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Atomic Structures of Periplasmic Binding Proteins and the High-Affinity Active Transport Systems in Bacteria [and Discussion]

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Atomic structures of periplasmic binding proteins and the high-affinity active transport systems in bacteria

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We have determined and refined the X-ray crystal structures of six periplasmic binding proteins that serve as initial receptors for the osmotic-shock sensitive, active transport of L-arabinose, D-galactose/D-glucose, maltose, sulphate, leucine/isoleucine/valine and leucine. The tertiary structures and atomic interactions between proteins and ligands show common features that are important for understanding the function of the binding proteins. All six structures are ellipsoidal, consisting of two similar, globular domains. The ligand-binding site is located deep in the cleft between the two domains. Irrespective of the nature of the ligand (e.g. saccharide, sulphate dianion or leucine zwitterion), the specificities and affinities of the binding sites are achieved mainly through hydrogen-bonding interactions. Binding of ligands induces a large protein conformational change. Three different structures have been observed among the binding proteins: unliganded 'open cleft', liganded 'open cleft', and liganded 'closed cleft'. Here we discuss the functions of binding proteins in the light of numerous crystallographic and ligand-binding studies and propose a mechanism for the binding protein-dependent, high-affinity active transport.

Introduction

In recent years our laboratory has been engaged in the determination of the X-ray structures of seven proteins that are found in the periplasmic space of gram-negative bacteria. These proteins (table 1) represent about a third of the entire family of binding proteins that serve as initial receptors of the osmotic-shock-sensitive, high-affinity active transport systems. (For the latest comprehensive review see Furlong (1987).) Table 1 indicates the considerable progress in structure determination of six of these proteins (shown in figure 1), and, most importantly, the co-workers who have been prominently associated with each. This paper, for the first time, focuses on the relation between tertiary structure and function of binding proteins. Space does not permit extensive discussion of other studies, but recent review articles (see, for example, Ames (1986); Furlong (1987)) more than rectify this deficiency.

Binding proteins (*ca.* two dozen) consist of a single polypeptide chain, ranging in size from 23 500 to 52 000 Da, with a single substrate-binding site. Substrates bound by these proteins include monosaccharides, oligosaccharides, tetrahedral oxyacids, amino acids, oligopeptides and other nutrients. Nevertheless, a remarkable common feature is the high affinity of their complexes with substrates; K_d values are usually in the micromolar range (see table 1) (Miller *et al.* 1983; Furlong 1987).

The kinetics of substrate binding in several binding proteins, which was determined by a stopped-flow rapid-mixing technique, indicate a rapid, second-order process (Miller *et al.* 1983). The association rate constants are similar (1×10^7–10^8 M^{-1} s^{-1}) even though the

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TABLE 1. BINDING PROTEINS: PROPERTIES AND STATUS OF X-RAY ANALYSES

(M.M., molecular mass (Da); *, primary substrate only; ABP, L-arabinose-binding protein; GBP, D-galactose/glucose-binding protein; MBP, D-maltose-binding protein; LIVBP, leucine/isoleucine/valine-binding protein; LBP, leucine-binding protein; SBP, sulphate-binding protein; PBP, phosphate-binding protein.)

protein	ABP	GBP	MBP
source	*E. coli* B/r	*E. coli* B/r	*E. coli* K12
M.M./residues	33600/306	34000/309	40500/370
specificity	L-arabinose = D-galactose > D-fucose	D-galactose = D-glucose	D-maltose ≈ maltodextrins
K_d (M)*	0.1×10^{-6}	0.4×10^{-6}	3.5×10^{-6}
structure status	1.7 Å refined	1.9 Å refined	2.3 Å refined
R-factor	0.15	0.15	0.25
co-workers	N. K. Vyas	N. K. Vyas and M. N. Vyas	J. C. Spurlino

protein	LIVBP	LBP	SBP	PBP
source	*E. coli* K12	*E. coli* K12	*S. typhimurium* LT2	*E. coli* K12
M.M./residues	36770/344	37900/346	34700/310	34400/321
specificity	leu = ile > val = thr	leucine	sulfate ≈ chromate	phosphate ≈ arsenate
K_d (M)*	1.0×10^{-6}	1.0×10^{-6}	0.2×10^{-6}	1.0×10^{-6}
structure status	2.4 Å refined	2.4 Å refined	1.7 Å refined	3.5 Å MIR
R-factor	0.16	0.16	0.15	—
co-workers	J. S. Sack and M. A. Saper	J. S. Sack	J. S. Sack and J. W. Pflugrath	H. Luecke

substrates tested were monopyranosides, large cyclic oligosaccharides, and amino acids. It is also noteworthy that the observed *in vitro* substrate specificities and affinities correspond well with the *in vivo* properties of each of the specific systems (Oxender 1972; Miller *et al.* 1983; Furlong 1987).

Focusing on the seven binding proteins under investigation in our laboratory (table 1) serves to highlight the diverse substrate specificities and binding properties of this family. These properties make this family of proteins an excellent system for comparative structure–function studies.

The L-arabinose-binding protein (ABP, see table 1 for key to abbreviations) also binds D-galactose and D-fucose (Hogg & Engelsberg 1969; Miller *et al.* 1983). The D-galactose-binding protein (GBP) actually binds the 4-epimer D-glucose slightly more tightly than D-galactose (Anraku 1968; Miller *et al.* 1983). The maltose-binding protein (MBP) has the most unusual saccharide-binding specificity as it can bind linear oligosaccharides of from two to seven α,(1–4)-linked glucosyl sugars, as well as cyclic α- and β-dextrins (Miller *et al.* 1983). MBP does not bind D-glucose. We have discovered that ABP, GBP, and MBP bind either the α or β anomeric form (Miller *et al.* 1983). Based on the well-refined ABP and GBP structures, the mechanisms for binding anomers and epimers have been clearly elucidated (Quiocho & Vyas 1984; Vyas *et al.* 1988). Note that GBP and MBP, but not ABP, are also essential components for chemotaxis (MacNab 1987).

In contrast to the sugar-binding proteins, the sulphate-binding protein (SBP) and the phosphate-binding protein (PBP) exhibit stringent specificities; the former binds only fully ionized tetrahedral sulphate, chromate and selenate dianions (Pardee 1966; Jacobson & Quiocho 1988), whereas the latter binds only the weak acids phosphate and arsenate (Medvenzky & Rosenberg 1970).

Finally, we have determined the structure of a leucine/isoleucine/valine-binding protein

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(LIVBP), which is able to recognize the three branched aliphatic amino acids and a leucine-binding protein (LBP), which recognizes only leucine (Anraku 1968; Furlong & Weiner 1970; Nazos *et al.* 1984). With the exception of the LIVBP and LBP pair, which shows about 80% sequence identity (Landick & Oxender 1985), the other binding proteins listed in table 1 have only about 10 to 20% sequence similarity (F. A. Quiocho, unpublished data).

In addition to the fast-reacting tight-binding substrate site, the binding proteins that participate in both active transport and chemotaxis have two other distinct sites or sets of sites; one set for interacting with the plasma-membrane bound protein components that actually pass the nutrient from the periplasm to the cytoplasm and another set for interacting with the cytoplasmic transmembrane signal transducer protein that triggers taxis toward chemical attractants (Ames 1986; MacNab 1987). It has been proposed that the membrane-bound proteins recognize and bind to the liganded form of the binding proteins in preference to the unliganded ones (Quiocho *et al.* 1977; Sack *et al.* 1989). This type of interaction must exist because binding proteins outnumber membrane components by several orders of magnitude. An implicit feature of this model is that the binding proteins undergo a ligand-induced conformational change, producing distinct structures for the substrate-loaded proteins.

The membrane-bound transport components usually consist of three proteins that appear to form a hetero-oligomer complex (Ames 1986; Furlong 1987; Higgins *et al.* 1989). A cartoon drawing of these complexes is shown in figure 2, but nothing is known about the nature of the interaction in the complex. Two proteins, being predominantly hydrophobic, are largely embedded in the membranes. The third protein, which is relative hydrophilic, is considered to be peripherally bound on the inner surface of the cytoplasmic membrane. It is the proposed unit for coupling energy to the binding protein-dependent active-transport systems, which appears to require ATP or a related metabolite (Berger & Heppel 1974). The structures of the membrane proteins are not known, but we predict, and hence have depicted (figure 2), that the hydrophilic protein that binds ATP will have a two-domain structure as found for periplasmic binding proteins and also observed for kinases and dehydrogenases (see below).

There are nearly two dozen binding proteins in the periplasm, each specific for a different substrate or set of related substrates, but all must perform the following tasks: bind substrate tightly, interact with the membrane-bound components and readily release substrate for transport. These constraints require a degree of similarity for all binding proteins, despite their observed differences in size, sequence, and specificity. Indeed, as will be shown, the three-dimensional structures of the binding proteins are very similar, even down to the atomic interactions between proteins and ligands. These common structural features lie at the core of understanding the mode of action of these proteins, as well as suggesting a possible mechanism for the binding protein-dependent active transport system (see below).

Tertiary structures

Common features

With the determination of the well-refined atomic structures of six periplasmic binding proteins (table 1), at least one from each major specificity group (i.e. sugars, oxyacid anions, and amino acids) and both unliganded and liganded conformations, basic structural and functional features shared by this family of proteins have emerged (figure 1). One dominant feature is the similarity of the tertiary structures of ABP, GBP, SBP, LIVBP, LBP and MBP.

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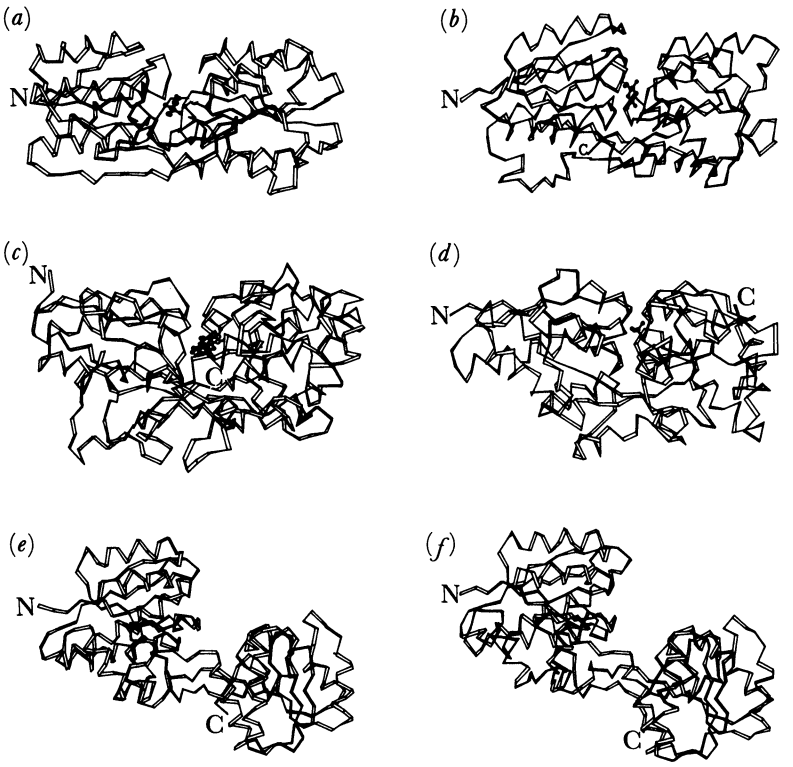

FIGURE 1. α-Carbon trace of the tertiary structures of six binding proteins. N, N-terminal end, C, C-terminal end. (*a*) L-arabinose-binding protein; (*b*) D-galactose-binding protein; (*c*) maltose-binding protein; (*d*) sulphate-binding protein; (*e*) leucine/isoleucine/valine-binding protein; (*f*) leucine-binding protein. Proteins in (*a*) to (*d*) contain ligand, bound and sequestered in the cleft between the two domains, whereas proteins (*e*) and (*f*) are of the unliganded open-cleft forms.

These binding proteins all share the following prominent structural features (figure 1) (Quiocho & Vyas 1985; Pflugrath & Quiocho 1988; Vyas *et al.* 1987, 1988; Sack *et al.* 1989*a*, *b*; J. C Spurlino & F. A. Quiocho, unpublished result). (i) Their common shape is a prolate ellipsoid with dimensions of about 70 Å† × 35 Å × 30 Å. (ii) They are composed of two distinct globular domains, designated N-domain and C-domain, as the former contains the amino or N-terminus and the latter the carboxyl or C-terminus. (iii) Approximately the first third of the chain constitutes the major part of the N-domain and the second third provides the bulk of the C-domain. The final segment meanders between the two domains. (iv) The two domains are connected by three separate short peptide segments. Although the three inter-domain segments are widely separated along the polypeptide chain, they are spatially close together, providing a boundary, base, or hinge, for the cleft between the two domains. (v) The deep cleft formed between the two domains contains the substrate-binding site. (vi) Although neither domain of the binding proteins is folded from one continuous peptide segment, the two domains exhibit similar packing of secondary structure (or 'supersecondary structure'); a central core of a pleated sheet, sandwiched by helices. We recognized 12 years ago (Quiocho *et al.* 1977) that this supersecondary structure is similar to the then so-called 'Rossmann' or nucleotide fold, which is found in kinases and dehydrogenases. Therefore, it is likely that the

† 1 Å = 10^{-10} m = 10^{-1} nm.

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membrane component that binds ATP or analogues will have domain structures similar to those of the binding proteins.

Among the sugar-binding proteins, ABP and GBP structures are very similar (figure 1) (N. K. Vyas & F. A. Quiocho, unpublished data). Least squares superimposing of binding-protein structures gave the surprising result that MBP is much closer to SBP than to ABP or GBP (J. C. Spurlino & F. A. Quiocho, unpublished result). As expected, as LIVBP and LBP have almost 80% sequence identity, their structures (figure 1) are virtually identical (Sack *et al.* 1989*b*).

The differences in the sizes of the binding proteins are reflected in their structures through a combination of factors, mainly the number and size of loops and, to some extent, the number and length of helices and strands. For example, in the LIVBP structure (344 residues), extra residues (38 more than ABP) are found in extended loops. We therefore predict that the structures of the other binding proteins, even those with molecular masses at the lower and upper ends of the range (about 25000 and 50000 Da respectively) will have similar overall structural features to those outlined above.

Open-unliganded, open-liganded and closed-liganded structures: conformational change

The structures of ABP, GBP, MBP, and SBP were determined with bound, endogenous substrate. However, it was necessary to remove leucine bound in LIVBP and LBP to obtain large crystals suitable for high resolution structure analysis (Sack *et al.*, 1989*a*, *b*). As shown in figure 1, the two domains of the LIVBP and LBP are well separated, rendering the cleft between the two domains open by as much as 18 Å and accessible to the bulk solvent. This open cleft structure contrasts with the close packing of the domains in the structures of ABP, GBP, MBP and SBP, where the substrate bound in each protein is buried and completely enclosed in the binding-site cleft (figure 1). Moreover, in the closed, liganded structures, both domains participate in binding the substrate.

Native LIVBP crystals grown in the absence of substrate and then soaked in a solution containing leucine do not change their crystal morphology. Structure refinement of the isomorphous LIVBP leucine-soaked crystal revealed a major portion of the binding site; the leucine is lodged exclusively in a cavity of one of the domains (the N-domain) facing the wide-open cleft (Sack *et al.* 1989*a*). Thus we have seen three different forms of the binding proteins: open unliganded, open liganded, and closed liganded.

Atomic interactions between binding proteins and substrates

Perhaps the most remarkable and totally unforeseen common feature of the binding proteins revealed by our high resolution structural studies is that substrate specificities of these proteins are conferred primarily by hydrogen bonds. As the substrates of these proteins are diverse – uncharged molecules (e.g. mono- and oligosaccharides), dianions (e.g. sulphate and phosphates) and zwitterions (e.g. amino acids) – this finding is truly remarkable. Not only is this finding consistent with the data showing that binding-protein–substrate complexes have similar K_d values (Miller *et al.* 1983; Furlong 1987), but it also illustrates the role of binding proteins in active transport (see below).

The highly refined structures of the liganded forms of ABP at 1.7 Å resolution (Quiocho & Vyas 1984) and GBP at 1.9 Å (Vyas *et al.* 1987, 1988) have revealed the mode of sugar binding

and paved the way for a thorough and general understanding of the atomic interactions between proteins and carbohydrates. As these interactions have been extensively described in original papers (Quiocho & Vyas 1984; Vyas *et al.* 1988) and recently reviewed at length in three recent articles (Quiocho 1986, 1988, 1989) the salient features are only summarized here. (i) As hydroxyl groups, which are stereospecific and highly exposed, constitute the major functional groups of carbohydrates, hydrogen bonds involving these groups are the main factors in conferring specificity and affinity to protein–carbohydrate interactions. (ii) Three types of hydrogen bond are formed, 'cooperative' hydrogen bonds, bidentate hydrogen bonds and networked hydrogen bonds. (iii) Sugar-binding sites are heavily populated by residues with polar, planar side chains with at least two functional groups capable of engaging in all three types of hydrogen bonds (e.g. Asn, Asp, Glu, Arg, His). (iv) Carboxylate side-chain residues are especially important in binding anomers and epimers. (v) Numerous van der Waals' contacts are formed, involving all the atoms of the bound saccharides. Most of these contacts result from hydrogen bonds, enabling more of the atoms of the polar residues to come within van der Waals' distance of sugar. (vi) Aromatic residues in the binding sites stack on the sugar ring, close to the hydrophobic patches of the monopyranosides.

Although hydrogen bonds were expected in binding protein–saccharide complexes, their importance in binding of charged substrates (e.g. sulphate dianion to SBP and leucine zwitterion to LIVBP) was completely unforeseen (Pflugrath & Quiocho 1985, 1988; Sack *et al.* 1989*a*). (We have also found that the side chain of the leucine bound to LIVBP is stacked with the side chain of a binding site leucine residue.) Despite the differences in the nature and extent of the uncompensated charges on these substrates, three key features are associated with the interactions with the ionic groups (Quiocho *et al.* 1987): (i) the interactions are mediated primarily via hydrogen bonds; (ii) the hydrogen bonds are formed chiefly with main-chain peptide units; the peptide NH groups with the sulphate dianion and both the peptide NH and CO groups with the α-carboxylate and ammonium groups, respectively, of the leucine; (iii) the peptide units associated with the charged substrates are in turn coupled to hydrogen-bond arrays, which lead to the bulk solvent. These common elements constitute the basis of our proposed mechanism for stabilization of charges on isolated ionic groups sequestered in proteins (Pflugrath & Quiocho 1985; Quiocho *et al.* 1987). The mechanism simply considers the dipoles from the hydrogen-bonding groups, especially those from the dipolar peptide units, to be effective in stabilizing the buried charges. Moreover, the hydrogen-bond arrays, arranged like electrical conductors, can dissipate the charge.

It is interesting to note that the hydrogen-bonding interactions in the binding protein–saccharide complexes depend entirely on amino acid side-chains, whereas the complexes of the SBP with sulphate and LIVBP with leucine rely héavily on main-chain peptide units. The heavy reliance on dipolar main-chain peptide groups reflects the need not only to offset the hydration energies of the ionic substrates, which are much greater than those of uncharged saccharides, but also to provide a means of stabilizing the charges on the ionic substrates.

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Binding protein-dependent, high-affinity active transport

Function of binding proteins

The binding proteins serve as initial receptors for active transport and confer specificity on the system. As specificity does not necessarily require tight ligand binding, it is intriguing that they bind ligands so tightly. We believe that this property is required for efficient transport (discussed below). As already noted above, the equilibrium dissociation constants of binding protein–substrate complexes *in vitro* are very similar to the Michaelis constants for active transport. Moreover, as discussed by Miller *et al.* (1983), the large ligand association rate constants of binding proteins allow a rapid response and confer, in part, the high sensitivity of both the corresponding transport and chemotaxis systems. Even if the concentration of ligands were 10^{-7} M in the periplasm, the half-time for binding would be less than 1 s.

Results of the crystallographic analysis leave no doubt that hydrogen bonds are mainly responsible for conferring specificity and ensuring correctness of fit of the substrates to the binding proteins. This conclusion is based on the understanding that hydrogen bonds are more highly directional than dispersion forces.

Other factors associated with binding protein–substrate affinity have also been considered (Quiocho 1988, 1989). For instance, hydrogen bonds are highly desirable, especially in active transport, because they are stable enough to impart the requisite affinity for a given ligand site but are of sufficiently low strength or have a low energy barrier to allow rapid ligand dissociation. The many hydrogen bonds between protein and nutrients observed in the closed, ligand forms mitigates any need for aqueous solvation of the substrates, overcoming the large hydration energies of these substrates, especially the ionic ones, in the initial step of active transport.

The binding proteins must undergo a ligand-induced conformational change, aptly described as a 'Venus' flytrap' or 'Pac-man' model (Quiocho *et al.* 1977; Newcomer *et al.* 1981*a*, *b*; Mao *et al.* 1982; Miller *et al.* 1983; Sack *et al.* 1989*a*). The existence of the three structural forms among the binding proteins suggests the following pathway (I–III, figure 2) to the formation of a productive protein–substrate complex. The ligand binds preferentially to one domain of the open form by virtue of the greater number of interactions (hydrogen bonds and van der Waals' contacts) than will be formed with the other domains. A bending motion about a hinge between the two domains causes the other domain to participate in binding and completely entrap the substrate. The process is fast and coupled to the dehydration of the substrate and the displacement of ordered water molecules that were hydrogen bonded to the polar residues in the binding site, giving rise to favourable entropy.

The closed, substrate-loaded form of the binding proteins presents a unique structure, which is clearly distinguishable from any of the open forms. This form interacts with membrane-bound components, thus initiating nutrient translocation or flagella motion. We further envisage that for the closed form of the binding protein to be recognized in preference to the open form, there must be at least two sites in the binding protein, one on each domain on the cleft side of the protein (figure 2) (Quiocho *et al.* 1977; Newcomer *et al.* 1981*a*, *b*; Sack *et al.* 1989*a*). There is one example of this: the MBP–Tar signal transducer interaction (J. C. Spurlino & F. A. Quiocho, unpublished data). This is also likely to exist in the interaction between LIVBP and membrane components (Sack *et al.* 1989*b*). Vyas *et al.* (1988) have further considered the nature of the interactions between binding protein and membrane components.

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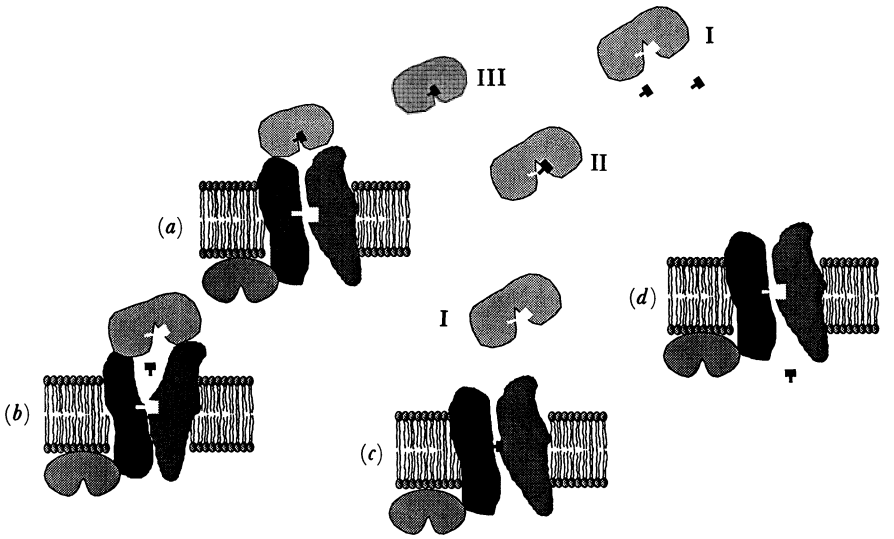

FIGURE 2 A proposed mechanism for the binding protein-dependent high-affinity active transport. See text for details. I, II, and III are the different conformations of binding proteins (located on the periplasmic side) as revealed by X-ray analysis. Further portrayed are three membrane-bound proteins; the one peripherally bound in the inner surface of the membrane, at the cytoplasmic side, is the ATP-binding protein. Based on crystallographic analysis, the ligand-binding sites in the binding proteins are schematically shown to be asymmetric; one domain interacts with the substrate more extensively than the other domain. To convey this idea further, nutrients ('T') are also depicted as being asymmetric (although not necessarily the case). One crucial feature of the proposed mechanism is the differential affinities of the ligand-binding sites of the system; high in the binding protein and low in the binding site(s) located in the membrane components. (Figure courtesy of Bruce Jackson.)

A mechanism for binding protein-dependent, high-affinity active transport

Our proposed mechanism for active transport assumes the existence and importance of at least two substrate binding sites in the entire system; one with high affinity at the uptake side of the plasma membrane and another with lower affinity at the discharge side, towards the cytoplasm (Quiocho & Vyas 1984; Quiocho 1986; Sack *et al.* 1989*a*). This mechanism attaches considerable importance to the features of binding proteins, especially the facts that these proteins are located in the periplasmic space or uptake side, and have extremely high affinity for nutrients. It should be underscored that normal, highly efficient transport has never been shown to occur in the absence of the binding proteins. Deletion of binding proteins by genetic manipulation leads to abolition of active transport (Ames 1986; Furlong 1987). Although pseudorevertants in the maltose transport system translocate ligands with only three membrane proteins and without a binding protein, the affinity of this unnatural system is several orders of magnitude less than that of the wild-type system (Shuman 1982). This finding, we believe, gives further evidence that binding proteins are essential for achieving the requisite kinetics and affinity for efficient transport against a high concentration gradient *in vivo*.

In our proposed mechanism, portrayed in figure 2, transport is initiated by interaction of the liganded, closed form of a binding protein (III) with the membrane components to give (*a*). The closed, liganded, high-affinity form (III) and the preceding liganded open (II) and unliganded open (I) forms of the binding protein are well established (*vide supra*). The two lobes are depicted in productive interaction in (*a*). Conformational changes propagate throughout

the entire system in synchrony with translocation. The substrate is released from the binding protein and transferred to an activated site or sites in the membrane components (*b*) and finally translocated to the cytoplasm (*c–d*), completing a cycle. Energy, which depends on a metabolite related to ATP, has to be somehow coupled to the binding protein-dependent transport systems.

Our proposed mechanism differs from those that assume the importance of pores formed by the membrane-bound protein components (Higgins *et al.* 1982; Boos 1982; Ames 1986) and others such as the model proposed for the accumulation of maltose (Shuman 1982; see also Ames (1986)).

Conformational change is a key element in all proposed mechanisms, including the one discussed here. Such a change could modulate the affinity of the ligand-binding site in the membrane. It could alter the orientation of the membrane site and thus provide a gating mechanism for unidirectional transport.

The membrane ligand site of each system could be in one protein or shared between two. However, the crucial assumption is that the membrane site has affinity much lower than that of the binding protein site, closer to the concentration of substrates in the cytoplasm. If this is the case (an estimate of $K_d \approx 10^{-3}$ M), the ratio of the affinity of the membrane sites and to the binding protein site is similar to the ratio of the internal substrate concentrations at equilibrium, experimentally found to be as large as 10^4 for the maltose transport system (Szmelcman *et al.* 1976).

By searching for homology between a peptide segment of ABP involved in sugar binding, as determined by X-ray analysis (Quiocho & Vyas 1984; Quiocho 1986), and a segment in the sequence of *lac* permease, a shock-insensitive active transport system, and by carrying out site-directed mutagenesis, Roepe & Kaback (1989) have been able to identify a putative sugar-binding site in the permease. This strategy could be applied to the binding protein dependent transport systems for possible identification of a substrate site in the membrane proteins. Studies on the pseudorevertants in the maltose transport system indicate the existence of a sugar-binding site in the membrane components (Shuman 1982).

In light of the finding that the similar high substrate affinity of the periplasmic binding proteins is achieved principally through hydrogen-bonding and van der Waals' interactions, it is realistic to assume that similar interactions determine or modulate the affinity, as well as specificity, of the ligand binding site(s) in the plasma membrane-bound protein components.

I am indebted to fellow co-workers for their unstinting efforts. Their individual contributions, which are recognized through publications (see references below) and table 1, are credited for our recent accomplishments and discoveries. Work from this laboratory was supported by the Howard Hughes Medical Institute and grants from the National Institutes of Health and the Welch Foundation.

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Discussion

C. F. Higgins (*Department of Biochemistry, University of Dundee, U.K.*). I would like to make two points relating to Dr Quiocho's talk. First, Dr Quiocho suggested on kinetic grounds that the membrane proteins of binding protein-dependent transport systems should possess low affinity substrate-binding sites.

First, there is already good genetic evidence that such sites exist. Giovanna Ames has characterized mutants of the histidine transport system, which are altered in substrate specificity and that alter the membrane components of the system (Payne *et al.* 1985). The best evidence is that of Howard Shuman who showed specific transport via the maltose system in the absence of a periplasmic protein (Treptow & Shuman 1985). The only reasonable interpretation of these data is that the membrane proteins themselves possess specific substrate-binding sites.

My second point relates to the role of the periplasmic binding protein. I argue against the view that the periplasmic protein is integral to the transport process. I suggest that the periplasmic protein is best viewed as a 'bolt-on' component, which clearly enhances the affinity and efficiency of transport but does not itself play an integral role in the mechanism of substrate translocation across the membrane; transport can occur in the absence of a periplasmic component. This idea is discussed in my paper for this symposium. The best evidence for this is Shuman's mutants, which allow active maltose accumulation in the absence of a periplasmic protein. In addition, the mammalian Mdr protein (which everybody believes to operate by a similar mechanism to the binding protein-dependent transport system), does not have an equivalent of a periplasmic component, implying that the binding protein is not essential for the translocation mechanism.

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F. A. Quiocho. What Professor Higgins pointed out concerning the presence of substrate-binding site in the membrane component is also noted in my chapter for this volume. Moreover, I further indicate, appropos the studies Professor Higgins cited, that although transport occurs in the absence of a periplasmic binding protein, the K_m of transport is several orders of magnitude less than that in the presence of the binding protein.

As to your second point, the binding proteins may not be playing an 'integral' role *per se*, but they are certainly present not only in Gram-negative but also in Gram-positive bacteria. In the Gram-positive bacterium *Streptococcus pneumoniae* and also in the mycoplasma *Mycoplasma hyorhinis*, the binding proteins are maintained at proximity of the cytoplasmic membrane by insertion of their N-terminal glyceride-cysteine into this membrane (Gilson *et al.* 1988). Furthermore, as discussed in many of our papers (see References) and in this paper, the binding proteins play several essential roles (including enhancing affinity and efficiency) in the 'high

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affinity active transport system'. One crucial role revealed by our studies is the ability of the high-affinity binding proteins to effectively dehydrate substrates, especially those with enormous hydration energy (e.g. sulphate). The binding protein-dependent active transport system is extremely efficient, able to carry out translocation against a very large gradient (see above). It is doubtful if this efficiency can be duplicated in the absence of the periplasmic binding protein (this is certainly true in the pseudorevertant maltose system) or perhaps in the Mdr protein. As several mechanisms have been proposed for the binding protein-dependent transport system, it may not be safe to assume that everybody believes or have settled on one mechanism. Which mechanism is it anyway?

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