opens the way to further elucidation of the protein structure by crystallization studies, the production of site-directed mutant proteins, and an increased understanding of the binding mechanism. Moreover, possibilities for large-scale production of recombinant proteins may open the way to various applications of these proteins. These proteins will be especially valuable for controlled release of insect pheromones and aliphatic compounds (Rothemund et al., 1999), e.g., in environment-friendly crop protection applications.

2. Immunosuppressant-Binding Proteins. This small group of intracellular proteins, which are also known as immunophilins, is capable of binding immunosuppressant drugs (Braun et al., 1995). The cyclophilins, which have a high affinity for the immunosuppressant cyclosporin A (Weber et al., 1991), form a subset of the immunophilins. Other group members include FK506-binding proteins and Jurkat T-cell phosphoproteins (Schreiber, 1991). Cyclophilins are abundant proteins found in both prokaryotic and eukaryotic organisms. The major human form has a molecular mass of 17.7 kDa. All immunophilins appear to be peptidyl-prolyl isomerases (Walsh et al., 1992), enzymes that catalyze the interconversion of the *cis*- and *trans*-rotamers of the peptidyl-prolyl amide bond in proteins and peptides, and should thus be considered as chaperones. They have not been discussed in the section on enzymes above because the catalytic activity does not concern the immunosuppressant, the ligand of interest for potential controlled release applications, but rather concerns prolyl-containing proteins and peptides that bind at a different site. The rotamase activity of cyclophilins is inhibited by cyclosporin A and that of the FK506-binding proteins is inhibited by FK506. Despite their similar enzymatic properties, the two protein types have dissimilar sequences and secondary and tertiary structures; human FK506-binding protein is smaller (11.8 kDa) (Schreiber, 1991; Walsh et al., 1992). The inhibition of isomerase activity is not the reason for immunosuppression and the inhibition of transcription of early T-cell response genes. Rather, allosteric inhibition of the intracellular protein serine phosphatase calcineurin by the cyclosporin-cyclophilin complex appears to be the key event. The ligands bound by immunophilins are relatively large, hydrophobic compounds. Cyclosporin A is a cyclic undecapeptide fungal metabolite that can undergo conformational changes. In the crystal state or in nonpolar solvents, it adopts a tightly folded structure very different from the cyclophilin-bound conformation. The crystal structures of various cylcosporin-cyclophilin complexes have now been elucidated (e.g., Kallen et al., 1998). The protein consists of two α-helices, a number of β-strands arranged in a barrel-like structure, and a number of loops. The immunosuppressant binds to a cleft on the outside of the barrel, opposite to the helices (Fig. 14). The rotamase activity and the ability to bind immunosuppressants have prompted many investigations. Site-directed mutagenesis of human recombinant cyclophilin showed that all four cysteine residues in the protein could be replaced with alanine without an effect on rotamase or binding activity. This rules out the participation of cysteine. As the interest in these proteins increases, the identification and characterization of new immunophilins will provide new understanding of the role of these proteins and their interaction with novel ligands, both natural and synthetic.

Human immunophilins could possibly be used as extracellular carriers of cyclosporins, to obtain enhanced solubility and decreased systemic toxicity of the immunosuppressants. Coupled to targeting devices like anti-T

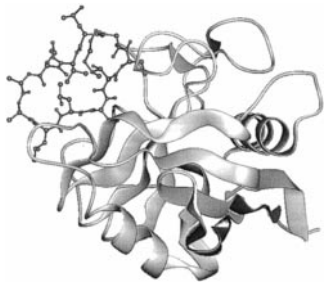

FIG. 14. Crystal structure of human cyclophilin A complexed with cyclosporin A. The structure was obtained from the Brookhaven protein database (PDB), as deposited by V. Mikol, J. Kallen, and M. D. Walkinshaw (see Mikol et al., 1993). The PDB data were processed using software from Quanta/Charmm Molecular Simulations Inc.

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cell antibodies, cyclophilins would probably allow a more targeted delivery of cyclosporins.

3. Phosphate- and Sulfate-Binding Proteins. This broad title brings together proteins from a number of diverse families, including members of the periplasmic proteins discussed in *Section II.C.* above, and is only useful from a functional (application-oriented) point of view. It has been reported (Copley and Barton, 1994) that approximately 50% of known proteins bind or process compounds containing phosphoryl groups (e.g., the periplasmic proteins discussed above), so generalization of the structures or characteristics of these proteins is impossible as well as meaningless. A number of PiBPs and SBPs have been characterized with respect to their binding sites and propensities for binding by Copley and Barton (1994). In more than 25% of these proteins, phosphate groups bound only to one amino acid, although binding could involve as many as seven amino acids. There was no typical phosphate-binding site, although positively charged and polar residues were found to be better at binding than were bulky nonpolar residues. PiBPs and SBPs could find application in agriculture for controlled release of nutrients. However, this would require the development of highly efficient, probably recombinant, production systems to make such an application economically feasible.

I. Comparative Overview of Protein Classes Surveyed in Section II

To facilitate the comparison of the protein groups reviewed above, the general parameters relevant to the development of controlled release systems have been compiled in Table 2. The information concerns the types of ligand that are bound, the specificity and versatility of the binding, the amenability/manageability (e.g., solubility, size, adaptability), and the availability of structural data from X-ray crystallography. Because in many cases it will be desirable or necessary to engineer the proteins of choice and subsequently to produce the modified proteins by overexpression in heterologous organisms, the availability of cDNA and the reported possibilities for production in heterologous (micro)organisms have also been included. Note that the table merely serves to provide a quick overview but could not possibly include all the relevant information. For example, we have classified the binding properties of lectins as "not versatile" because they only bind carbohydrates, but for an application of carbohydrate transport, the binding characteristics of lectins as a group of proteins could be considered as suitably versatile. For more details about the protein groups featured in Table 2, the reader is referred to the corresponding subsections of the survey in *Section II*.

III. Discussion and Perspective

A. Aspects Intrinsic to Ligand-Selective (High-Affinity) Binding Proteins

Binding proteins offer great potential for the development of systems for controlled release of small molecules. 1) They naturally function to bind small molecules and ions, usually with high specificity. 2) As a family of complexing agents, they bind an extremely wide variety of molecules (e.g., hydrophobic aliphatic and aromatic molecules, amino acids, smaller and larger peptides, fatty acids, drugs, alcohols, carbohydrates, inorganic

TABLE 2
Overview of ligand-binding proteins

Characteristics[b]	Protein Classes[a]									
	A	B	C	D	E	F	G	H	I	J
cDNA[c]	+	+	+	+	+	+	+	+	+	+
Structure[d]	X-ray	X-ray	X-ray	X-ray	X-ray	X-ray	X-ray	NMR	X-ray	X-ray
Mol/biol adaptability[e]	+	+	+	+	+	+	+	+	−	+
Versatility[f]	−	+	+	−	+	+	+	−	−	−
Ligand combination[g]	−	−	−	−	+	+/−	−	−	−	−
Availability[h]	+	+	−	−	+	+	+	−	−	−
Size (kDa)	15	18	20–50	25	65	150	various	14	18	various
Hydrophobic ligands[i]		+	+		+	+	+	+		
Inorganic ligands[i]			+		+		+			+
Amino acid ligands[i]	+		+		+	+	+			
Carbohydrate ligands[i]			+	+		+	+			
Specific ligands[j]	biotin								CyFK[j]	

[a] Protein classes: A, biotin-binding proteins; B, hydrophobic transporter molecules; C, periplasmic-binding proteins; D, lectins; E, serum albumins; F, immunoglobulins proteins; G, enzymes; H, insect pheromone-binding; I, immunosuppressant-binding proteins; J, sulphate-/phosphate-binding proteins.
[b] In all cases, the positive or negative rating of a protein group refers to the properties of the group and not of individual proteins. A + is given if at least one group member possesses a particular attribute.
[c] A + denotes the reported availability of cDNA.
[d] Where the existence of X-ray data is reported here, it is not necessarily an indication of the ready availability of these data. NMR indicates that the structural studies reported so far are based on NMR data.
[e] A + denotes a reported production of recombinant protein.
[f] A + is given in cases where the protein group is capable of binding a broad range of compounds. This is only a general appreciation, and protein groups with a low (−) rating may be sufficiently versatile for certain applications.
[g] Serum albumin is the only protein reported to have multiple binding sites. The production of divalent antibodies is theoretically possible.
[h] A + denotes the commercial availability of at least one class member.
[i] A + denotes the protein class binds this ligand type.
[j] A + denotes the binding of a ligand specific to that protein class. CyFK, cyclosporins/FK506.

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ions, etc.). 3) As a result of high-affinity binding, they can screen small ligand molecules from incompatible environments. They can thus mask toxicity and bitterness, prevent unwanted chemical reactions of the ligand, etc. 4) Many of them possess binding properties that are susceptible to useful environmental stimuli and controlling parameters, allowing the design of sophisticated mechanisms of controlled release and uptake of ligands. Finally, 5) modern protein engineering and biotechnological production techniques now allow the production of adapted and partially or fully redesigned protein molecules. Proteins can be optimized with respect to their structural, functional, binding, and responsive properties, and the spectrum of possible ligands can be extended. The variety of protein systems that is available allows the engineer to choose and adapt triggering mechanisms that are tuned to the particular physiological or nonphysiological application for which they have to be used. These new possibilities offered by protein-based systems are beyond the scope of the possibilities of traditional systems. Obviously, and partly in analogy to the inverse targeting described by Balthasar and Fung (1995, 1996), free, encapsulated, or immobilized proteins can also be used in systems for controlled *removal* (uptake) of small molecules rather than controlled *release*.

In *Section II*, several examples of existing delivery systems based on ligand-binding proteins have been given. In light of the ideas developed in this review, the most relevant systems involving noncovalent interactions between carrier proteins and relatively small ligands are 1) BsAbs, which combine selective, high-affinity substrate binding with targeting functions (e.g., Berg et al., 1991; Fanger et al., 1992; Makrides, 1998; Merchant et al., 1998), 2) hormone-binding antibodies that enhance hormone activity (e.g., Aston et al., 1989), 3) serum albumin-borne hydrophobic agents and hydrophobized hormones (e.g., Chen et al., 1987; Doughty et al., 1995; Larroque et al., 1996; Markussen et al., 1996), 4) antibody-avidin fusion proteins in combination with biotinylated active agents (e.g., Bickel et al., 1993; Shin et al., 1997; Penichet et al., 1999), 5) cationized serum albumin-avidin conjugates for delivery of biotinylated agents across the blood-brain barrier (e.g., Kang and Pardridge, 1994), and 6) negative targeting of methotrexate by antibodies (Balthasar and Fung, 1995, 1996). The combination of avidin conjugates with targeting molecules and biotinylated active agents, as in example (4) and (5) above, has in principle a very broad application potential both in pharmacology as well as in crop protection, or elsewhere, next to BsAb. Similarly, LBPs and serum albumins have in principle broad potential for application in controlled delivery of hydrophobic agents. Instead of targeting with the help of the Fab function of antibodies (Fanger et al., 1992; Vingerhoeds et al., 1994) or with the help of cationization or anionization of albumin or other proteins (Kumagai et al., 1987; Meijer and van der Sluijs, 1989; Franssen et al., 1994; Halmos et al., 1997), glycosylation of ligand-carrying proteins may also be used as a cell receptor targeting device (e.g., Molema and Meijer, 1994).

The mode of binding and possible binding-associated conformational changes should be taken into account when considering the modification of the binding properties of a particular protein. In some proteins (for example, the lectins), the binding site is a shallow surface depression, which accommodates the ligand with little change in the protein conformation. In fact, it is often the ligand conformation that is important in this process. In the hydrophobic transporter molecules, the ligand, generally a fatty acid or retinol analog, is bound deeply in a barrel-shaped pocket. Also, in this case, there is virtually no difference in the structure of the apo and holo forms of the protein. In the absence of the hydrophobic ligand, solvent molecules occupy the large binding site. In other proteins, quite subtle conformational changes occur on binding of the ligand (e.g., when biotin binds to avidin and streptavidin; see below). Large conformational adjustments occur, for example, in the periplasmic binding proteins, which consist of two lobes connected by a hinge region. On binding, the protein undergoes a large rigid body movement of one of the lobes relative to the other to entrap the ligand. Similarly, various enzymes are subject to significant conformational changes on binding of their substrate (e.g., Bennett and Steitz, 1980; Schulz et al., 1990). The principle of such an induced fit is illustrated in the cartoon in Fig. 15. It should be noted that not only binding proteins but also some ligands change their conformation on binding to their binding protein, for example, oligo- or polysaccharides bound to lectins, peptides bound to periplasmic binding proteins, and cyclosporins bound to immunosuppressant-binding proteins.

Systems based on specific, high-affinity ligand-protein complexes can in several cases be ideal for development of self-regulated systems that rely on environmental stimuli or feedback as the trigger for ligand release. This trigger can involve an increase in concentration of competing ligands (triggering the release) or a decrease of

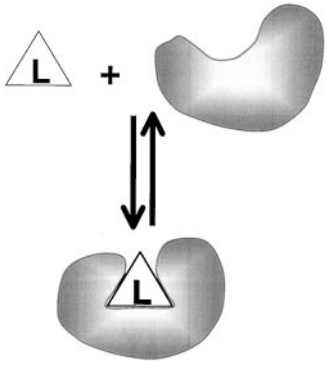

FIG. 15. Principle of induced fit: on binding of the ligand (L), the conformation of the binding pocket (and thus of the binding protein) changes to induce tight fit (tight binding).

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the ambient pH (e.g., as reaction products are formed). Thus, a simple and elegant idea was the use of lectins (encapsulated, thus immunologically shielded; see *Sections B* and *C* below) for the delivery of glycosylated insulin, with glucose displacing the insulin in a concentration-dependent manner (Brownlee and Cerami, 1979; Jeong et al., 1985). Another approach has been reported by Stayton et al. (1995), who covalently coupled a stimulus-responsive polymer to the bacterial protein streptavidin. The resulting protein-polymer complex binds biotin, the natural ligand of streptavidin, only at temperatures below 32°C. Above this temperature, the polymer collapses and blocks binding. Such a reversible inhibition of binding could be possibly used in some areas of controlled release. Disulfide-linked aggregates of ligand-binding proteins can be used in cases where appropriately reducing conditions in the target environment may serve to trigger the depolymerization of the ligand-binding protein, which could be exploited as a prerequisite for any subsequent release of ligand.

B. Complex Systems Incorporating Ligand-Selective (High-Affinity) Binding Proteins

The simplest ligand-binding protein-based systems are those in which 1) the protein-ligand complex is added as a powder or solution to the target environment, 2) the resulting change of conditions initiates release, and 3) the continuous presence of the carrier protein does not cause problems like immunogenic responses or unwanted removal of the vehicle protein. Molecules that serve to trigger the release may be present naturally in the destination environment or co-added with the protein-ligand complex.

The binding protein can also be combined with other components to build more complex agent-carrying systems. Where removal of the binding protein is necessary after release or, conversely, after binding of the ligand, it is possible to couple the protein to an insoluble support. This will enable its removal by filtration or passive settling. By encapsulating the specific binding protein-ligand complex within a polymeric (proteinaceous) matrix, the binding (carrier) protein can be shielded from the environment. Moreover, it allows the development of dual-stimulus-responsive systems. The first stimulus (e.g., an increase of relative humidity, temperature, pH, ionic strength, or proteolytic degradation of the matrix) serves to release the ligand-binding protein from the matrix, whereas the second stimulus (e.g., specific protease activity or displacement by competitive ligands) enables the release of the ligand from the binding. Changes in temperature can, for example, induce melting of the vehicle (e.g., fat-based systems) (Pothakamury and Barbosa-Cánovas, 1995; Risch and Reineccius, 1995), swelling of polymer-based systems (Karsa and Stephenson, 1993), or phase transition of phospholipid bilayers in the case of temperature-sensitive liposomes (Merlin, 1991; Vingerhoeds et al., 1994). Also, pressure activation or mechanical destruction of the capsule or matrix can induce release, for example, the release of flavors during the chewing of gum (Risch and Reineccius, 1995) or release of ink when writing on carbonless carbon paper (Karsa and Stephenson, 1993).

Two classes of responsive polymeric delivery systems have been developed (Kost and Langer, 1992), the externally regulated and the self-regulated systems. The externally regulated systems are controlled by externally applied triggers, i.e., triggers, such as a magnetic field, ultrasound, or irradiation, that are generated outside the local environment of the ligand-carrier system. Self-regulated systems are potentially more useful because they are subject to environmental feedback; the presence or absence of a particular stimulus (e.g., local pH, local temperature, local presence of interacting enzymes, etc.) acts to control the release of the active compound (Brownlee and Cerami, 1979; Jeong et al., 1985). A number of such systems has been reviewed by Kost and Langer (1992). In most cases, the system is controlled by pH or by competitive binding of other ligands, displacing the active compound from the carrier.

In addition to the possibility of shielding the binding protein from the environment and the possibility for additional (dual) triggering of release of ligand (agent), inclusion of high-affinity agent-binding proteins in microcapsules offers another advantage. It can be used to obtain a better control of *slow* release than would be possible with free binding protein or with capsules devoid of binding protein. Binding proteins can offer selectivity and high affinity for the agent as well as potential triggering mechanisms (e.g., pH- or protease-sensitive binding sites). High binding affinity can keep the free (available) ligand concentration suitably low at all times. However, the kinetics of release will usually be intrinsically fast. In combination with the diffusion barrier of the capsule or matrix, high-affinity binding to the binding protein or low-affinity high-capacity binding will result in significant retardance of release of the agent from the capsule or matrix, compared with the protein-free capsule. This is illustrated by the model results shown in Fig. 16.

C. Parameters That Influence the Choice of Specific Ligand-Binding Proteins

When developing controlled delivery systems that involve ligand-binding proteins, it is essential that the ligand-binding protein be carefully chosen. Depending on the application, high ligand specificity or, conversely, high versatility of the carrier protein may be more important. To a certain extent, these parameters can be manipulated by protein engineering, but the characteristics of the natural protein on which the development is based will largely dictate the characteristics that can eventually be reached.

The natural ligand specificity of different proteins and protein groups is very different. For example, lectins

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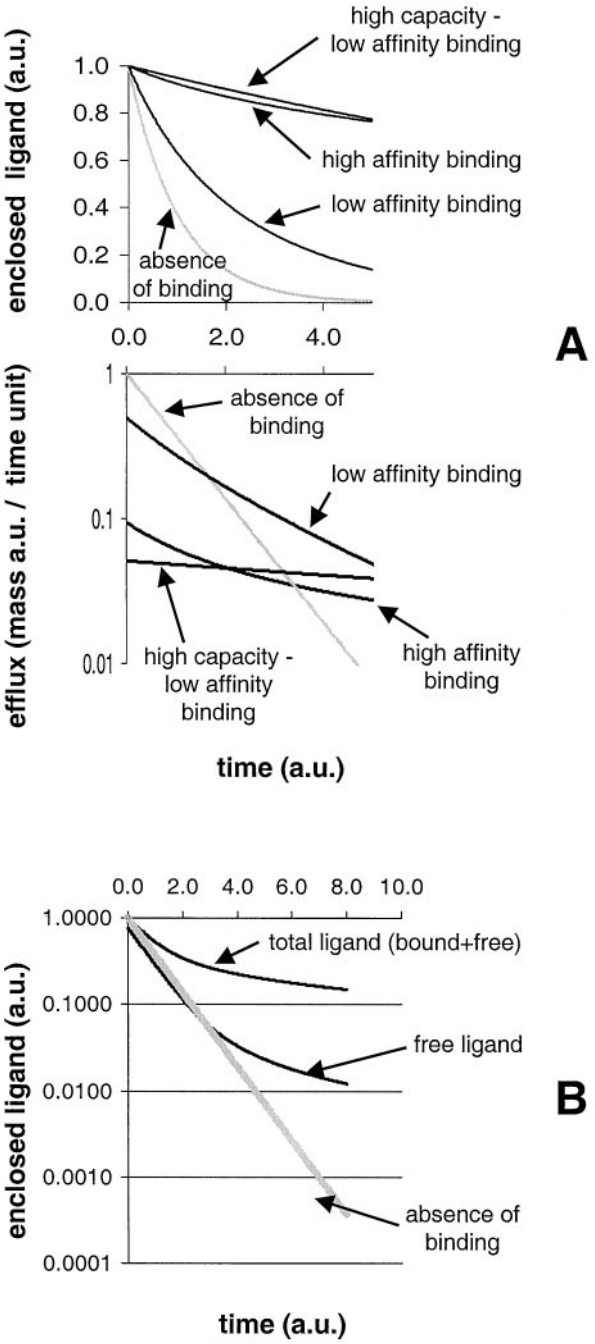

FIG. 16. Binding of encapsulated agent to high-affinity sites inside the capsule or high-capacity binding to low-affinity sites inside the capsule results in significantly slower (sustained) release of the agent from the capsule, compared with free (unbound) encapsulated agent. The situation addressed in this figure is essentially as depicted in the lower scheme of Fig. 2 or the right scheme of Fig. 17. The figure shows the results of a set of simple model (theoretical) experiments assuming 1) binding of the agent to saturable sites that can hold a single agent molecule and 2) first-order kinetics of diffusion across the capsule boundary (i.e., the flux is linearly related to the concentration difference across the boundary). Panels A (top) and B both show the time course of the total amount of agent (free plus protein-bound) remaining in the internal compartment. Panel A (bottom) shows the flux of the agent (amount of agent passing per time unit) through the capsule boundary. The gray lines consistently refer to a control situation in which no binding of the agent can occur and all agent is free (absence of binding protein). The various quantities and parameters were expressed in the same self-consistent units throughout all situations tested (e.g., moles, seconds, meters, square meters, cubic meters, etc.), the exact choice of units being essentially arbitrary. The settings were as follows: total internal ligand at start, 1 arbitrary unit (a.u.) (e.g., 1 mol); external agent concentration, 0 at time 0; external volume, infinite; internal volume, 1 a.u. (e.g., 1 m^3; thus, the starting local concentration of total internal agent could be seen as 1 mM); low-capacity binding corresponds to a total number of 1 a.u. (e.g., 1 mol) of internal binding sites; high-capacity binding corresponds to 10 a.u. internal binding sites; "high"-affinity binding, K_d = 0.01 mass a.u.·(volume a.u.)$^{-1}$ (e.g., 0.01 mol/m^3, or 10 μM); "low"-affinity binding, K_d = 0.5 mass a.u.·(volume a.u.)$^{-1}$; the clearance of agent through the capsule boundary was consistently 1 (volume a.u.)^3·(time a.u.)$^{-1}$ (e.g., 1 m^3s^{-1}). Note that the kinetics of release are often quasi-monoexponential, irrespective of binding (several traces in the lower logarithmic chart of panel A). However, if at time 0 a significant part of the internal ligand is free (thus, the amount of internal binding sites is limiting) and the binding affinity of the internal binding site is high, the release becomes clearly biphasic. This is shown already for high-affinity binding (K_d = 0.01 mass a.u.·(volume a.u.)$^{-1}$) in the logarithmic chart of panel A. However, it is especially clear in the logarithmic chart of panel B, where the number of internal binding sites was only 0.25 a.u. The initial amount of internal agent = 1 a.u. in all experiments; thus at least 75% of the internal agent was free at time 0 in panel B. In panel B, like in panel A, K_d = 0.01 mass a.u.·(volume a.u.)$^{-1}$ (high-affinity binding). This illustrates that ligand-binding proteins, as part of more complex controlled release systems, offer additional possibilities to manipulate and control the release of active agent. This is especially true if the binding proteins are chosen to be sensitive to certain triggering stimuli, for example, pH, certain ions, displacer molecules acting allosterically or competitively, temperature changes, etc. (for example, see the application described in Fig. 9).

bind only carbohydrate molecules, and the individual members of the lectin group often display high specificity for only one type of carbohydrate. At the other end of the versatility spectrum are the serum albumins. A serum albumin can bind a rather diverse range of hydrophobic compounds. This essentially broadens the potential spectrum of applications for these proteins. Compared with lectins and serum albumins, the ligand-binding proteins from the periplasmic space of Gram-negative bacteria occupy an intermediate position. As a family, they bind perhaps the most diverse range of ligands from small oxyanions to large macromolecules such as oligopeptides and carbohydrates. However, the individual binding proteins may be very specific for their particular ligand. From this same point of view, enzymes can be considered as a collection of unrelated proteins grouped under a functional heading, which together bind a diverse range of ligands (substrates), each with high specificity. Similarly, the Ig family can bind a virtually infinite number of ligand molecules, although each individual protein displays high specificity.

Other obviously important parameters that are relevant to the choice of protein are solubility, (thermo)stability and stability in the final formulated product, sensitivity to environmental stimuli (e.g., changes of pH and temperature), and commercial availability of the protein. To facilitate a comparison of various ligand-binding protein groups, their binding properties and general biochemical characteristics are described in more detail in the survey in *Section II*.

To use proteins as transporters of molecules other than their natural ligands, it will often be necessary to modify key aspects of their structure and properties. The possibility to produce and use recombinant proteins is often vital to the development of protein-mediated con-

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trolled release systems. Crystallographic data coupled with the production of site-directed mutant proteins have, in many cases, lead to detailed insight into the nature of the protein's binding site and structurally important amino acid residues. The availability of such structural and genetic data of a particular protein will in many cases be essential to the potential application of that (type of) ligand-binding protein in controlled release systems. In some cases, it has been possible to identify regions of the protein away from the binding site that are essential for maintaining the overall structure and binding characteristics or are performing other important functions. Conversely, it is obvious that other regions will exist that are not essential for ligand binding and could possibly be dispensed with, depending on the application. An example could be domain IA of HSA, which appears to play no part in ligand binding and structure maintenance. The use of recombinant methods of protein production also enables novel properties to be introduced, such as a modified binding site for new ligands, a modified affinity for an effective displacement of the active compound by a structural analog, or proteolytically sensitive sites for protease-triggered release of ligands. As far as fast and efficient methods exist to test a desired protein function (e.g., increased binding affinity, sensitivity to pH, proteolytic sensitivity, etc.) and as far as these can be applied to fast screening procedures, novel methods such as gene shuffling and directed evolution can be used even without knowledge of the protein structure (e.g., Stemmer, 1994; Moore et al., 1997; Crameri et al., 1998). However, in cases where a large number of simultaneous requirements, including biocompatibility, are essential, this will be less easily applicable, at least for the time being.

Finally, one should take into account the possible toxicity, stability, and elimination rates of the protein of choice in environments relevant to the application (e.g., tissues and body compartments). For many therapeutic applications, proteins like HSA, which is a natural component of the blood and can be expected to be nontoxic and nonimmunogenic, could be the carrier of choice (Chen et al., 1987; Wolkoff, 1987; Gupta and Hung, 1989). Lectins (but also many other proteins) are potentially dangerous in this respect even when they are sequestered in an encapsulating matrix.

In pharmaceutical applications, many parameters determine the bioavailability. The route of delivery is an important variable in this respect (Banerjee et al., 1991 and other articles in Lee, 1991; Banga, 1995). For example, oral administration of protein-drug complexes for intestinal delivery requires that the (drug binding site of) protein carrier is resistant to gastric enzymes and acid. This can be accomplished by choosing naturally resistant proteins, by encapsulating the protein in a resistant matrix, and sometimes by genetically modifying the protein. Parenteral and "nonparenteral" (e.g., nasal, transdermal) routes of protein administration are similarly subject to enzymatic barriers (Lee, 1986; Banerjee et al., 1991). A general overview of enzymatic barriers can be found in Lee et al. (1991). Also, physical barriers such as the intestinal epithelium and the endothelium (notably the blood-brain barrier), which are governed by cellular processes like endocytosis, can cause serious problems (Baker et al., 1991). However, if an i.v. injected drug-protein complex serves to mask systemic toxicity of the drug (or to prevent a loss of drug to nontarget tissues), it can be advantageous if the complex does not leave the blood stream and therefore does not reach unwanted sites. It may, for example, release the drug when this drug is taken over by high-affinity receptors facing the bloodstream (e.g., Meijer and van der Sluijs, 1989) or at sites where the pH is aberrant or the carrier protein is degraded or endocytosed. Uptake of carrier protein at unwanted sites can sometimes be easily circumvented. Thus, uptake of albumin microspheres containing the anticancer drug 5-fluorouracil by the reticuloendothelial system was avoided by presaturation of these sites with drug-free microspheres (Sugibayahshi et al., 1979).

In addition to enzymatic and physical barriers, competition for the drug between the carrier protein and other drug-binding sites in the body is an important parameter determining bioavailability (MacKichan, 1989; Kompella and Lee, 1991; Banga, 1995). These sites can be located on plasma proteins like albumin or α_1-acid glycoprotein, which bind (lipophilic) anions and cations, respectively (Creasey, 1979; Cascieri et al., 1988; Kakutani et al., 1988; Meijer and van der Sluijs, 1989; Aguirre et al., 1996), or on extra- or intracellular proteins outside the plasma compartment (Faed, 1981). The relative importance of binding sites other than the carrier protein may be appreciated when drugs are displaced from the carrier (Stöckel et al., 1988; MacKichan, 1989). The increase of free drug concentration after (induced) drug displacement from a high-affinity but low-capacity carrier protein will be only marginal if other drug-binding sites in the local environment buffer away the free drug (MacKichan, 1989). The binding affinity, the drug dissociation rate, and the binding capacity of the carrier protein influence the ratio of free and bound drug. The ensemble of binding events inside and outside the bloodstream influences the apparent volume of distribution and thereby the rate of elimination of the drug and/or drug-carrier complex (Faed, 1981; Kompella and Lee, 1991; Banga, 1995), e.g., resulting from hepatic uptake or renal (glomerular) filtration. When the clearance at a certain site is "restrictive" (rate limiting), only the free drug is removed. This will happen if, for example, the drug-binding affinity and capacity of the carrier are high and the drug dissociation rate is slow relative to the residence time of the drug-carrier complex at the site of clearance (Rowland, 1984; MacKichan, 1989; Meijer and van der Sluijs, 1989). Under these conditions and especially if the volume of distribution is relatively

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small, drug binding to the carrier protein will retard the clearance and increase the half-life of the drug, compared with a situation in which the drug is entirely free (Faed, 1981; Ko et al., 1995). In agreement, a decreased clearance was observed to result from unintended binding of various agents to serum proteins (albumin or α_1-acid glycoprotein) after introduction of the drug into the body. This went hand in hand with a decreased activity of the agent (Cascieri et al., 1988; Kakutani et al., 1988, Stöckel et al., 1988; Dudley et al., 1990; Sugihara et al., 1993; Nowak and Shaw, 1995; Aguirre et al., 1996). When the (plasma) clearance at a certain site is nonrestrictive, the blood flow is rate limiting, and both free and bound drug are efficiently cleared (Rowland, 1984; MacKichan, 1989; Meijer and van der Sluijs, 1989). If only the plasma compartment is taken into account, drug binding to a carrier protein will in that case have minimal effect on drug clearance. However, if such binding would decrease the drug redistribution to other body compartments (i.e., decrease the apparent volume of distribution), it could actually increase the elimination rate of the drug.

Obviously, these effects will only occur if the carrier protein is not rapidly removed from the plasma by elimination and/or degradation. The half-lives of proteins and peptides vary widely between proteins, species, and individuals. They can be as short as a few minutes or as long as several hours or even days (Lee, 1986; Davis et al., 1991; Kompella and Lee, 1991; Banga, 1995). Often, the elimination of foreign proteins, peptides, or drugs is according to a quasi-multiexponential elimination process. The shortest phase is sometimes overlooked due to relatively large sampling intervals, e.g., as discussed by Kompella and Lee (1991). This, in addition to other factors, makes it difficult to compare half-life values from different studies and to come up with generalized recommendations for the choice of carrier proteins. A short half-life of the drug-carrying protein will be problematic if the aim is slow, sustained release of drug. However, if targeting, masking of toxic effects, and/or stabilization of the drug is the goal, a short plasma half-life may not be a real problem, especially if the removal from the plasma is due to uptake at the target site. It should also be noted that many free drugs have a short half-life in the order of a few minutes (e.g., Creasey, 1979; McMartin, 1992). Thus, complexation of the drug to a proteinaceous carrier can indeed have a positive effect on its half-life and retard its elimination. Especially when natural plasma proteins like albumin are used, the half-life of the carrier protein will not be a problem. Unexpected effects can help to decrease the elimination of the carrier protein from the blood. RBP, which can be used as a high-affinity carrier for certain hydrophobic drugs, binds to transthyretin, another plasma protein (Monaco et al., 1995). In contrast to free RBP, this complex is too large to allow renal (glomerular) filtration and fast clearance from the blood.

To evaluate the suitability of a particular protein as a drug carrier, the balance sheet of the advantages (e.g., decreased drug oxidation, decreased systemic drug toxicity) and disadvantages (e.g., immunogenicity of the carrier, inability to enter certain compartments, sensitivity of the carrier to proteolytic enzymes) should be drawn up for the carrier-bound drug compared with the free drug. It should be noted that most disadvantages mentioned above are associated with pharmaceutical, biomedical, or cosmetics applications but essentially not with crop protection or with industrial, office, or household applications. It should also be realized that even when ligand-binding proteins are used for medical applications, viz., for the targeting and controlled release of drugs, the targeting and binding/release functions can often be separated. Problems such as protein stability in the body and protein clearance can be addressed by using multicomponent systems of which the drug-binding and drug-stabilizing protein is only one part (Fig. 17). It is well documented that clearance, degradation, and toxicity of proteins in the body can effectively be decreased by chemical modification, i.e., covalent attachment of small groups (e.g., *N*-acetylneuraminyl, glucuronyl, lactosyl, acetyl, acyl), by attachment of polymers like polyethylene glycol or dextran (Lee, 1986; Davis et al., 1991; Banga, 1995), or by encapsulation (Brownlee and Cerami, 1979; Jeong et al., 1985; Fischel-Ghodsian et al., 1988; Jefferey et al., 1993; Tabata et al., 1993, Kim et al., 1994).

D. Conclusion

As has been indicated above, various applications have been proposed that highlight the unique properties and advantages of ligand-selective binding proteins, and several applications are in the course of development. It

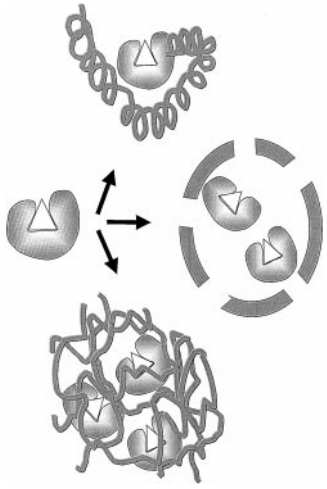

FIG. 17. Principle of immunological shielding of the agent-carrying carrier protein by covalently attached polymers (e.g., polyethylene glycol) (top), by encapsulation (right), or by incorporation in a polymer matrix (e.g., in a microsphere) (bottom). The bound ligand is schematically represented by the white triangle; the carrier protein is represented by the kidney-shaped structure.

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can thus be only a matter of time before such proteins are forming the basis of a large number of controlled release formulations and targeting systems. With respect to controlled delivery and uptake, there seem to be many as yet unexplored possibilities for binding proteins. We hope that this review will serve as a first source of information for those aiming at the development of new protein-based systems.

IV. Summary

The stabilization of valuable and/or labile active agents in challenging environments, the masking of unwanted properties such as bitterness or toxicity, and the targeting and controlled delivery of these agents under specific conditions are common problems in pharmacology, food science, crop protection, biotechnology, and industrial chemical processes. The concept of using ligand-selective high-affinity carriers for these purposes is relatively new, and such carriers would be of great value. Because ligand-binding proteins can be made to bind various low-molecular weight active agents, they can be used for controlled delivery of such agents. The structural and ligand-binding properties of a number of relevant protein families are reviewed in light of their possible suitability for incorporation in novel protein-based controlled delivery systems for appropriate natural or unnatural (atypical) ligands. Included are biotin-binding proteins, LBPs, bacterial periplasmic binding proteins, lectins, serum albumins, immunoglobulins, inactivated enzymes, insect PBPs, immunosuppressant-binding proteins, PiBPs, and SBPs.

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REFERENCES

Aguirre C, Troconiz IF, Valdivieso A, Jimenez RM, Gonzalez JP, Calvo R and Rodriguez-Sasiain JM (1996) Pharmacokinetics and pharmacodynamics of penbutolol in healthy and cancer subjects: Role of altered protein binding. *Res Commun Mol Pathol Pharmacol* **92:**53–72.

Ahearn JM and Fearon DT (1989) Structure and function of the complement receptors, CR1 (CD35), and CR2 (CD21). *Adv Immunol* **46:**183–219.

Airenne KJ, Sarkkinen P, Punnonen EL and Kulomaa MS (1994) Production of recombinant avidin in *Escherichia coli. Gene* **144:**75–80.

Åkerström B and Lögdberg L (1990) An intriguing member of the lipocalin protein family: α_1-Microglobulin. *Trends Biochem Sci* **15:**240–243.

Albin GW, Horbett TA, Miller SR and Ricker NL (1987) Theoretical and experimental studies of glucose sensitive membranes. *J Control Release* **6:**267–291.

Allan GG and Neogi SAN (1971) Synthetic polymers furnishing controlled release of a biologically active component during degradation. US Patent 184259.

Ames GFL (1986) Bacterial periplasmic transport systems: Structure, mechanism and evolution. *Annu Rev Biochem* **55:**397–425.

Aston R, Cowden WB and Ada GL (1989) Antibody-mediated enhancement of hormone activity. *Mol Immunol* **26:**435–446.

Bagshawe KD, Springer CJ, Searle F, Antoniw P, Sharma SK, Melton RG and Sherwood RF (1988) A cytotoxic agent can be generated selectively at cancer sites. *Br J Cancer* **58:**700–703.

Baker J, Hidalgo IJ and Borchardt RT (1991) Intestinal epithelial and vascular endothelial barriers to peptide and protein delivery, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 359–390, Marcel Dekker, New York.

Balthasar JP and Fung HL (1995) High-affinity rabbit antibodies directed against methotrexate: Production, purification, characterization, and pharmacokinetics in the rat. *J Pharm Sci* **84:**2–6.

Balthasar JP and Fung HL (1996) Inverse targeting of peritoneal tumors: Selective alteration of the disposition of methotrexate through the use of anti-methotrexate antibodies and antibody fragments. *J Pharm Sci* **85:**1035–1043.

Banaszak L, Winter N, Xu Z, Bernlohr DA, Cowan S and Jones TA (1994) Lipid-binding proteins: A family of fatty acid and retinoid transport proteins. *Adv Protein Chem* **45:**89–151.

Banerjee PS, Hosny EA and Robinson JR (1991) Parenteral delivery of peptide and protein drugs, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 487–544, Marcel Dekker, New York.

Banga AK (1995) *Therapeutic Peptides and Proteins*. Technomic Publishing Company, Lancaster, Pennsylvania.

Batt CA, Brady J and Sawyer L (1994) Design improvements of β-lactoglobulin. *Trends Food Sci Technol* **5:**261–265.

Batt CA, Rabson LD, Wong DWS and Kinsella JE (1990) Expression of recombinant bovine β-lactoglobulin in Escherichia coli. *Agric Biol Chem* **54:**949–955.

Bennett WS Jr and Steitz TA (1980) Structure of a complex between yeast hexokinase A and glucose. *J Mol Biol* **140:**183–230.

Berendsen HJC (1996) Bio-molecular dynamics comes of age. *Science (Wash DC)* **271:**954–955.

Berg J, Lötscher E, Steimer KS, Capon DJ, Baenziger J, Jäck HM and Wabl M (1991) Bispecific antibodies that mediate killing of cells infected with human immunodeficiency virus of any strain. *Proc Natl Acad Sci USA* **88:**4723–4727.

Bickel U, Yoshikawa T, Landaw EM, Faull KF and Pardridge WM (1993) Pharmacologic effects in vivo in brain by vector-mediated peptide drug delivery. *Proc Natl Aca Sci USA* **90:**2618–2622.

Binnie RA, Zhang H, Mowbray S and Hermodson MA (1992) Functional mapping of the surface of *Escherichia coli* ribose-binding protein: Mutations that affect chemotaxis and transport. *Protein Sci* **1:**1642–1651.

Björkman AJ, Binnie RA, Zhang H, Cole BL, Hermodson MA and Mowbray SL (1994) Probing protein-protein interactions—The ribose-binding protein in bacterial transport and chemotaxis. *J Biol Chem* **269:**30206–30211.

Blondeau K, Boze H, Jung G, Moulin G and Galzy P (1994) Physiological approach to heterologous human serum albumin production by *Kluyveromyces lactis* in chemostat culture. *Yeast* **10:**1297–1303.

Böcskei Z, Groom CR, Flower DR, Wright CE, Phillips SEV, Cavaggioni A, Findlay JBC and North ACT (1992) Pheromone binding to two rodent urinary proteins revealed by X-ray crystallography. *Nature (Lond)* **360:**186–188.

Boye JI, Ismail AA and Alli I (1996) Effects of physicochemical factors on the secondary structure of β-lactoglobulin. *J Dairy Res* **63:**97–109.

Braun W, Kallen J, Mikol V, Walkinshaw MD and Wüthrich K (1995) Three-dimensional structure and actions of immunosuppressants and their immunophilins. *FASEB J* **9:**63–72.

Brossmer R, Wagner M and Fischer E (1992) Specificity of the sialic acid-binding lactin from the snail *Cepaea hortensis. J Biol Chem* **267:**8752–8756.

Brownlee M and Cerami A (1979) A glucose-controlled insulin-delivery system: Semisynthetic insulin bound to lectin. *Science (Wash DC)* **206:**1190–1191.

Campanacci V, Longhi S, Nagnan-Le Meillour P, Cambillau C and Tegoni M (1999) Recombinant pheromone binding protein 1 from Mamestra brassicae (MbraPBP1). Functional and structural characterization. *Eur J Biochem* **264:**707–716.

Canfield R (1963) The amino acid sequence of egg white lysozyme. *J Biol Chem* **238:**2698–2707.

Carter DC and Ho JX (1994) Structure of serum albumin. *Adv Protein Chem* **45:** 153–203.

Cascieri MA, Saperstein R, Hayes NS, Green BG, Chicchi GG, Applebaum J and Bayne ML (1988) Serum half-life and biological activity of mutants of human insulin-like growth factor I which do not bind to serum binding proteins. *Endocrinology* **123:**373–381.

Chatel JM, Bernard H, Clement G, Frobert Y, Batt CA, Galvachin J, Peltre G and Wal JM (1996) Expression, purification and immunochemical characterization of recombinant bovine beta-lactoglobulin, a major cow milk allergen. *Mol Immunol* **33:**1113–1118.

Chen Y, Willmott N, Anderson J and Florence AT (1987) Comparison of albumin and casein microspheres as a carrier for doxorubicin. *J Pharm Pharmacol* **39:**978–985.

Chester KA and Hawkins RE (1995) Clinical issues in antibody design. *Trends Biotechnol* **13:**294–300.

Chilkoti A, Schwartz BL, Smith RD, Long CJ and Stayton PS (1995a) Engineered chimeric streptavidin tetramers as novel tools for bioseparations and drug delivery. *Biotechnology (NY)* **13:**1198–1204.

Chilkoti A, Tan PH and Stayton PS (1995b) Site-directed mutagenesis studies of the high-affinity streptavidin-biotin complex: Contributions of tryptophan residues 79, 108 and 120. *Proc Natl Acad Sci USA* **92:**754–1758.

Cho Y, Batt CA and Sawyer L (1994a) Probing the retinol-binding site of bovine β-lactoglobulin. *J Biol Chem* **269:**1102–11107.

Cho Y, Gu W, Watkins S, Lee SP, Kim TR, Brady JW and Batt CA (1994b) Thermostable variants of bovine β-lactoglobulin. *Protein Eng* **7:**263–270.

Chuo WH, Tsai TR, Hsu SH and Cham TM (1996) Preparation and in-vitro evaluation of nifedipine loaded albumin microspheres cross-linked by different glutaraldehyde concentrations. *Int J Pharm* **144:**241–245.

Cohen AI, Sim JSY, Van Horn MH, Gordesky SE and Gordon SI (1977) Sustained release pesticidal composition. US Patent 4007258.

Cohen AI, Sim JSY, Van Horn MH, Gordesky SE and Gordon SI (1979) Sustained release pharmaceutical composition. US Patent 4007258.

Copley RR and Barton GJ (1994) A structural analysis of phosphate and sulphate-binding sites in proteins. *J Mol Biol* **242:**321–329.

Cowan SW, Newcomer ME and Jones TA (1993) Crystallographic studies on a family of cellular lipophilic transporter proteins—refinement of P2 myelin protein and the structure determination and refinement of cellular retinol-binding protein in complex with all trans retinol. *J Mol Biol* **230:**1225–1246.

Crameri A, Raillard SA, Bermudez E and Stemmer WP (1998) DNA shuffling of a family of genes from diverse species accelerates directed evolution. *Nature (Lond)* **391:**288–291.

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Creasey WA (1979) *Drug Disposition in Humans—the Basis of Clinical Pharmacology*. Oxford University Press, New York.

Creighton TE (1993) *Proteins—Structures and Molecular Properties*. W. H. Freeman and Co., New York.

Crommelin DJA, Bergers JJ and Zuidema J (1992) Antibody-based drug targeting approaches: Perspectives and challenges, in *Medicinal Chemistry for the 21st Century* (Wermuth CG, Koga N, König H and Metcalf BW eds) pp 351–365, Blackwell, Oxford.

Curry S, Mandelkow H, Brick P and Franks N (1998) Crystal-structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat Struct Biol* **5:**827–835.

Dale GL (1980) Resealed erythrocytes as drug carriers, in *Drug Delivery Systems—Characteristics and Biomedical* (Juliano RL ed) pp 237–252, Oxford University Press, New York.

Danty E, Briand L, Michard-Vanhee C, Perez V, Arnold G, Gaudemer O, Huet D, Huet JC, Ouali C, Masson C and Pernollet JC (1999) Cloning and expression of a queen pheromone-binding protein in the honeybee: An olfactory-specific, developmentally regulated protein. *J Neurosci* **19:**7468–7475.

Davies DR and Padlan EA (1990) Antibody-antigen complexes. *Annu Rev Biochem* **59:**439–473.

Davis FF, Kazo GM, Nucci ML and Abuchowski A (1991) Reduction of immunogenicity and extension of circulating half-life of peptides and proteins, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 831–864, Marcel Dekker, New York.

Dean DA, Hor LI, Shuman HA and Nikaido H (1992) Interaction between maltose-binding protein and the membrane-associated maltose transporter complex in *Escherichia coli. Mol Microbiol* **6:**2033–2040.

DelaFuente G, Lagunas R and Sols A (1970) Induced fit in yeast hexokinase. *Eur J Biochem* **16:**226–233.

Denton H, Smith M, Husi H, Uhrin D, Barlow PN, Batt CA and Sawyer L (1998) Isotopically labeled bovine β-lactoglobulin for NMR studies expressed in *Pichia pastoris. Protein Expr Purif* **14:**97–103.

de Ropp JS, Chen Z and La Mar GN (1995) Identification of residues in the aromatic substrate binding site of horseradish peroxidase by ^{1}H NMR studies on isozymes. *Biochemistry* **34:**13477–13484.

de Wolf FA, van der Bent A, Pollaart KMEP, Vingerhoeds MH, Muresan S, Werten MWT, Mooibroek A and Derksen JTP (1998) Expression of β-lactoglobulin in *Pichia pastoris*, *in EC Framework IV Symposium Yeast as a Cell Factory* (Osseweijer P and van Dijken JP eds) p122, Delft University of Technology, Delft, the Netherlands.

Dong A, Matsuura J, Allison SD, Chrisman E, Manning MC and Carpenter JF (1996) Infrared and circular dichroism spectroscopic characterization of structural differences between β-lactoglobulin A and B. *Biochemistry* **35:**1450–1457.

Dosio F, Brusa P, Crosasso P, Arpicco S and Cattel L (1997) Preparation, characterization and properties in-vitro and in-vivo of a paclitaxel albumin conjugate. *J Control Release* **47:**293–304.

Doughty JC, Anderson JH, Willmott N and McArdle CS (1995) Intra-arterial administration of adriamycin-loaded albumin microspheres for locally advanced breast cancer. *Postgrad Med J* **71:**47–49.

Du G, Ng CS and Prestwich GD (1994) Odorant binding by a pheromone-binding protein: Active site mapping by photoaffinity labeling. *Biochemistry* **33:**4812–4819.

Du G and Prestwich GD (1995) Protein structure encodes the ligand-binding specificity in pheromone-binding proteins. *Biochemistry* **34:**8726–8732.

Dudley MN, Blaser J, Gilbert D and Zinner SH (1990) Significance of "extravascular" protein binding for antimicrobial pharmacokinetics in an in vitro capillary model of infection. *Antimicrob Agents Chemother* **34:**98–101.

Dufour E, Genot C and Haertlé T (1994) β-lactoglobulin-binding properties during its folding changes studied by fluorescence spectroscopy. *Biochim Biophys Acta* **1205:**105–112.

Dumay EM, Kalichevsky MT and Cheftel JC (1994) High-pressure unfolding and aggregation of β-lactoglobulin and the baroprotective effects of sucrose. *J Agric Food Chem* **42:**1861–1868.

Dunten P and Mowbray SL (1995) Crystal structure of the dipeptide binding protein from Escherichia coli involved in active transport and chemotaxis. *Protein Sci* **4:**2327–2334.

Einspahr H, Parks EH, Suguna K, Subramanian E and Suddath FL (1986) The crystal structure of pea lectin at 3.0-A resolution. *J Biol Chem* **261:**16518–16527.

Endicott JA, Sarangi F and Ling V (1991) Complete cDNA sequences encoding the Chinese hamster ovary P-glycoprotein gene family. *DNA Seq* **2:**89–101.

Endrizzi JA, Breddam K and Remington SJ (1994) 2.8-Å Structure of yeast serine carboxypeptidase. *Biochemistry* **33:**11106–11120.

Faed EM (1981) Protein binding of drugs in plasma, interstitial fluid and tissues: Effect on pharmacokinetics. *Eur J Clin Pharmacol* **21:**77–81.

Fanger MW, Morganelli PM and Guyre PM (1992) Bispecific antibodies. *Crit Rev Immunol* **12:**101–124.

Fedorov AA, Joseph-McCarthy D, Fedorov E, Sirakova D, Graf I and Almo SC (1996) Ionic interactions in crystalline bovine pancreatic ribonuclease A. *Biochemistry* **35:**15962–15979.

Felgner PL (1990) Particulate systems and polymers for *in vitro* and *in vivo* delivery of polynucleotides. *Adv Drug Del Rev* **5:**163–167.

Fersch KE and Stearns WE (1976) Controlled release fertilizer. US Patent 673791.

Fersht AR (1987) Dissection of the structure and activity of the tyrosyl-tRNA synthetase by site-directed mutagenesis. *Biochemistry* **26:**8031–8037.

Fischel-Ghodsian F, Brown L, Mathiowitz E, Brandenburg D and Langer R (1988) Enzymatically controlled drug delivery. *Proc Natl Acad Sci USA* **85:**2403–2406.

Fiume L, Bassi B, Busi C, Mattioli A, Spinosa G and Faulstich H (1986) Galactosylated poly(L-lysine) as a hepatotropic carrier of 9-β-D-arabinofuranosyladenine 5′monophosphate. *FEBS Lett* **203:**203–206.

Flower DR, North ACT and Attwood TK (1993) Structure and sequence relationships in the lipocalins and related proteins. *Protein Sci* **2:**753–761.

Fothergill MD and Fersht AR (1991) Correlations between kinetic and X-ray analyses of engineered enzymes: Crystal structures of mutants Cys 224 Gly-35 and Tyr 224 Phe-34 of tyrosyl-tRNA synthetase. *Biochemistry* **30:**5157–5164.

Franssen EJF, Moolenaar F, de Zeeuw D and Meijer DKF (1994) Drug targeting to the kidney with low-molecular-weight proteins. *Adv Drug Deliv Rev* **14:**67–88.

Garibotti M, Christiansen H, Schmale H and Pelosi P (1995) Porcine VEG proteins and tear prealbumins. *Chem Senses* **20:**69–76.

Geisow MJ (1992) Human serum albumin structure—solved. *Trends Biotechnol* **10:**335–337.

Gerlt JA (1987) Relationships between enzymatic catalysis and active site structure revealed by applications of site-directed mutagenesis. *Chem Rev* **87:**1079–1105.

Giebel LB, Cass RT, Milligan DL, Young DC, Arze R and Johnson CR (1995) Screening of cyclic peptide libraries identifies ligands that bind streptavidin with high affinities. *Biochemistry* **34:**15430–15435.

Gong QH, Fukuda T, Parkison C and Cheng SY (1988) Nucleotide sequence of a full length cDNA clone encoding a mouse cellular thyroid hormone binding protein disulfide isomerase and the beta subunit of prolyl-4-hydroxylase. *Nucleic Acids Res* **16:**1203.

Green NM (1990) Avidin and streptavidin. *Methods Enzymol* **184:**51–67.

Grubmüller H, Heymann B and Tavan P (1996) Ligand binding: Molecular mechanics calculation of the streptavidin-biotin rupture force. *Science (Wash DC)* **271:**997–999.

Gupta PK and Hung CT (1989) Albumin microspheres II: Applications in drug delivery. *J Microencapsul* **6:**463–472.

Hage DS, Noctor TAG and Wainer IW (1995) Characterization of the protein binding of chiral drugs by high-performance affinity chromatography interactions of R- and S-ibuprofen with human serum albumin. *J Chromatogr A* **693:**23–32.

Halmos T, Santarromana M, Herscovici J and Scherman D (1997) Brain drug delivery through the blood-brain barrier transport systems—attempted strategies and issues. *STP Pharma Sciences* **7:**37–42.

Hamilton-Wessler M, Ader M, Dea M, Moore D, Jorgensen PN, Markussen J and Bergman RN (1999) Mechanism of protracted metabolic effects of fatty acid acylated insulin, NN304, in dogs: Retention of NN304 by albumin. *Diabetologia* **42:**1254–1263.

Hattori M, Ametani A, Katakura Y, Shimizu M and Kaminogawa S (1993) Unfolding/refolding studies on bovine β-lactoglobulin with monoclonal antibodies as probes. *J Biol Chem* **268:**22414–22419.

Hattori M, Watabe A and Takahashi K (1995) β-lactoglobulin protects β-ionone related compounds from degradation by heating, oxidation and irradiation. *Biosci Biotechnol Biochem* **59:**2295–2297.

Haunerland NH, Jacobson BL, Wesenberg G, Rayment I and Holden HM (1994) Three-dimensional structure of the muscle fatty-acid-binding protein isolated from the desert locust *Schistocerca gregaria. Biochemistry* **33:**12378–12385.

He XM and Carter DC (1992) Atomic structure and chemistry of human serum albumin. *Nature (Lond)* **358:**209–215.

Helaakoski T, Vuori k, Myllylae R, Kivirikko L and Pihlajaniemi T (1989) Molecular cloning of the alpha subunit of human prolyl-4-hydroxylase: The complete cDNA-derived amino acid sequence—evidence for alternative splicing of RNA transcripts. *Proc Natl Acad Sci USA* **86:**4392–4396.

Hiles ID, Gallagher MP, Jamieson DJ and Higgins CF (1987) Molecular characterization of the oligopeptide permease of *Salmonella typhimurium. J Mol Biol* **195:**125–142.

Hing AW, Tjandra N, Cottam PF, Schaefer J and Ho C (1994) An investigation of the ligand-binding site of the glutamine-binding protein of *Escherichia coli* using rotational-echo double-resonance NMR. *Biochemistry* **33:**8651–8661.

Hirabayashi H, Nishikawa M, Takakura Y and Hashida M (1996) Development and pharmacokinetics of galactosylated poly-L-glutamic acid as a biodegradable carrier for liver-specific drug delivery. *Pharm Res* **13:**880–884.

Hoedemaeker FJ, van Eijsden RR, Díaz CL, de Pater BS and Kijne JW (1993) Destabilization of pea lectin by substitution of a single amino acid in a surface loop. *Plant Mol Biol* **22:**1039–1046.

Hoffman A and Ziv E (1997) Pharmacokinetic considerations of new insulin formulations and routes of administration. *Clin Pharmacokinet* **33:**285–301.

Hong Li, Hardin CC and Foegeding EA (1994) NMR studies of thermal denaturation and cation-mediated aggregation of β-lactoglobulin. *J Agric Food Chem* **42:**2411–2420.

Hsieh DST, Langer R and Folkman J (1981) Magnetic modulation release of macromolecules from polymers. *Proc Natl Acad Sci USA* **78:**1863–1867.

Iametti S, Cairoli S, De Gregori B and Bonomi F (1995) Modifications of high order structures upon heating of β-lactoglobulin: Dependence on the protein concentration. *J Agric Food Chem* **43:**53–58.

Iobst ST, Wormald MR, Weis WI, Dwek RA and Drickamer K (1994) Binding of sugar ligands to Ca^{2+}-dependent animal lectins. *J Biol Chem* **269:**15505–15511.

Jain RK (1989) Delivery of novel therapeutic agents in tumors: Physiological barriers and strategies. *J Natl Cancer Inst* **81:**570–576.

Jakoby MG, Covey DF and Cistola DP (1995) Localization of tolbutamide-binding sites on human serum albumin using titration calorimetry and heteronuclear 2-D NMR. *Biochemistry* **34:**8780–8787.

Jefferey H, Davis SS and O'Hagan D (1993) The preparation and characterization of poly(lactide-co-glycolide) microparticles II. The entrapment of a model protein using a (water-in-oil)-in water emulsion solvent evaporation technique. *Pharm Res* **10:**362–368.

Jeong SY, Kim SW, Holmberg DL and McRea JC (1985) Self-regulating insulin delivery systems III—*in vivo* studies. *J Controll Release* **2:**143–152.

Jeyarajah S and Allen JC (1994) Calcium binding and salt-induced structural changes of native preheated β-lactoglobulin. *J Agric Food Chem* **42:**80–85.

Jones ML and Kurzban GP (1995) Noncooperativity of biotin binding to tetrameric streptavidin. *Biochemistry* **34:**11750–11756.

Jordan ET and Goldstein IJ (1994) The sequence of a second member of the lima bean lectin gene family and the expression and characterization of recombinant lectin in *Escherichia coli. J Biol Chem* **269:**7674–7681.

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PHARM REV

PHARMACOLOGICAL REVIEWS

Jordan ET and Goldstein IJ (1995) Site-directed mutagenesis studies on the lima bean lectin. *Eur J Biochem* **230:**958–964.
Joshi AT (1996) Controlled release of volatile substances. US Patent 686730.
Kakutani T, Sumimoto E and Hashida M (1988) Effect of plasma protein binding on drug disposition in muscle tissue: Application of statistical moment analysis and network theory to in situ local single-pass perfusion system. *J Pharmacokinet Biopharm* **16:**129–149.
Kallen J, Mikol V, Taylor P and Walkinshaw MD (1998) X-ray structures and analysis of 11 cyclosporin derivatives complexed with cyclophilin A. *J Mol Biol* **283:**435–449.
Kang CH, Shin WC, Yamagata Y, Gokcen S, Ames GFL and Kim S-H (1991) Crystal structure of the lysine-, arginine-, ornithine-binding protein (LAO) from *Salmonella typhimurium* at 2.7-Å resolution. *J Biol Chem* **266:**23893–23899.
Kang YS and Pardridge WM (1994) Brain delivery of biotin bound to a conjugate of neutral avidin and cationized human albumin. *Pharm Res* **11:**1257–1264.
Kang YS, Saito Y and Pardridge WM (1995) Pharmacokinetics of [^{3}H]-biotin bound to different avidin analogues. *J Drug Target* **3:**159–165.
Karsa DR and Stephenson RA (1993) *Encapsulated and Controlled Release*. Royal Chemical Society, Cambridge, England.
Karsa DR and Stephenson RA (1996) *Chemical Aspects of Drug Delivery Systems*. Royal Chemical Society, Cambridge, England.
Kashiwagi K, Ymaguchi Y, Sakai Y, Kobayashi H and Igarashi K (1990) Identification of the polyamin-induced protein as a periplasmic oligopeptide binding protein. *J Biol Chem* **265:**8387–8391.
Katakura Y, Totsuka M, Ametani A and Kaminogawa S (1994) Tryptophan-19 of β-lactoglobulin, the only residue completely conserved in the lipocalin superfamily, is not essential for binding retinol, but relevant to stabilizing bound retinol and maintaining its structure. *Biochim Biophys Acta* **1207:**58–67.
Katoh S and Watanabe K (1995) Immunoadsorption of impurities contained in recombinant human serum albumin secreted from yeast. *J Ferment Bioeng* **79:** 545–548.
Katz BA (1995) Binding to protein targets of peptidic leads discovered by phage display: Crystal structures of streptavidin-bound linear and cyclic peptide ligands containing the HPQ sequence. *Biochemistry* **34:**15421–15429.
Kehres DG and Hogg RW (1992) *Escherichia coli* K12 arabinose-binding protein mutants with altered transport properties. *Protein Sci* **1:**1652–1660.
Kennedy JF, Palva PMG, Corella MTS, Cavalcanti MSM and Coelho LCBB (1995) Lectins, versatile proteins of recognition: A review. *Carbohydr Polym* **26:**219–230.
Kim CK, Im EB, Lim SJ, Oh YK and Han SK (1994) Development of glucose-triggered pH-sensitive liposomes for a potential insulin delivery. *Int J Pharm* **101:**191–197.
Kim TR, Goto Y, Hirota N, Kuwata K, Denton H, Wu SY, Sawyer L and Batt CA (1997) High-level expression of bovine β-lactoglobulin in *Pichia pastoris* and characterization of its physical properties. *Protein Eng* **10:**1339–1345.
Knight AT, Anderson TP and Ahmetagic MA (1995) Biodegradable controlled release amylaceous material matrix. US Patent 454093.
Ko HC, Almon RR and Jusko WJ (1995) Effect of corticosteroid binding globulin on the pharmacokinetics of prednisolone in rats. *Pharm Res* **12:**902–904.
Kock K, Ahlers C and Schmale H (1994) Structural organization of the genes for rat von Ebner's gland proteins 1 and 2 reveals their close relationship to lipocalins. *Eur J Biochem* **221:**905–916.
Kohita H, Matsushita Y and Moriguchi I (1994) Binding of carprofen to human and bovine serum albumins. *Chem Pharm Bull* **42:**937–940.
Kompella UB and Lee VHL (1991) Pharmacokinetics of peptide and protein drugs, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 392–484, Marcel Dekker, New York.
Korsching S (1991) Sniffing out odorant receptors. *Trends Biochem Sci* **16:**277–278.
Kost J and Langer R (1992) Responsive polymer systems for controlled delivery of therapeutics. *Trends Biotechnol* **10:**127–131.
Kost J, Leong K and Langer R (1989) Ultrasound-enhanced polymer degradation and release of incorporated substances. *Proc Natl Acad Sci USA* **86:**7663–7666.
Kreuter J (1992) *Colloidal Drug Delivery Systems*. Marcel Dekker, New York.
Kruhoffer M, Bub A, Cieslak A, Adermann K, Kunstyr I, Forssmann W and Magert H (1997) Gene expression of aphrodisin in female hamster genital tract segments. *Cell Tissue Res* **287:**153–160.
Kuderna JG and Saliman PM (1974) Depletion indicator for controlled release pesticide formulations. US Patent 464869.
Kumagai AK, Eisenberg J and Pardridge WM (1987) Absorptive-mediated endocytosis of cationized albumin and a β-endorphin-cationized albumin chimeric peptide by isolated brain capillaries. *J Biol Chem* **262:**15214–15219.
Kurtzhals P, Havelund S, Jonassen I and Markussen J (1997) Effect of fatty acids and selected drugs on the albumin binding of a long-acting, acylated insulin analogue. *J Pharm Sci* **86:**1365–1368.
Kuzin AP, Liu H, Kelly JA and Knox JR (1995) Binding of cephalothin and cephotaxime to D-Ala-D-ALa-peptidase reveals a functional basis of a natural mutation in a low-affinity penicillin-binding protein and in extended-spectrum β-lactamases. *Biochemistry* **34:**9532–9540.
LaLonde JM, Bernlohr DA and Banaszak LJ (1994a) X-ray crystallographic structures of adipocyte lipid-binding protein complexed with palmitate and hexadecanesulfonic acid. Properties of cavity binding sites. *Biochemistry* **33:**4885–4895.
LaLonde JM, Levenson MA, Roe JJ, Bernlohr DA and Banaszak LJ (1994b) Adipocyte lipid-binding protein complexed with arachidonic acid. *J Biol Chem* **269:** 25339–25347.
Larroque C, Pelegrin A and van Lier JE (1996) Serum albumin as a vehicle for zinc phtalocyanine: Photodynamic activities in solid tumor models. *Br J Cancer* **74:** 1886–1890.
Lassen D, Lücke C, Kveder M, Mesgarzadeh A, Schmidt JM, Specht B, Lezius A, Spener F and Rüterjans H (1995) Three-dimensional structure of bovine heart fatty-acid-binding protein with bound palmitic acid, determined by multidimensional NMR spectroscopy. *Eur J Biochem* **230:**266–280.
Lebbe C, Reichen J, Wartna E, Sagesser H, Poelstra K and Meijer DKF (1997) Targeting naproxen to non-parenchymal liver cells protects against endotoxin-induced liver damage. *J Drug Target* **4:**303–310.
Leckband DE, Schmitt FJ, Israelachvili JN and Knoll W (1994) Direct force measurements of specific and nonspecific protein interactions. *Biochemistry* **33:**4611–4624.
Lee VHL (1986) Peptide and protein drug delivery: Opportunities and challenges. *Pharm Int* **7:**208–212.
Lee VHL (1991) *Peptide and Protein Drug Delivery*. Marcel Dekker, New York.
Lee VHL, Traver RD and Taub ME (1991) Enzymatic barriers to peptide and protein drug delivery, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 303–358, Marcel Dekker.
Leung SHS, Nagai T and Machida Y (1991) Mucoadhesive dosage forms for peptide and protein drug delivery, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 741–767, Marcel Dekker, New York.
Lewis DFV and Lake BG (1995) Molecular modelling of members of the P4502A subfamily: Application to studies of enzyme specificity. *Xenobiotica* **25:**585–598.
Linvah O, Bayer EA, Wilchek M and Sussman JL (1993) Three-dimensional structures of avidin and the avidin-biotin complex. *Proc Natl Acad Sci USA* **90:**5076–5080.
Lis H and Sharon N (1986) Lectins as molecules and as tools. *Annu Rev Biochem* **55:**35–67.
Lobsanov YD, Gitt MA, Leffler H, Barondes SH and Rini JM (1993) Crystal-structure of the human dimeric S-lac lectin, L-14-II, in complex with lactose at 2.9 Ångströms resolution. *J Biol Chem* **268:**27034–27038.
Lubbe AS, Bergemann C, Riess H, Schriever F, Reichardt TP, Possinger K, Matthias M, Dorken B, Hermann F, Gurtler R, Hohenberger P, Haas N, Sohr R, Sander B, Lemke AJ, Ohlendorf D, Huhnt W and Huhn D (1996) Clinical experiences with magnetic drug targeting: A phase I study with 4′ epidoxorubicin in 14 patients with advanced solid tumors. *Cancer Res* **56:**4686–4693.
MacKichan JJ (1989) Protein binding drug displacement interactions—fact or fiction? *Clin Pharmacokinet* **16:**65–73.
Macleod A, Fedio WM and Ozimek L (1995) Aggregation of β-lactoglobulin as a function of pH and temperature. *Milchwiss* **50:**666–669.
Magert HJ, Cieslak A, Alkan O, Luscher B, Kauffels W and Forssmann WG (1999) The golden hamster aphrodisin gene. Structure, expression in parotid gland of female animals, and comparison with a similar murine gene. *J Biol Chem* **274:** 444–450.
Makrides SC (1998) Therapeutic inhibition of the complement system. *Pharmacol Rev* **50:**59–88.
Malpeli G, Stoppini M, Zapponi MC, Folli C and Berni R (1995) Interactions with retinol and retinoids of bovine cellular retinol-binding protein. *Eur J Biochem* **229:**486–493.
Markussen J, Havelund S, Kurtzhals P, Andersen AS, Halstrom L, Hasselager E, Larsen UD, Ribel U, Schaffer L, Vad K and Jonassen I (1996) Soluble fatty acid acylated insulins bind to albumin and show protracted action in pigs. *Diabetologia* **39:**281–288.
Matsuura JE and Manning MC (1994) Heat induced gel formation of β-lactoglobulin: A study on the secondary and tertiary structure as followed by circular dichroism spectroscopy. *J Agric Food Chem* **42:**1650–1656.
McMartin C (1992) Pharmacokinetics of peptides and proteins: Opportunities and challenges. *Adv Drug Res* **22:**41–106.
Meijer DKF and van der Sluijs P (1989) Covalent and noncovalent protein binding of drugs: Implications for hepatic clearance, storage, and cell-specific drug delivery. *Pharm Res* **6:**105–118.
Merchant AM, Zhu Z, Yuan JQ, Goddard A, Adams CW, Presta LG and Carter P (1998) An efficient route to human bispecific IgG. *Nat Biotechnol* **16:**677–681.
Merlin J (1991) Encapsulation of doxorubicin in thermosensitive small unilamellar vesicle liposomes. *Eur J Cancer* **27:**1026–1030.
Mestetsky TS (1973) Adhesive tape coated with a controlled release coating composition. US Patent 405764.
Mi FL, Wong TB and Shyu SS (1997) Sustained release of oxytetracycline from chitosan microspheres prepared by interfacial acylation and spray hardening methods. *J Microencapsul* **14:**577–591.
Mikol V, Kallen J, Pflügl G and Walkinshaw MD (1993) X-ray structure of a monomeric cyclophilin A—cyclosporin A crystal complex at 2.1 Ångströms resolution. *J Mol Biol* **234:**1119–1130.
Mitchell DC (1986) Protein compounds. US Patent 4568665.
Mock DM, Lankford GL and Mock NI (1995) Biotin accounts for only half of the total avidin-binding substances in human serum. *J Nutr* **125:**941–946.
Molema G and Meijer DKF (1994) Targeting of drugs to various blood cell types using (neo-)glycoproteins, antibodies and other protein carriers. *Adv Drug Deliv Rev* **14:**25–40.
Monaco H, Zanotti G, Spadon P, Bolognesi M, Sawyer L and Eliopoulos E (1987) *J Mol Biol* **197:**695–706.
Monaco HL, Rizzi M and Coda A (1995) Structure of a complex of two plasma proteins: Transthyretin and retinol-binding protein. *Science (Wash DC)* **268:**1039–1041.
Moore JC, Jin HM, Kuchner O and Arnold FH (1997) Strategies for the in vitro evolution of protein function: Enzyme evolution by random recombination of improved sequences. *J Mol Biol* **272:**336–347.
Mowbray SL (1992) Ribose and glucose-galactose receptors—Competitors in bacterial chemotaxis. *J Mol Biol* **227:**418–440.
Moy VT, Florin EL and Gaub HE (1994) Intermolecular forces and energies between ligands and receptors. *Science (Wash DC)* **266:**257–259.
Muckerheide A, Apple RJ, Pesce AJ and Michael JG (1987) Cationization of protein antigens. *J Immunol* **138:**833–837.
Müller HN and Skerra A (1993) Functional expression of the uncomplexed serum retinol-binding protein in *Escherichia coli*. *J Mol Biol* **230:**725–732.
Murray IA, Cann PA, Day PJ, Derrick JP, Sutcliffe MJ, Shaw WV and Leslie AGW (1995) Steroid recognition by chloramphenicol acyltransferase: Engineering and

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PHARMACOLOGICAL REVIEWS

structural analysis of a high affinity fusidic acid binding site. *J Mol Biol* **254:**993–1005.

Nagahora H, Harata K, Muraki M and Jigami Y (1995) Site-directed mutagenesis and sugar-binding properties of the wheat germ agglutinin mutants Tyr73Phe and Phe116Tyr. *Eur J Biochem* **233:**27–34.

Narayani R and Rao KP (1996) Solid tumor chemotherapy using injectable gelatin microspheres containing free methotrexate and conjugated methotrexate. *Int J Pharm* **142:**25–32.

Ndi EE, Swanson BG, Barbosa-Cánovas GV and Luedecke LO (1996a) Rheology and microstructure of β-lactoglobulin/sodium polypectate gels. *J Agric Food Chem* **44:**86–92.

Ndi EE, Swanson BG, Dunker AK and Luedecke LO (1996b) Relation of β-lactoglobulin-sodium polypectate aggregation to bulk macromolecular concentration. *J Food Sci* **61:**69–73.

Nelson RA (1953) The immune-adherence phenomenon: An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science (Wash DC)* **118:**733–737.

Nelson RA (1955) The immune-adherence phenomenon: A hypothetical role for erythrocytes in defence against bacteria and viruses. *Proc R Soc Med* **49:**55–58.

Newcomer ME (1995) Retinoid-binding proteins: Structural determinants important for function. *FASEB J* **9:**229–239.

Nickitenko AV, Trakhanov S and Quiocho FA (1995) 2 Å resolution structure of DppA, a periplasmic dipeptide transport/chemosensory receptor. *Biochemistry* **34:**16585–16595.

Nowak I and Shaw LM (1995) Mycophenolic acid binding to human serum albumin: Characterization and relation to pharmacokinetics. *Clin Chem* **41:**1011–1017.

Oh BH, Ames GFL and Kim SH (1994a) Structural basis for multiple ligand specificity of the periplasmic lysine-, arginine-, ornithine-binding protein. *J Biol Chem* **269:**26323–26330.

Oh BH, Kang CH, DeBondt H, Kim SH, Nikaido K, Joshi AK and Ames GFL (1994b) The bacterial periplasmic histidine-binding protein. *J Biol Chem* **269:**4135–4143.

Oh BH, Pandit J, Kang CH, Nikaido K, Gokcen S, Ames GFL and Kim S-H (1993) Three dimensional structure of the periplasmic lysine/arginine/ornithine-binding protein with and without a ligand. *J Biol Chem* **268:**11348–11355.

Ohla GA, Trakhanov S, Trewhella J and Quiocho FA (1993) Leucine/isoleucine/valine-binding protein contracts upon binding of ligand. *J Biol Chem* **268:**16241–16247.

O'Neill TE and Kinsella JE (1987) Binding of alkanone flavours to β-lactoglobulin: Effects of conformational and chemical modification. *J Agric Food Chem* **35:**770–774.

Ozaki M, Morisaki K, Idei W, Ozaki K and Tokunaga F (1995) A putative lipophilic stimulant carrier protein commonly found in the taste and olfactory systems. A unique member of the pheromone-binding protein superfamily. *Eur J Biochem* **230:**298–308.

Papiz MZ, Sawyer L, Eliopoulos EE, North ACT, Findlay JBC, Sivaprasadarao R, Jones TA, Newcomer ME and Kraulis PJ (1986) The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature (Lond)* **324:**383–385.

Pardridge WM, Triguero D, Buciak JL and Yang J (1990) Evaluation of cationized rat albumin as a potential blood-brain barrier drug transport vector. *J Pharmacol Exp Ther* **255:**893–899.

Pedersen AO, Mensberg KLD and Kragh-Hansen U (1995) Effects of ionic strength and pH on the binding of medium-chain fatty acids to human serum albumin. *Eur J Biochem* **233:**395–405.

Penichet ML, Kang YS, Pardridge WM, Morrison SL and Shin SU (1999) An antibody-avidin fusion protein specific for the transferrin receptor serves as a delivery vehicle for effective brain targeting: Initial applications in anti-HIV antisense drug delivery to the brain. *J Immunol* **163:**4421–4426.

Peumans WJ and Van Damme EJM (1996) Prevalence, biological activity and genetic manipulation of lectins in foods. *Trends Food Sci Technol* **7:**132–138.

Pevsner J, Hou V, Snowman AM and Snyder SH (1990) Odorant binding protein. Characterization of ligand binding. *J Biol Chem* **265:**6118–6125.

Pinnaduwage P and Huang L (1992) Stable target-sensitive immunoliposomes. *Biochemistry* **31:**2850–2855.

Pirrung MC and Huang CY (1996) A general method for the spatially defined immobilization of biomolecules on glass surfaces suing "caged" biotin. *Bioconjug Chem* **7:**317–321.

Pothakamury UR and Barbosa-Cánovas GV (1995) Fundamental aspects of controlled release in foods. *Trends Food Sci Technol* **6:**397–406.

Poznansky MJ and Cleland LG (1980) Biological macromolecules as carriers of drugs and enzymes, in *Drug Delivery Systems—Characteristics and Biomedical* (Juliano RL ed) pp 253–315, Oxford University Press, New York.

Poznansky MJ and Juliano RL (1984) Biological approaches to the controlled delivery of drugs: A critical review. *Pharmacol Rev* **4:**277–336.

Prestwich GD (1993) Bacterial expression and photoaffinity labeling of a pheromone-binding protein. *Protein Sci* **2:**420–428.

Pugliese L, Malcovati M, Coda A and Bolognesi M (1994) Crystal structure of apo-avidin from hen egg-white. *J Mol Biol* **235:**42–46.

Qi X, Holt C, McNulty D, Clarke DT and Jones G (1995) Limited interpretation of changes in the FTIR spectrum of β-lactoglobulin with temperature. *Biochem Soc Trans* **23:**612S.

Quiocho FA, Spurlino JC and Rodseth LE (1997) Extensive features of tight oligosaccharide binding revealed in high-resolution structures of the maltodextrin transport/chemosensory receptor. *Structure* **5:**997–1015.

Redl B, Holzfeind P and Lottspeich F (1992) cDNA cloning and sequencing reveals human tear prealbumin to be a member of the lipophilic-ligand carrier protein superfamily. *J Biol Chem* **267:**20282–20827.

Rees AR, Staunton D, Webster DM, Searle SJ, Henry AH and Pedersen JT (1994) Antibody design: Beyond the natural limits. *Trends Biotechnol* **12:**199–206.

Reist CJ, Combs MJ, Croft BY and Taylor RP (1993) Antigens pre-bound to the primate erythrocyte complement receptor via cross-linked bispecific monoclonal antibody heteropolymers are rapidly cleared from the circulation. *Eur J Immunol* **23:**3021–3027.

Richieri GV, Ogata RT and Kleinfeld AM (1994) Equilibrium constants for the binding of fatty acids with fatty acid-binding proteins from adipocyte, intestine, heart and liver measured with the fluorescent probe ADIFAB. *J Biol Chem* **269:**23918–23930.

Risch SJ and Reineccius GA (1995) Encapsulation and controlled release of food ingredients, in *American Chemical Society Symposium Series* vol 590, pp 1–214 American Chemical Society, Washington, DC.

Rocha TL, Paterson G, Crimmins K, Boyd A, Sawyer L and Fothergill-Gilmore LA (1996) Expression and secretion of recombinant ovine β-lactoglobulin in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. *Biochem J* **313:**927–932.

Rothemund S, Liou YC, Davies PL, Krause E and Sönnichsen FD (1999) A new class of hexahelical insect proteins revealed as putative carriers of small hydrophobic ligands. *Structure* **7:**1325–1332.

Rowland M (1984) Protein binding and drug clearance. *Clin Pharmacokinet* **9:**10–17.

Sacchettini JC and Gordon JI (1993) Rat intestinal fatty acid-binding protein. *J Biol Chem* **268:**18399–18402.

Sanders LM (1991) Controlled delivery systems for peptides, in *Peptide and Protein Drug Delivery* (Lee VHL ed) pp 785–806, Marcel Dekker, New York.

Sano T and Cantor CR (1990) Expression of a cloned streptavidin gene in *Escherichia coli*. *Proc Natl Acad Sci USA* **87:**142–146.

Sano T and Cantor CR (1995) Intersubunit contacts made by tryptophan 120 with biotin are essential for both strong biotin binding and biotin-induced tighter subunit association of streptavidin. *Proc Natl Acad Sci USA* **92:**3180–3184.

Sano T, Pandori MW, Chen X, Smith CL and Cantor CR (1995) Recombinant core streptavidins. A minimum-sized core streptavidin has enhanced structural stability and higher accessibility to biotinylated macromolecules. *J Biol Chem* **270:**28204–28209.

Scapin G, Gordon JI and Sacchettini JC (1992) Refinement of the structure of recombinant rat intestinal fatty acid-binding apoprotein at 1.2-Å resolution. *J Biol Chem* **267:**4253–4269.

Schaeffer HA and Brooks GJ (1992) Methods of improved skin care and the treatment of dermatological conditions. US Patent 5124313.

Schechter B, Chen L, Arnon R and Wilcheck M (1999) Organ selective delivery using a tissue-directed streptavidin-biotin system targeting 5-fluorouridine via TNP-streptavidin. *J Drug Target* **6:**337–348.

Scher HB (1999) *Controlled-Release Delivery Systems for Pesticides*. Marcel Dekker, New York.

Schreiber SL (1991) Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science (Wash DC)* **251:**283–287.

Schulz GE, Muller CW and Diederichs K (1990) Induced-fit movements in adenylate kinases. *J Mol Biol* **213:**627–630.

Scrimshaw NS and Murray EB (1995) Nutritional value and safety of "single cell protein", in *Enzymes, Biomass, Food and Feed* (Reed G and Nagodawithana TW vol eds), *Biotechnology 9* (Rehm HJ, Reed G, Pühler A and Stadler P eds) pp 221–237, Weinheim, New York.

Segalman DJ and Wallace JS (1995) Controlled release of molecular components of dendrimer/bioactive complexes. US Patent 415352.

Senderoff RI, Sheu MT and Sokoloski TD (1991) Fibrin based drug delivery systems. *J Parenteral Sci Technol* **45:**2–6.

Senter PD, Saulnier MG, Schreiber GJ, Hirschberg DJ, Brown JP, Hellström I and Hellström KE (1988) Anti-tumour effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate. *Proc Natl Acad Sci USA* **85:**4842–4846.

Sha RS, Kane CD, Xu Z, Banaszak LJ and Bernlohr DA (1993) Modulation of ligand-binding affinity of the adipocyte lipid-binding protein by selective mutation. *J Biol Chem* **268:**7885–7892.

Shaanan B, Lis H and Sharon N (1991) Structure of a legume lectin with an ordered N-linked carbohydrate in complex with lactose. *Science (Wash DC)* **254:**862–866.

Sharff AJ, Rodseth LE, Spurlino JC and Quiocho FA (1992) Crystallographic evidence of a large ligand-induced hinge-twist motion between the two domains of the maltodextrin-binding protein involved in active transport and chemotaxis. *Biochemistry* **31:**10657–10663.

Sharff AJ, Rodseth LE, Szmelcman S, Hofnung M and Quiocho FA (1995) Refined structures of two insertion/deletion mutants probe function of the maltodextrin-binding protein. *J Mol Biol* **246:**8–13.

Sharon N (1993) Lectin-carbohydrate complexes of plants and animals: An atomic view. *Trends Biochem Sci* **18:**221–226.

Shier WT (1979) Lectins as drug carriers, in *Drug Carriers in Biology* (Gregoriadis G ed) pp 43–70, Academic Press, London.

Shilton BH and Mowbray SL (1995) Simple models for the analysis of binding protein-dependent transport systems. *Protein Sci* **4:**1346–1355.

Shin SU, Wu D, Ramanathan R, Pardridge WM and Morrison SL (1997) Functional and pharmacokinetic properties of antibody-avidin fusion proteins. *J Immunol* **158:**4797–4804.

Sivaprasadarao A and Findlay JBC (1994) Structure-function studies on human retinol-binding protein using site-directed mutagenesis. *Biochem J* **300:**437–442.

Spurlino JC, Lu GY and Quiocho FA (1991) The 2.3-Å resolution structure of the maltose- or maltodextrin-binding protein, a primary receptor of bacterial active transport and chemotaxis. *J Biol Chem* **266:**5202–5219.

Stanfield RL and Wilson IA (1994) Antigen-induced conformational changes in antibodies: A problem for structural prediction and design. *Trends Biotechnol* **12:**275–279.

Stayton PS, Shimoboji T, Long C, Chilkoti A, Chen G, Harris JM and Hoffman AS (1995) Control of protein-ligand recognition using a stimuli-responsive polymer. *Nature (Lond)* **378:**472–474.

Steinbrecht RA (1996) Are odorant-binding proteins involved in odorant discrimination? *Chem Senses* **21:**719–727.

Stemmer WP (1994) Rapid evolution of a protein in vitro by DNA shuffling. *Nature (Lond)* **370:**389–391.

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Stöckel K, Trüb V, Dubach UC and McNamara PJ (1988) Effect of probenecis on the elimination and protein binding of ceftriaxone. *Eur J Clin Pharmacol* **34:**151–156.
Storm G, Koppenhagen FJ, Heeremans ALM, Vingerhoeds MH, Woodle MC and Crommelin DJA (1995) Liposomal delivery of peptides and proteins. *J Lipid Res* **5:**481–489.
Stryer L (1988) *Biochemistry*. W. H. Freeman and Co., New York.
Sugibayahshi K, Morimoto Y, Nadai T, Kato Y Hasegawaw A and Arita T (1979) Drug carrier property of albumin microspheres in chemotherapy: 2. Preparation and tissue distribution in mice of microsphere entrapped 5-fluorouracil. *Chem Pharm Bull* **27:**204–209.
Sugihara N, Furuno K, Kita N, Murakami T and Yata N (1993) The influence of increased plasma protein binding on the disposition of quinidine in turpentine-treated rats. *Biol Pharm Bull* **16:**63–67.
Tabata Y, Gutta S and Langer R (1993) Controlled delivery systems for proteins using polyanhydride microspheres. *Pharm Res* **10:**487–496.
Tam R and Saier MH Jr (1993) Structural, functional and evolutionary relationships among extracellular solute-binding receptors of bacteria. *Microbiol Rev* **57:**320–346.
Taylor RP and Ferguson PJ (1995) Primate erythrocyte (E) complement receptor (CR1) as an anchor site for bispecific-based therapies to clear pathogens or autoantibodies safely from the circulation. *J Hematother* **4:**357–362.
Taylor RP, Martin EN, Reinagel ML, Nardin A, Craig M, Choice Q, Schlimgen R, Greenbaum S, Incardona NL and Ochs HD (1997) Bispecific monoclonal antibody complexes facilitate erythrocyte binding and liver clearance of a prototype particulate pathogen in a monkey model. *J Immunol* **159:**4035–4044.
Taylor RP, Reist CJ, Sutherland WM, Otto A, Labuguen RH and Wright EL (1992) In vivo binding and clearance of circulating antigen by bispecific heteropolymer-mediated binding to primate erythrocyte complement receptor. *J Immunol* **148:**2462–2468.
Taylor RP, Sutherland WM, Reist CJ, Webb DJ, Wright EL and Labuguen RH (1991) Use of heteropolymeric antibodies to attach antigens to the C3b receptor of human erythrocytes: A potential therapeutic treatment. *Proc Natl Acad Sci USA* **88:**3305–3309.
Thies C and Bissery M (1984) Biodegradable microspheres for parenteral administration, in *Biomedical Applications of Microencapsulation* (Lim F ed) pp 53–74, CRC Press, Boca Raton, FL.
Thompson JR, Bratt JM and Banaszak LJ (1995) Crystal structure of cellular retinoic acid-binding protein 1 shows increased access to the binding cavity due to formation of an intermolecular β-sheet. *J Mol Biol* **252:**433–446.
Thompson LD and Weber PC (1993) Construction and expression of a synthetic streptavidin-encoding gene in *Escherichia coli*. *Gene* **136:**243–246.
Tomlinson E (1987) Theory and practise of site-specific drug delivery. *Adv Drug Del Rev* **1:**187–198.
Trouet A, Masquelier M, Baurain R and Deprez-De Campeneere D (1982) A covalent linkage between daunorubicin and proteins that is stable in serum and reversible by lysosomal hydrolases, as required for a lysosomotropic drug-carrier conjugate: In-vitro and in-vivo studies. *Proc Natl Acad Sci USA* **79:**626–629.
Vajda S, Weng Z, Rosenfeld R and DeLisi C (1994) Effect of conformational flexibility and solvation on receptor-ligand binding free energies. *Biochemistry* **33:**13977–13988.
Van Eijsden RR, Hoedemaeker FJ, Díaz CL, Lugtenberg BJJ, de Pater BS and Kijne JW (1992) Mutational analysis of pea lectin. Substitution of Asn^{125} for Asp in the monosaccharide-binding site eliminates mannose/glucose-binding activity. *Plant Mol Biol* **20:**1049–1058.
Verhoeyen ME, van der Logt CPE, Beggs TS and Davis PJ (1995) Antibody fragments for controlled delivery of therapeutic agents. *Biochem Soc Trans* **23:**1067–1072.
Vingerhoeds MH, Haisma HJ, van Muijen M, van de Rijt RBJ, Crommelin DJA and Storm G (1993) A new application for liposomes in cancer therapy: Immunoliposomes bearing enzymes (immuno-enzymosomes) for site-specific activation of prodrugs. *FEBS Lett* **336:**485–490.
Vingerhoeds MH, Storm G and Crommelin DJA (1994) Immunoliposomes *in vivo*. *Immunomethods* **4:**259–272.
Vinogradov S, Batrakova E, Li S and Kabanov A (1999) Polyion complex micelles with protein-modified corona for receptor-mediated delivery of oligonucleotides into cells. *Bioconjug Chem* **10:**851–860.
Wagner CR and Benkovic SJ (1990) Site-directed mutagenesis: A tool for enzyme mechanism dissection. *Trends Biotechnol* **8:**263–270.
Walsh CT, Zydowsky LD and McKeon FD (1992) Cyclosporin A, the cyclophilin class of peptidylproyl isomerases, and the blockade of T cell signal transduction. *J Biol Chem* **267:**13115–13118.
Wang Z, Choudhary A, Ledvina PS and Quiocho FA (1994) Fine tuning the specificity of the periplasmic phosphate transport receptor. *J Biol Chem* **269:**25091–25094.
Weber C, Wider G, von Freyberg B, Traber R, Braun W, Widmer H and Wüthrich K (1991) The NMR structure of cyclosporin A bound to cyclophilin in aqueous solution. *Biochemistry* **30:**6563–6574.
Weber PC, Pantoliano MW and Thompson LD (1992) Crystal structure and ligand-binding studies of a screened peptide complexed with streptavidin. *Biochemistry* **31:**9350–9354.
Weber PC, Pantoliano MW, Simons DM and Salemme FR (1994) Structure-based design of synthetic azo-benzene ligands for streptavidin. *J Am Chem Soc* **116:**2717–2724.
Weiner HL (1994) Oral tolerance. *Proc Natl Acad Sci USA* **91:**10762–10765.
Wilchek M and Bayer EA (1990) Avidin-biotin technology. *Methods Enzymol* **184:**5–13.
Wilkin JM, Dubus A, Joris B and Frèrè JM (1994) The mechanism of action of DD-peptidases: The role of Threonine-299 and -301 in the Streptomyces R61 DD-peptidase. *Biochem J* **301:**477–483.
Willet CS and Harrison RG (1999) Pheromone binding proteins in the European and Asian corn borers: No protein change associated with pheromone differences. *Insect Biochem Mol Biol* **29:**277–284.
Winter NS, Bratt JM and Banaszak LJ (1993) Crystal structure of holo and apo-cellular retinol-binding protein II. *J Mol Biol* **230:**1247–1259.
Withenshaw JD, Chadwick MA and Wilson WJ (1996) Agglomerated active with controlled release. US Patent 710340.
Wolf A, Shaw EW, Nikaido K and Ames GFL (1994) The histidine-binding protein undergoes conformational changes in the absence of ligands as analyzed with conformation-specific monoclonal antibodies. *J Biol Chem* **269:**23051–23058.
Wolf A, Shaw EW, Oh BH, DeBondt H, Joshi AK and Ames GFL (1995) Structure/function analysis of the periplasmic histidine-binding protein. *J Biol Chem* **270:**16097–16106.
Wolkoff AW (1987) The role of an albumin receptor in hepatic organic anion uptake: The controversy continues. *Hepatology* **7:**777–779.
Woolf TB (1998) Simulations of fatty acid-binding proteins suggest sites important for function. I. Stearic acid. *Biophys J* **74:**681–693.
Wootan MG, Bass NM, Bernlohr DA and Storch J (1990) Fatty acid-binding sites of rodent adipocyte and heart fatty acid-binding proteins: Characterization using fluorescent fatty acids. *Biochemistry* **29:**9305–9311.
Wu SY, Perez MD, Puyol P and Sawyer L (1999) Beta-lactoglobulin binds palmitate within its central cavity. *J Biol Chem* **274:**170–174.
Xu Z, Bernlohr DA and Banaszak LJ (1993) The adipocyte lipid-binding protein at 1.6-Å resolution. *J Biol Chem* **268:**7874–7884.
Xu Z, Buelt MK, Banaszak LJ and Bernlohr DA (1991) Expression, purification and crystallization of the adipocyte lipid-binding protein. *J Biol Chem* **266:**14367–14370.
Yao N, Trakhanov S and Quiocho FA (1994) Refined 1.89-Å structure of the histidine-binding protein complexed with histidine and its relationship with many other active transport/chemosensory proteins. *Biochemistry* **33:**4769–4779.
Yolles S (1973) Controlled release flavor compositions. US Patent 331324.
Yoshikawa T and Pardridge WM (1992) Biotin delivery to brain wit a covalent conjugate of avidin and a monoclonal antibody to the transferrin receptor. *J Pharmacol Exp Ther* **263:**897–903.
Yu BG, Kwon IC, Kim YH, Han DK, Park KD and Jeong SY (1996) Development of a local antibiotic delivery system using fibrin glue. *J Control Release* **39:**65–70.
Zanotti G, Berni R and Monaco HL (1993a) Crystal structure of liganded and unliganded forms of bovine plasma retinol-binding protein. *J Biol Chem* **268:**10728–10738.
Zanotti G, Malpeli G and Berni R (1993b) The interaction of N-ethyl retinamide with plasma retinol-binding protein (RBP) and the crystal structure of the retinoid-RBP complex at 1.9-Å resolution. *J Biol Chem* **268:**24873–24879.
Zanotti G, Marcello M, Malpeli G, Folli C, Sartori G and Berni R (1994) Crystallographic studies on complexes between retinoids and plasma retinol-binding protein. *J Biol Chem* **269:**29613–29620.
Zanotti G, Scapin G, Spadon P, Veerkamp JH and Sacchettini JC (1992) Three-dimensional structure of recombinant human muscle fatty acid-binding protein. *J Biol Chem* **267:**18541–18550.

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