Invited Trends Article

# Sialic Acid-Binding Proteins: Characterization, Biological Function and Application

Fu-Yue Zeng

Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Hermann-Rein-Straße 3, D-W-3400 Göttingen, Bundesrepublik Deutschland

and

Hans-Joachim Gabius

Institut für Pharmazeutische Chemie der Universität, Abteilung Glykobiochemie und Angewandte Tumorlektinologie, Marbacher Weg 6, D-W-3550 Marburg, Bundesrepublik Deutschland

Z. Naturforsch. **47c**, 641–653 (1992); received May 11, 1992

The last decade has witnessed steadily growing support for the notion that the carbohydrate portion of glycoconjugates is not merely an inert structural addition to the protein or lipid backbone. Considerable attention has been given to the chemical composition of glycosidic residues in such heteropolymers. Sialic acids are frequently occurring components of oligosaccharide side chains in glycoconjugates of most higher animals and a few microorganisms. They appear to play an important role as ligands in glycobiological interactions. Mediation of a proposed protein-carbohydrate recognition will necessarily involve a binding protein with the respective specificity. Such proteins thus are able to serve as receptors for certain types of carbohydrate moieties like sialic acids *in vivo*. Various members of this class of proteins have already proven their value as analytical tools in studying expression and localization of defined sialoglycoconjugates. These proteins attract much attention due to both their functions *in situ* and their potential as laboratory tools in glycoconjugate research in areas like biochemistry or histology. We present a survey of the purification, characterization and application of this class of proteins to illustrate the status of knowledge and the current directions of research in this field.

## Introduction

Molecular recognition between two different types of determinants is involved in mediation of the physiological functions of the participating molecules. In addition to protein-protein or protein-nucleic acid interactions, the ligand properties of the carbohydrate part of cellular glycoconjugates are now increasingly appreciated [1–3]. Consequently, different determinants of naturally occurring carbohydrate sequences are under scrutiny in this respect. Due to their location terminally at the carbohydrate main or side chains of glycoconjugates, sialic acids deserve special attention within the quest to unravel the assumed code, determined by carbohydrate sequences.

The sialic acids are a family of about 30 derivatives of neuraminic acid, generated by addition of different substituents at the amino group or at the hydroxyl groups at C4, C7, C8 or C9 of the backbone. Various sialic acid derivatives exhibit an interesting species and tissue distribution, whose physiological relevance is not yet fully understood. For example, N-acetylneuraminic acid (Neu5Ac), the most common neuraminyl derivative, and its hydroxylated derivative N-glycoloylneuraminic acid (Neu5Gc) occur exclusively or together with other sialic acids in tissue type-dependent quantities in almost all higher animals [4–6]. O-Acetyl groups are attached to certain positions in both Neu5Ac and Neu5Gc. 9-O-Ac–Neu5Ac (Neu-5,9-$Ac_2$) is mainly found in different tissues, sera and saliva of man, in gangliosides of various vertebrates and in erythrocytes of mouse, rat, rabbit and Rhesus monkey, whereas 4-O-Ac–Neu5Ac/Gc can be detected in different tissues from horse, donkey and the Australian monotreme Echidna [4–6].

The ubiquitous occurrence of sialic acids in oligosaccharide chains of mammalian glycoconjugates prompts the pertinent question concerning their functional relevance. They appear to play an important role in many biological recognition

Reprint requests to Prof. H.-J. Gabius.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/0900–0641 $ 01.30/0

mechanisms at the level of proteins and cells [7–9]. Enzymatic cleavage of sialic acid from human blood clotting factor IX for example results in a loss of its activity, and the infection of host cells by pathogenic agents can be caused by a specific interaction between cell surface sialyl residues and viral hemagglutinin/sialidase molecules or bacterial agglutinins [3–5, 10]. Moreover, sialic acids are also involved in the binding of a variety of toxins to cells, emphasizing their contribution to molecular recognition [5, 11]. The monitoring of their presence also deserves attention in pathological processes, *e.g.* sialidosis, in which sialoglycoconjugates accumulate due to the lack of sialidase [12, 13]. These arbitrarily chosen examples underscore the physiological relevance of this substance group for a remarkable variety of processes, warranting definition of the presence of this epitope and any modulation during physiological processes like differentiation or inflammation with specific probes. This reason is one incentive to turn to sialic acid-binding proteins, viewed as molecular tools.

The sialic acid-specific lectins or antibodies have potential applications as specific probes to investigate the role of cell surface carbohydrates during development, differentiation and malignant transformation of cells, as indicated for other types of sugar receptors [1–3]. These lectins or antibodies could be used as powerful tools for the purification and characterization as well as the study of the distribution and localization of many sialic acid-containing biomolecules, *e.g.* glycoproteins, gangliosides and polysaccharides. Immobilized lectin from *Maackia amurensis* has been used to separate a mixture of complex Asn-linked oligosaccharide chains containing (α2-3)-linked terminal sialic acid [14]; influenza C virus hemagglutinin has successfully been employed to detect the different forms of two murine glycophorins and O-acetylated gangliosides [15] and the S protein of bovine coronavirus can reliably detect Neu-5,9-$Ac_2$-containing glycoconjugates [10]. *Limax flavus* lectin has been used to detect the distribution of sialic acid residues in various tissues of rat [16].

Sialic acid-binding lectins are mainly found in a variety of invertebrates, but also in many bacteria and viruses and in a few plants. The first known sialic acid-binding lectin was isolated from the hemolymph of the horseshoe crab, *Limulus polyphemus* [18]. Up to now more than 20 sialic acid-binding lectins have been isolated and characterized from various sources [17, 19]. In addition to these lectins, the recently discovered mammalian sialic acid-binding cell adhesion molecules, termed selectins, sialic acid-specific autoantibodies (cold agglutinins) and monovalent sialic acid-binding proteins clearly support the notion that sialic acids can indeed be ligands for a recognitive protein-carbohydrate interplay. The studies on these proteins will contribute to the understanding of the role of such a binding site in different classes of proteins.

The following sections are intended to describe the occurrence of sialic acid-binding proteins in organisms of various branches of the evolutionary tree and ways to detect their presence as well as to present insights into their characterization and application in basic and applied glycoscience.

## Occurrence

### *Vertebrates*

Only a few sialic acid-binding lectins have been found in vertebrates, *e.g.* in the uterus [20] and brain [21] of rat or in frog egg [22], and in Tunicates [23], as summarized in Table I. A sialic acid-binding protein (calcylin) has been isolated from several human tissues [24] and bovine heart [25]. Certain cell adhesion molecules like sialoadhesin and the selectins [endothelial cell-leukocyte adhesion molecule 1 (ELAM-1) and 140 kDa granule membrane protein (GMP 140)], too, show binding specificity for sialoglycoconjugates, *i.e.* the sialyl-Lewis$^x$ structure [26–28]. In addition to these proteins that can be distinguished from immunoglobulins, sialic acid-specific autoantibodies have been isolated from patients with cold agglutinin disease [29, 30], from human placenta that has been proven to be an immunoglobulin G ([31], Zeng and Gabius, unpublished results) and from mouse cell lines [32–35]. A summary of their individual binding preferences to certain sialic acids (type of modification and linkage in the oligosaccharide chain) is given in Table II.

### *Invertebrates*

Sialic acid-binding lectins are ubiquitous among invertebrates (Table I). They are at present already described for hemolymph of American [36], Indian [37], and Japanese [38] horseshoe crabs, a marine

Table I. The molecular properties, sugar specificities and purification methods of some sialic acid-binding lectins.

| Source | Molecular weight Native | Subunit | Carbohydrate content [%] | Specificity | Purification | Ref. |
|---|---|---|---|---|---|---|
| a) Vertebrates | | | | | | |
| Rat uterus | | 30 & 28 | n.d. | Neu5Ac | fetuin-Sepharose | [20] |
| Rat brain | | | | Neu5Ac, Neu5Gc | | [21] |
| Frog egg | 12.5 | 12.5 | 0 | sialoglycoproteins | Sephadex G-75, cellulose-hydroxy-apatite and CM-cellulose | [22] |
| Human placenta (sarcolectin) | 190 | 65 | 0 | Neu5Ac, Neu5Gc | Sephacryl S-200, DEAE-cellulose | [82] |
| b) Invertebrates | | | | | | |
| American horseshoe crab *(Limulus polyphemus)* | 335 | 18 | 4 | Neu5Ac(α2-3)GalNAc > Neu5Ac(α2-6)GalNAc > Neu5Ac | BSM-Sepharose | [36] |
| Indian horseshoe crab *(Carcinoscorpius rotunda)* | 440 | 28 | 5.8 | Neu5Ac(α2-6)Gal ≫ Neu5Ac(α2-3)Gal > Neu5Ac > Neu5Gc | BSM-Sepharose | [37] |
| Japanese horseshoe crab *(Tachypleus tridentatus)* | 420 | 42 | n.d. | Neu5Ac, Neu5Gc | BSM-Sepharose and cellofine GC 700 | [38] |
| Marine crab *(Cancer antennarius)* | 70 | 35 | n.d. | 9-O-Ac-Neu5Ac > 4-O-Ac-Neu5Ac > Neu5Ac > Neu5Gc | BSM-Sepharose | [39] |
| Saharan scorpion *(Androctonus australis)* | | | | sialyllactose > Neu5Ac > Neu5Gc | | [41] |
| Arizona lethal scorpion *(Centruroides sculpturatus)* | | | | Neu5Ac, Neu5Gc | | [42] |
| Whip scorpion *(Masticoproctus giganteus)* | | | | Neu5Ac | | [43] |
| Indian scorpion *(Heterometrus granulomanus)* | 500 | 15 | n.d. | Neu5Ac(α2-3)Lac > Neu5Ac > Neu5Gc | ESSG-Sepharose | [44] |
| Scorpion *(Paruroctonus mesaensis)* | | | | sialic acid-containing glycoproteins | | [19] |
| American spider *(Aphonopelma cepaeahortensis)* | | | | sialoglycoproteins | | [45] |
| Pacific oyster *(Crassostrea gigas)* | | | | Neu5Ac, sialoglycoproteins | | [57] |
| American lobster 1 *(Homarus americanus)* | 19S | 55 | n.d. | Neu5Ac, Neu5Gc | gel filtration and gel electrophoresis | [47] |
| American lobster 2 | 70 | 70 | n.d. | Neu5Ac | fetuin-Sepharose | [19] |
| Black tiger prawn *(Peneaus monodon)* | 420 | 27 | n.d. | Neu5Ac | fetuin-Sepharose | [49] |
| Fresh water prawn *(Macrobrachium rosenbergii)* | | | | Neu5Ac | | [48] |
| Sea snail *(Dolabella)* | | | | Neu 5Ac | | [51] |
| African snail *(Achatina fulica)* | 240 | 15 | 21 | Neu 5Ac(α2-3)Gal > Neu 5 Ac (α2-6)Gal > Neu 5 Ac > Neu 5 Gc | fetuin-Sepharose | [52] |
| Garden snail *(Cepaea hortensis)* | 80 | 23 & 16 | n.d. | Neu 5 Ac > Neu 5 Gc | | [53] |
| Apple snail *(Pila globosa)* | 440 | 190, 145 & 105 | 25 | Neu5Gc | BSM-Sepharose | [54] |
| Sea slug *(Limax flavus)* | 44 | 22 | | Neu5Ac > Neu5Gc | BSM-Sepharose | [55] |
| c) Plants | | | | | | |
| Wheat germ seed *(Triticum vulgare)* | 36 | 18 | 0 | GlcNAc > Neu5Ac | fetuin-Sepharose | [58] |
| Elderberry *(Sambucus nigra)* | 140 | 34 & 38 | 5.6 | Neu5Ac(α2-6)Gal/GalNAc > Neu5Ac(α2-3)Gal/GalNAc | BSM-Sepharose | [60] |
| Elderberry *(Sambucus sieboldiana)* | | 23 to 34 | n.d. | mucin | fetuin- and mucin-Sepharose | [61] |
| *Maackia amurensis* seed (MAL) | 130 | 33 | n.d. | Neu5Ac(α2-3)Gal(β1-4)Glc/GlcNAc | laminin-Sepharose | [14] [62] |

Abbreviations: n.d., not determined; BSM, bovine submaxillary mucin; ESGG, equine submandibular gland glycoprotein; Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycoloylneuraminic acid; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine.

Table II. A summary of sialic acid-specific lectin-like proteins and autoantibodies (cold agglutinins).

| Source | Sugar specificity | Ref. |
|---|---|---|
| *a) From bacteria* | | |
| *Mycoplasma pneumoniae* | sialoglycoproteins | [64] |
| *Pseudomonas aeruginosa* | Neu 5 Ac | [65] |
| *Escherichia coli* | Neu 5 Ac(α 2-3)Gal(β 1-3)GalNAc | [66] |
| Adhesin K 99 | sialoglycoconjugates | [67] |
| *Streptococcus sanguis* | Neu 5 Ac | [68] |
| Pertussis toxin | Neu 5 Ac(α 2-6)Gal | [89] |
| Cholera toxin | sialic acid-containing gangliosides | [19] |
| *b) From viruses* | | |
| Influenza A virus | Neu 5 Ac-containing glycoconjugates | [19] |
| Influenza B virus | Neu 5 Ac-containing glycoconjugates | [19] |
| Influenza C virus | O-acetylated sialic acids | [15] |
| Sendai virus | Neu 5 Ac-containing glycoconjugates | [19] |
| Polyoma virus | Neu 5 Ac(α 2-3)Gal(β 1-3)GalNAc | [19] |
| *c) From human or animal sources* | | |
| Bovine heart (calcyclin) | Neu 5 Gc, Neu 5 Ac | [25] |
| Human erythrocytes (cold agglutinin disease) | | |
| Anti-$Pr_2$ (Cd, Fl, Sa) Autoantibodies | Neu 5 Ac(α 2-3)Gal(β 1-4)GlcNAc/Glc | [30] |
| Human serum autoantibodies (Waldenström macroglobulinemia) | Neu 5 Ac-containing glycoproteins | [29] |
| Human placenta IgG | O-acetylated sialic acids | [31] |
| Hybridoma cell lines (monoclonal antibody) | Neu 5 Ac(α 2-3)Gal or Neu 5 Ac(α 2-6)Gal | [32, 33] |
| B 49 cell lines (monoclonal antibody) | O-acetylated sialic acids | [34] |
| Autoimmune NZB mouse strain (monoclonal antibody) | sialoglycoconjugates | [35] |
| Murine tissue macrophage | Neu 5 Ac(α 2-3)Gal(β 1-3)GalNAc | [26] |
| Cell adhesion molecules (ELAM-1, GMP 140) | sialyl $Lewis^x$ | [27, 28] |

crab [39] and coconut crab [40], in Saharan scorpion [41], the Arizona lethal scorpion [42], the whip scorpion [43], the Indian scorpion [44] and an American spider [45] as well as in some other species of scorpion [46]. Sialic acid-binding lectins have been reported to be expressed in many species of Mandibulata, *e.g.* American lobster [47], fresh water prawn [48], black tiger prawn [49] and beetles [50], and in many species of Mollusca, *e.g.* snails [51–54], sea slug [55], sea mussel [56] and pacific oyster [57]. As can also be seen from Table I, few of these lectins have been purified and biochemically characterized, leaving a large territory to be carefully mapped.

*Plants*

Up to now, only a few plants have been found to contain sialic acid-binding lectins. Wheat germ agglutinin was the first plant lectin, known to have specificity for sialic acids [58, 59]. Two sialic acid-binding lectins were isolated from elderberry bark [60, 61]. A leukoagglutinin from the seeds of the leguminous plant *Maackia amurensis* also specifically binds to sialic acid-containing glycoproteins [14, 62]. Interestingly, it has recently been demonstrated that many legume lectins can also interact with sialic acids besides their strong reactivity to the saccharides, defining their nominal specificity in hemagglutination [63].

*Bacteria*

Many sialic acid-binding lectin-like proteins have been detected on the cell surface of bacteria, such as *Mycoplasma* [64], *Pseudomonas aeruginosa* [65], *Escherichia coli* [66, 67], *Streptococcus sanguis* and *Streptococcus mutans* [68]. Bacterial exotoxins, such as the cholera and the pertussis toxin, too, exhibit this property [19]. Carbohydrate specificities of such prokaryotic receptors are exemplified in Table II.

*Viruses*

Viruses constitute no exception concerning the ability to specifically bind sialic acids. The virus family Orthomyxoviridae, encompassing the influenza viruses, and other families such as the Paramyxoviridae, Picornaviridae, Papovaviridae, Reoviridae and Adenoviridae have been shown to be a source of sialic acid-specific hemagglutinins [5, 15, 17, 19].

## Carrier-immobilized sialic acids as tools to detect respective receptors

Labeled carbohydrate ligands have been employed as appropriate instruments for the detection of respective binding sites in tissues and cells. In this respect, neoglycoproteins and neoglycoenzymes have been applied [1, 69–72]. These tools can conveniently be prepared by chemically conjugating sugars to non-glycosylated carrier proteins, usually bovine serum albumin (BSA) [72]. For histochemical detection of sialic acid-binding proteins in tissues and cells sialic acid derivatives are attached to biotinylated or radioactively labeled bovine serum albumin, enabling investigators to specifically localize sialic acid receptors on the cell surface or in the tissue section. Using this tool, evidence has been gathered that sialic acid-binding proteins are present in human placenta [73]. Staining, too, was detected in nuclei of epithelial cells of distal convoluted tubes of human kidney [24].

Prompted by these glycohistochemical results, we have isolated two sialic acid-binding proteins from human placenta and other human tissues [24, 31]. Apparently, glycohistochemical evidence will provide a reliable basis for receptor isolation. Moreover, the successful application of histochemical tools serves as a guideline to select suitable ligands for affinity chromatography, facilitating straightforward attempts for the purification of the respective receptors. Their availability enables investigators to gain access to antibodies that are specific for individual receptor types. These specific antibodies raised against purified sialic acid-binding proteins can detect or quantitatively determine the distribution of the sialic acid receptors in tissues or cells [25]. The presence of calcyclin, a protein with the ability to bind sialic acids, in various leukemic cells, determined with an ELISA, corroborated determinations of the level of the mRNA that was cloned due to its pronounced cell cycle-dependent regulation [25, 80].

## Purification

In order to study their molecular properties, physiological functions and potential applications, access to purified proteins is needed. By taking advantage of their property of sialic acid-binding affinity, most of these proteins can be purified using immobilized sialoglycoproteins, *e.g.* fetuin, bovine (sheep or equine) submaxillary mucin, combined with conventional gel chromatography. Because their sugar-binding activities are usually dependent on the presence of $Ca^{2+}$ [17], the proteins can be specifically adsorbed onto resins exposing immobilized sialoglycoproteins in the presence of $Ca^{2+}$ at the optimal pH for binding. After washing off unbound proteins, they can be specifically eluted with EDTA or EGTA [14, 20, 74, 75]. This simple isolation procedure often results in sufficiently purified preparations. By using fetuin-Sepharose affinity chromatography that can be combined with an asialofetuin-Sepharose precolumn that removes any proteins with affinity to the polypeptide part or any other region in the sugar sequence of the glycoprotein we have isolated to homogeneity a sialic acid-binding protein from different types of human tissues and bovine heart [24, 25]. Besides taking advantage of the $Ca^{2+}$-dependence, pH or temperature shifts can be useful to elute the proteins [29, 31]. In addition to the withdrawal of $Ca^{2+}$ or shifting the pH a few lectins have been eluted by sugar, *e.g.* sialic acid [31, 55], D-glcNAc [76], and D-manNAc [77]. Some sialic acid-binding proteins have also been solely purified by conventional protein purification methods, *e.g.* the frog egg lectin was purified by Sephadex G-75 and

ion exchange chromatography [22]. An *Escherichia coli* lectin [66] and mammalian sarcolectin [78] were also isolated by consecutive performance of gel filtration and anion exchange chromatography. Recently, a Neu5Gc-specific lectin from apple snail was purified on BSM-Sepharose, eluted by the addition of 15 mM $Ca^{2+}$ [54]. Remarkably, this lectin exhibited a reduced affinity to its ligands in the presence of $Ca^{2+}$. Information on the purification methods is included in Table I.

## Molecular characteristics and sugar specificity

Phenomenologically, no generally applicable rule for the molecular characteristics can be discerned within this family. Some sialic acid-binding proteins consist of more than one subunit of molecular weight in the range from 15 to 35 kDa. But very little is known about detailed characteristics of these subunits and their interaction in forming the native proteins. These proteins can easily contain a high content of acidic residues, most of them are glycoproteins and in some lectins carbohydrate groups may also play an important role in their biological activity [74]. Several of these proteins appear to consist of a single subunit, *e.g.* frog egg lectin is composed of a single subunit of 111 residues, whose amino acid sequence has been determined [79]. Calcyclin is a 10.3 kDa protein, as predicted from its cDNA [80]. For the members of the recently described selectin family, whose detailed sequence information is available, domains can be correlated to properties: N-terminal lectin domain that mediates adhesion by binding carbohydrate ligands on the opposing cells, epidermal growth factor-like domain and variable numbers of C-terminal complement-like regulatory units that appear to influence the integrity of the carbohydrate-binding domain [81].

The optimum pH for stability and biological activity of most of these proteins lies between 7 and 9. Chemical modification studies on several proteins showed that basic residues (arginine or lysine) may play a key role in their binding to sialic acids [74, 82]. Results of such experiments with three mammalian sialic acid-binding proteins illustrate this conclusion (Table III). The secondary structures of a few lectins ($Achatinin_H$ [83], limulin [84] and wheat germ agglutinin [85]) have been studied by determination of circular dichroism. Three-dimensional structures of wheat germ agglutinin [86] and the complex of influenza virus hemagglutinin with sialic acids [87] have been elucidated by X-ray crystallography. Three-dimensional structural studies on other sialic acid-binding lectins have not been reported so far.

The sugar-binding specificity of lectins is usually determined by measuring the ability of a sugar (1) to inhibit the hemagglutination caused by the lectin, (2) to inhibit the precipitation of the lectin by polysaccharides or glycoproteins or (3) to inhibit the binding of the lectin to glycosubstances. The higher the affinity for the sugar, the less is needed for inhibition. The hemagglutination inhibition test is one of the most frequently used methods to determine the sugar specificity of the lectin. Be-

Table III. Effect of amino acid modification by group-specific reagents on binding of biotinylated fetuin by three mammalian proteins.

| Chemical treatment | Residues modified | Calcyclin | Sarcolectin | Aprotinin |
|---|---|---|---|---|
| Native protein | | 100[a] | 100 | 100 |
| O-Methylisourea | lysine | 40 | 20 | 60 |
| Citraconic anhydride | lysine, N-terminal $NH_2$ group | 40 | 0 | 90 |
| Cyclohexane-1,2-dione | arginine | 40 | 10 | 25 |
| Phenylglyoxal | arginine | 60 | 25 | 50 |
| N-acetylimidazole | tyrosine | 100 | 20 | 20 |
| Diethylpyrocarbonate | histidine | 100 | 35 | * |
| Ester-carbodiimide | carboxyl | 100 | 90 | 100 |

[a] Relative binding capacity, given as percentage.
* No histidine present.

cause erythrocytes from different species contain several kinds of sialic acid derivatives in varying amounts [4, 6, 38], their agglutinability by sialic acid-specific lectins can exhibit notably different patterns. For example, the marine crab lectin [39] agglutinates only rabbit, mouse, rat and horse erythrocytes, which contain Neu-5,9-$Ac_2$ or Neu-4,5-$Ac_2$, but not human erythrocytes, which lack O-acetylated Neu5Ac. $Achatinin_H$ agglutinates only rabbit, rat and guinea pig erythrocytes, which contain Neu-5,9-$Ac_2$. Some lectins, *e.g.* limulin [75], the lectin from the Indian scorpion [44], the Japanese horseshoe crab [38, 76], lobster [47] and sea slug [75], agglutinate erythrocytes from many animals. The *Maackia amurensis* leukoagglutinin (MAL) agglutinates cells of a mouse lymphoma line, but not erythrocytes [62]. Sialic acid-binding lectins do not agglutinate sialidase-treated erythrocytes, emphasizing the importance of the removed moieties for the process of agglutination.

The cell agglutinating properties of the lectins are a consequence of their binding to at least two adjacent cells. Therefore, monovalent carbohydrate-binding proteins that do not form aggregates in solution can apparently not agglutinate the erythrocytes. Their sugar specificities need to be measured by an enzyme-linked immunoadsorbent assay [89] or by a solid-phase assay using biotinylated sialoglycoproteins or neoglycoproteins [25, 31]. The latter provides a simple and reliable method to determine the carbohydrate-binding properties of the lectins. In this respect, neoglycoproteins again prove their versatility. Using biotinylated neuraminic acid-BSA as probe, the binding specificity of an antibody fraction from human placenta has been measured in solid-phase assays, which revealed consistency with that assessed by the hemagglutination-inhibition test [31]. We also evaluated the sugar-binding properties of three other mammalian proteins that bind fetuin with dependence on sialic acids (calcyclin, sarcolectin and aprotinin) in order to disclose, if detectable, differences in their individual specificities by a solid-phase assay, using biotinylated fetuin as a probe [25, 82]. Inhibition of binding of fetuin by glycosubstances, as compiled in Table IV, revealed differences in the sugar-binding specificity of the three proteins. Asialofetuin failed to bind to these proteins. As an internal control the binding of biotinylated calcyclin to (neo)glycoproteins in a solid-phase assay had similarly been determined [25]. For calcyclin the binding to fetuin was most effectively reduced by Neu5Gc within a panel of negatively charged sugars. Bovine submaxillary mucin proved more effective than neoglycoproteins in this case. However, such results do not necessarily imply that the tissue ligands will bind to the operationally defined sialic acid-binding proteins by such determinants. Conversely, ability to exhibit this binding property in the applied assay can translate into a productive interaction with negatively charged amino acid clusters in proteins. We could so far only isolate calcyclin-binding proteins that apparently are bound by protein-protein interactions, defining the affinity to glyceraldehyde-3-phosphate dehydrogenase with a $K_D$ value of 110 nM in Scatchard analysis. A ligand for sarcolectin, the migration inhibitory factor (MIF), similarly is recognized by such an interaction (Zeng, Kratzin and Gabius, in preparation). These results unmistakably underscore that caution needs to be exercised within extrapolation of measurable properties from an artificial assay system to the physiological *in situ* situation.

Some sialic acid-binding proteins show a higher affinity for the N-glycoloyl group than the N-acetyl group, *e.g.* calcyclin [25] and the lectin from *Pila globosa* snail [54]. Some of them display a high specificity for O-acetylated sialic acid derivatives, *e.g.* the marine crab lectin exhibits a pronounced affinity for 9-O-Ac (33-fold) and 4-O-Ac (11-fold) derivatives compared to Neu5Ac [39]. $Achatinin_H$ binds rather strongly to Neu-5,9-$Ac_2$ compared to its affinity to Neu5Ac. Hemagglutinins from corona virus [10] and from influenza C virus [15] as well as human antibody [31] show strong affinity for O-acetylated sialic acids.

Some plant lectins have an eminent specificity for oligosaccharides with a certain type of linkage of the sialic acid moiety to other sugars, *e.g.* *Maackia amurensis* leukoagglutinin is highly specific for Neu5Ac/Gc(α2-3)Gal(β1-4)GlcNAc/Glc, the elderberry bark lectin binds 1600–10,000-fold stronger to Neu5Gc(α2-6)Gal than to galactose [14]. Recently, Harada *et al.* [61] have isolated a lectin from elderberry *Sambucus sieboldiana,* which fails to bind to any of the sialoglycoproteins except mucin. Several bacterial surface lectins similarly exhibit a notable sugar-binding specificity. A lectin from *Escherichia coli* displays a remarkable

Table IV. Inhibition of fetuin binding of calcyclin, sarcolectin and aprotinin by sugars and glycosubstances in a solid phase assay.

| Sugars or glycosubstances | Concentration required for 50% inhibition [mM or mg/ml] Calcyclin | Sarcolectin | Aprotinin |
|---|---|---|---|
| Neu 5 Ac[a] | >100 | 50 | 50 (40%) |
| Neu 5 Gc[a] | 3 | 15 (25%) | 15 |
| Sialyllactose[a] | 25 | 25 | 25 |
| Lactose[a] | 50 | >100 | 100 (20%) |
| Melibiose[a] | >100 | >100 | >100 |
| Galactose[a] | >100 | >100 | 100 (40%) |
| Glucose[a] | >100 | >100 | 100 (40%) |
| Mannose[a] | 50 (20%) | >100 | 100 (30%) |
| Fucose[a] | >50 | >100 | >50 |
| Galactosamine[a] | >50 | 50 (10%) | 15 |
| Glucosamine[a] | >100 | 100 (30%) | 20 |
| GalNAc[a] | >50 | 100 | 100 |
| GlcNAc[a] | 100 | >100 | 50 (40%) |
| Galacturonic acid[a] | 100 (20%) | 100 | 50 (40%) |
| Glucuronic acid[a] | 50 (20%) | 20 | 50 (40%) |
| Man-6-p[a] | 25 | 20 (10%) | 25 |
| Gal-6-p[a] | 30 | 50 (20%) | 12 |
| Glc-6-p[a] | 40 | 40 | 10 |
| Fetuin[b] | 0.20 | 0.65 | 0.50 |
| Asialofetuin[c] | >10 | >10 | >10 |
| BSM[b] | 0.10 | 1.5 | >1.5 |
| $\alpha_1$-acid glycoprotein[c] | 5.0 (20%) | 1.0 | 5.0 |
| Sialic acid-BSA[b] | 0.15 | 0.75 (20%) | 0.75 (30%) |
| Glucuronic acid-BSA[b] | 0.15 | 0.75 (30%) | 0.75 (40%) |
| Man-6-P-BSA[b] | 0.12 | 0.75 (20%) | 0.75 (30%) |
| Gal-6-P-BSA[b] | 0.12 | 0.75 (30%) | 0.75 (10%) |
| GlcNAc-BSA[b] | 0.15 (20%) | >0.75 | 0.75 (40%) |
| Lactose-BSA[b] | 0.15 (25%) | >0.75 | 0.75 (30%) |
| Heparin[c] | 5.0 (20%) | 1.0 | 1.0 |
| Fucoidan[c] | 2.0 | 1.0 | 5.0 |
| Dextran sulfate[c] | 1.5 | 1.5 | 2.0 |

[a] mM; [b] mM (concentration is given in terms of sialic acid or saccharide); [c] mg/ml. Abbreviations: BSM, bovine submaxillary mucin; BSA, bovine serum albumin; galNAc, N-acetylgalactosamine; glcNAc, N-acetylglucosamine; glc-6-p, glucose-6-phosphate; gal-6-p, galactose-6-phosphate; man-6-p, mannose-6-phosphate, from [25, 82].

affinity for 4-O-Ac-Neu 5 Ac, which is notably reduced with 7-O- or 9-O-acetylation [88]. This kind of specificity may affect the choice of the host and tissue in infection and colonization, pointing to the clinical relevance of this finding. Viruses, too, bind to acetylated sialic acids, as already noted. Similarities of lectins from different organisms are not restricted to this case. The pertussis toxin, for example, shows a similar specificity as the elderberry bark lectin [89].

Many lectins bind sialoglycoconjugates stronger than simple mono- or disaccharides, *e.g.* bovine submaxillary mucin has been found to be the best inhibitor for the lectins from the marine crab, the American and Japanese horseshoe crabs, lobster and sea slug as well as see snails. Equine submaxillary mucin binds stronger to limulin, carcinoscorpin and marine crab lectin than free sialic acids [17, 19]. In addition, other sialic acid-containing glycoproteins (*e.g.* fetuin, human chorionic gonadotropin, $\alpha_1$-acid glycoprotein, serotransferrin, lactoferrin, fibrinogen *etc.*) and gangliosides have also been found to be good ligands of sialic acid-binding proteins [25, 44, 75]. This extent of binding also allows investigators to infer information on the preferred type of modification and linkage.

Several monoclonal autoantibodies (cold agglutinins) recognize sialic acid-containing determinants [90]. Anti-$Pr_2$ is reactive with
Neu5Ac(α2-3)Gal(β1-4)GlcNAc/Glc or
Neu5Ac(α2-3)Gal and
Neu5Ac(α2-8)Neu5Ac(α2-3)Gal sequences in gangliosides of the neolacto and the ganglio series. Anti-Gd, anti-Sa and anti-Fl react with the Neu5Ac(α2-3)Gal(β1-4)Glc/GlcNAc sequences in glycolipids of the neolacto series and GM3 [30]. Human monoclonal IgM(κ) from the serum of a patient with Waldenström macroglobulinemia specifically binds to N-acetylneuraminic acid residues of sialoglycoproteins in human cells and to gangliosides from dog cells [29]. Monoclonal antibodies from hybridoma cell lines have a specificity for Neu5Ac(α2-3)Gal or Neu5Ac(α2-6)Gal sequences [32, 33]. Monoclonal antibodies from the autoimmune NZB mouse strain are specific for weakly immunogenic polysaccharides of *E. coli* K1 and group B *Meningococci* [35].

As already indicated earlier, it has been demonstrated that a mouse protein, termed sialoadhesin, recognizes Neu5Ac(α2-3)Gal(β1-3)GalNAc sequences in either glycoproteins or gangliosides [26]. Two members of the recently discovered selectin family (ELAM-1 and GMP140) display capacity to bind to sialyl-$Le^a$ and sialyl-$Le^x$ structures [27, 28, 81].

## Biological activities and functions

The carbohydrate chains of membrane glycoproteins and glycolipids (in eukaryotes) or of polysaccharides (in prokaryotes) are accessible on the surface. Complementary lectins may mediate cell-cell interactions in some processes such as fertilization, embryogenesis, cell migration, cell adhesion or microbial infection by binding respective carbohydrates on opposing cells, thereby playing a key role in the control of various normal and pathological processes in living organisms [1–3, 69, 92–98]. Lectin-carbohydrate interactions are also of particular relevance in immune cell recognition and communication [3, 99].

Cell agglutination is one of most important and easily detectable properties of lectins of different specificities including all sialic acid-binding lectins. Some investigations have revealed that tumor cells often contain large amounts of sialic acids on their surface. Some cancer-specific antigens are sialic acid-containing gangliosides [100]. This can help to explain the observation that certain types of transformed cells are more easily agglutinated by these lectins than normal cells, *e.g.* frog egg lectin preferentially agglutinates cancer cells [101]. The lectins from the hemolymph of the scorpions *Androctonus australis* and *Centruroides sculpturatus* exhibit higher titers with leukemic cells than with normal human lymphocytes [42, 102]. Similarly, tumor cells are more sensitive in certain cases to lectin toxicity than non-malignant cells [103, 104].

Sialic acid residues are also important constituents of the lymphocyte cell membrane. Several investigations have underscored that the modification of sialyl residues on cell surfaces can cause lymphoblastogenesis and proliferation [105]. A few sialic acid-binding lectins have proven to exhibit mitogenic activity for lymphocytes. The lectin limulin from *Limulus polyphemus* shows a mitogenic activity for both human and mouse lymphocytes, in mice its mitogenicity is directed towards both B and T lymphocytes [106]. Selectivity for B and T lymphocytes can also be found within this class of lectins. The lectin from lobster hemolymph is a B, but not T cell mitogen and wheat germ agglutinin also acts as a weak T cell mitogen [107]. In contrast, $Achatinin_H$ was found to induce proliferation of purified T lymphocytes and rat thymocytes, but is not mitogenic towards B lymphocytes [107]. The mitogenic activity is suppressed by the sialic acid-containing disaccharide, Neu5Ac(α2-6)N-acetyl-D-galactosaminitol, a strong inhibitor of this lectin [107].

Sialic acid-binding lectins on the bacterial surface may play a substantial role in the choice of the host and tissue in infection and colonization. Sialic acid-binding lectins from the hemolymph of invertebrates may functionally be equivalent to vertebrate antibodies in defence mechanisms [56, 108]. They can even facilitate phagocytosis [109]. Frog egg lectin may be involved in fertilization and development in the frog embryo [79]. A recent, already mentioned discovery concerns the area of mammalian cell adhesion. Sequence comparison of newly identified cell surface proteins to known lectins has been helpful to discern an important addition to the family of lectins. These studies on a distinct group of cell adhesion molecules, termed LEC-CAMs or selectins, have emphasized that

they mediate cell adhesion by binding a sialylated carbohydrate structure. Leukocyte trafficking and recruitment to sites of inflammation may directly involve the recognition of sialylated carbohydrate structures by a selectin on cell surfaces [27]. It is thus tempting to speculate about the perspective of clinical application of sialoglycoconjugates as anti-inflammatory drugs.

When considering the biological functions of lectins, it should not be overlooked that they may contain other functional domains besides the one focused upon in this article. Indeed, evidence has accumulated that many lectins have a second type of binding site that is specific for non-carbohydrate ligands [110]. Discoidin, a galactose-specific lectin, has another distinct site, established by the tripeptide Arg–Gly–Asp that interacts with a receptor protein, what results in mediation of cell-substratum adhesion, and the elastin receptor also contains a carbohydrate-binding and a protein-binding site (elastin) [110]. Some well-known receptor proteins, *e.g.* insulin-like growth factor II receptor [111], which is identical to a mannose-6-phosphate receptor [112], and some cytokine molecules, *e.g.* IL-2 [113], specifically bind to both proteins and carbohydrates. Evidence for two different carbohydrate-binding sites on the same molecule has also been provided. The occupation of one binding site by sialic acid on a galactose-recognizing macrophage receptor even modulates the binding of the other carbohydrate ligand [114].

## Applications

Lectins have been widely used for the study of carbohydrate structure in solution and on cell surfaces. They are increasingly applied in biochemistry, cell biology, histochemistry, immunology and related areas. They have also found potential applications in cancer diagnosis and therapy.

Immobilized sialic acid-binding lectins or antibodies have been successfully used for the isolation, purification and structural characterization of sialoglycoconjugates. Immobilized elderberry bark lectin has been applied for the fractionation of sialylated oligosaccharides, glycopeptides and glycoproteins from brain [115]. Immobilized *Maackia amurensis* leukoagglutinin has been instrumental in separating a mixture of complex Asn-linked oligosaccharide chains containing (α2-3)-linked terminal sialic acids [14]. Immobilized carcinoscorpin can fractionate the sialoglycoproteins from sheep and rat brain [116, 117]. Mucin-specific bark lectin from elderberry *Sambucus sieboldiana* has been successfully employed to purify mucin from crude preparations of porcine stomach or extracts of porcine submaxillary glands [61]. Recently, the potency of viral hemagglutinins to serve as an analytical tool for identifying O-acetylated sialoglycoconjugates directly after Western blotting of glycoproteins and thin layer chromatography of gangliosides has been illustrated [10, 15].

In addition to sialic acid-binding lectins, sialic acid-specific monoclonal antibodies have also been widely helpful to detect and localize sialoglycoconjugates in various tissues and cells. The monoclonal antibodies directed to the α2-3 or α2-6 sialylated $Le^a$ antigen mapped the tissue distribution of these two kinds of sialylated derivatives of Lewis A antigen in patients with cancers of the digestive system [118]. The monoclonal antibody D1.1 that specifically binds to O-acetylated sialic acids enables to study the extent of O-acetylation of sialic acids in human malignant melanoma cells [34]. In addition to lectins with preference to certain linkage types antisialooligosaccharide antibodies will similarly detect this characteristic in complex carbohydrates [119].

Sialic acid-binding lectins or antibodies histochemically and cytochemically localize the sialoglycoconjugates in various cells and tissues. Mazzuca *et al.* have first reported the histochemical localization of sialic acid-containing glycoproteins in goblet cells and serous cells from human bronchial mucosa by peroxidase-labeled limulin [120]. Light microscopical detection of sialoglycoproteins is certainly possible in any material with this technique, as shown for various animal tissues [121, 122]. The gold complexes of the elderberry bark lectin detect the expression of Neu5Ac(α2-6)Gal/GalNAc sequences in Lowicryl K4M-embedded sections of rat tissues by light and electron microscopy [123]. Lectins have also been employed for the study of the process of intracellular glycosylation [124].

## Perspectives

A growing number of proteins with specificity for sialic acids is under thorough investigation, with special emphasis placed on viral and bacterial

agglutinins. Characterization of their ligands enables an understanding and a rational design of compounds to interfere with the lectin-dependent adherence of pathogenic organisms. Similar assessment for mammalian proteins is expected to shed light on the desired, precise delineation of their physiological relevance, *e.g.*, in cell adhesion. This task is an attractive challenge, highlighted by the recent detection of the selectin family and its role for cellular recognition especially in the context of inflammatory processes. When viewed as laboratory tools, certain members of this group have already proven their value in glycoconjugate research. The determination of the expression of individual derivatives of the diverse groups of sialic acids is a crucial step in ascribing functional relevance to their presence and to the apparently intricate regulation of their patterns of covalent modification. Certain organisms that lack sialic acids even profit from the ability of animal cells to synthesize this sugar for their own advantage. Acquisition on the cell surface of protozoan parasites of sialic acid-containing epitopes, which are crucial for the invasion of mammalian cells, due to the activity of a *trans*-sialidase has been discovered recently [125, 126].

In order to understand glycobiological interactions comprehensively only complementary research on the protein side (receptor) as well as on the carbohydrate portion with particular emphasis on sialic acids (ligand) will provide the desired insights. They can then hopefully benefit research in different clinical aspects, *e.g.*, treatment of inflammatory diseases, of tumors like Neu-5,9-$Ac_2$-expressing melanomas, or of bacterial and viral infections.

*Acknowledgements*

We wish to express our sincere gratitude to Prof. S. S. Joshi and Mrs. S. Mann for helpful advice and to the Dr.-M.-Scheel-Stiftung für Krebsforschung for generous financial support.

[1] H.-J. Gabius, Angew. Chem., Int. Ed. Engl. **27,** 1267–1276 (1988).
[2] H.-J. Gabius and S. Gabius, Naturwissenschaften **77,** 505–514 (1991).
[3] N. Sharon and H. Lis, Science **246,** 227–234 (1989).
[4] R. Schauer, Adv. Carbohydr. Chem. Biochem. **40,** 131–234 (1982).
[5] R. Schauer (ed.), Sialic Acids, Cell Biol. Monogr. **10,** Springer Verlag, Heidelberg 1982.
[6] R. Schauer and T. Yamakawa (eds.), Proceedings of the Japanese-German Symposium on Sialic Acids, Kieler Verlag Wissenschaft und Bildung, Kiel 1988.
[7] R. Schauer, A. K. Shukla, C. Schröder, and E. Müller, Pure Appl. Chem. **56,** 907–921 (1984).
[8] A. G. Morell, G. Gregoriadis, I. H. Scheinberg, J. Hickman, and G. Ashwell, J. Biol. Chem. **246,** 1461–1467 (1971).
[9] S. I. Chavin and S. M. Weidner, J. Biol. Chem. **259,** 3387–3390 (1984).
[10] B. Schultze, H.-J. Gross, R. Brossmer, and G. Herrler, J. Virol. **65,** 6232–6237 (1991).
[11] A. Rosenberg and C. L. Schengrund, Biological Roles of Sialic Acid, Plenum Press, New York 1976.
[12] K. M. Erbil, J. D. Jones, and G. G. Klee, Cancer **55,** 404–409 (1985).
[13] R. Schauer, Trends Biochem. Sci. **10,** 357–360 (1985).
[14] W. C. Wang and R. D. Cummings, J. Biol. Chem. **263,** 4576–4585 (1988).
[15] J. C. Manuguerra, C. DuBois, and C. Hannoun, Anal. Biochem. **194,** 425–432 (1991).
[16] J. Roth, J. M. Lucocq, and P. M. Charest, J. Histochem. Cytochem. **32,** 1167–1176 (1984).
[17] C. Mandal and C. Mandal, Experientia **46,** 433–441 (1990).
[18] J. J. Marchalonis and G. M. Edelman, J. Mol. Biol. **32,** 453–465 (1968).
[19] M. Wagner, Adv. Lectin Res. **3,** 36–81 (1990).
[20] M. Chowdhury, M. Sarkar, and C. Mandal, Biochem. Biophys. Res. Commun. **130,** 1301–1307 (1985).
[21] M. Popoli and A. Mengano, Neurochem. Res. **13,** 63–67 (1988).
[22] F. Sakakibara, H. Kawauchi, G. Takayanagi, and H. Ise, Cancer Res. **39,** 1347–1352 (1979).
[23] R. K. Wright and E. L. Cooper, Comp. Biochem. Physiol. **79B,** 269–277 (1984).
[24] H.-J. Gabius, A. Bardosi, S. Gabius, K. P. Hellmann, M. Karas, and H. Kratzin, Biochem. Biophys. Res. Commun. **163,** 506–512 (1989).
[25] F.-Y. Zeng and H.-J. Gabius, Arch. Biochem. Biophys. **289,** 137–144 (1991).
[26] P. R. Crocker, S. Kelm, C. DuBois, B. Martin, A. S. McWilliam, D. M. Shotton, J. C. Paulson, and S. Gordon, EMBO J. **10,** 1661–1669 (1991).
[27] D. D. True, M. S. Singer, L. A. Lasky, and S. D. Rosen, J. Cell Biol. **111,** 2757–2764 (1990).
[28] M. J. Polley, M. L. Phillips, E. Wayner, E. Nudelman, A. K. Singhal, S. Hakomori, and J. C. Paulson, Proc. Natl. Acad. Sci. U.S.A. **88,** 6224–6228 (1991).
[29] C. M. Tsai, D. V. Zopf, R. K. Yu, R. Wistar, Jr., and V. Ginsburg, Proc. Natl. Acad. Sci. U.S.A. **74,** 4591–4595 (1977).
[30] K. I. Uemura, D. Roelcke, Y. Nagai, and T. Feizi, Biochem. J. **219,** 865–874 (1984).
[31] H. Ahmed and H.-J. Gabius, J. Biol. Chem. **264,** 18673–18678 (1989).
[32] T. Tai, L. Sze, I. Kawashima, R. E. Saxton, and R. F. Irie, J. Biol. Chem. **262,** 6803–6807 (1987).
[33] S. Hakomori, C. M. Patterson, E. Nudelman, and

K. Sekiguchi, J. Biol. Chem. **258,** 11819–11822 (1983).
[34] D. A. Cheresh, A. P. Varki, N. M. Varki, W. B. Stallcup, J. Levine, and R. A. Reisfeld, J. Biol. Chem. **259,** 7453–7459 (1984).
[35] M. Frosch, I. Görgen, G. J. Boulnois, K. N. Timmis, and D. Bitter-Suermann, Proc. Natl. Acad. Sci. U.S.A. **82,** 1194–1198 (1985).
[36] A. C. Roche and M. Monsigny, Biochim. Biophys. Acta **371,** 242–254 (1974).
[37] S. Bishayee and T. Dorai, Biochim. Biophys. Acta **623,** 89–97 (1980).
[38] S. Shimizu, M. Ito, and M. Niwa, Biochim. Biophys. Acta **500,** 71–79 (1977).
[39] M. H. Ravindranath, H. H. Higa, E. L. Cooper, and J. C. Paulson, J. Biol. Chem. **260,** 8850–8856 (1985).
[40] E. Cohen, Trans. N. Y. Acad. Sci. **30,** 427–443 (1968).
[41] E. Cohen, G. H. U. Ildi, Z. Brahmi, and J. Minowada, Dev. Comp. Immun. **3,** 429–440 (1979).
[42] G. R. Vasta and E. Cohen, Dev. Comp. Immun. **6,** 219–230 (1982).
[43] G. R. Vasta and E. Cohen, J. Invert. Path. **43,** 333–342 (1984).
[44] H. Ahmed, B. P. Chatterjee, S. Kelm, and R. Schauer, Biol. Chem. Hoppe-Seyler **367,** 501–506 (1986).
[45] G. R. Vasta and E. Cohen, Dev. Comp. Immun. **8,** 515–522 (1984).
[46] G. R. Vasta and E. Cohen, Comp. Biochem. Physiol. **77B,** 721–727 (1984).
[47] J. L. Hall and D. T. Rowlands, Jr., Biochemistry **13,** 821–827 (1974).
[48] G. R. Vasta, G. W. Warr, and J. J. Marchalonis, Dev. Comp. Immun. **7,** 13–20 (1983).
[49] S. Ratanapo and M. Chulavatnatol, Comp. Biochem. Physiol. **97B,** 515–520 (1990).
[50] K. Umetsu, S. Kosaka, and J. Suzuki, J. Biochem. **95,** 239–245 (1984).
[51] I. Ishiyama, A. Takatsu, W. Gielen, and G. Uhlenbruck, Hematologia **6,** 109–112 (1972).
[52] S. Basu, M. Sarkar, and C. Mandal, Mol. Cell. Biochem. **71,** 149–157 (1986).
[53] M. Wagner, Acta Histochem. **71,** 35–39 (1982).
[54] S. Swarnakar, P. S. Chowdhury, and M. Sarkar, Biochem. Biophys. Res. Commun. **178,** 85–94 (1991).
[55] R. L. Miller, J. F. Collawn, Jr., and W. W. Fish, J. Biol. Chem. **257,** 7574–7580 (1982).
[56] S. W. Hardy, T. C. Fletcher, and L. M. Gerrie, Trans. Biochem. Soc. **4,** 473–475 (1976).
[57] G. R. Vasta, J. T. Sullivan, T. C. Cheng, J. J. Marchalonis, and G. W. Warr, J. Invert. Path. **40,** 367–377 (1982).
[58] I. J. Goldstein, S. Hammarstroem, and G. Sundblad, Biochim. Biophys. Acta **405,** 53–61 (1975).
[59] M. Monsigny, A. C. Roche, C. Sene, R. Maget-Dana, and F. Delmotte, Eur. J. Biochem. **104,** 147–153 (1980).
[60] N. Shibuya, I. J. Goldstein, W. F. Broekaert, M. Nsimba-Lubaki, B. Peeters, and W. J. Peumans, J. Biol. Chem. **262,** 1596–1601 (1987).
[61] H. Harada, M. Kondo, M. Yanagisawa, and S. Sunada, Anal. Biochem. **189,** 262–266 (1990).
[62] R. N. Knibbs, I. J. Goldstein, R. M. Ratcliffe, and N. Shibuya, J. Biol. Chem. **266,** 83–88 (1991).
[63] A. Ayouba, C. Chatelain, and P. Rouge, FEBS Lett. **289,** 102–104 (1991).
[64] M. Banai, S. Razin, W. Bredt, and I. Kahane, Infect. Immun. **30,** 628–634 (1980).
[65] H. L. Ko, J. Beuth, J. Sölter, H. Schroten, G. Uhlenbruck, and G. Pulverer, Infection **15,** 237–240 (1987).
[66] T. Moch, H. Hoschützky, J. Hacker, J. K. D. Kröncke, and K. Jann, Proc. Natl. Acad. Sci. U.S.A. **84,** 3462–3466 (1987).
[67] T. Wadström, P. O. Sjöberg, and M. Lindahl, in: Lectins: Biology, Biochemistry, and Clinical Biochemistry (T. C. Bog-Hansen and J. Breborowicz, eds.), **Vol. 4,** pp. 417–425, de Gruyter Verlag, Berlin 1985.
[68] M. J. Levine, M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li, and T. Van Dyke, Infect. Immun. **19,** 107–115 (1978).
[69] H.-J. Gabius, Biochim. Biophys. Acta **1071,** 1–18 (1991).
[70] J. Schrevel, D. Gros, and M. Monsigny, Progr. Histochem. Cytochem. **14,** 1–255 (1981).
[71] H.-J. Gabius and A. Bardosi, Progr. Histochem. Cytochem. **22,** 1–63 (1991).
[72] C. P. Stowell and Y. C. Lee, Adv. Carbohydr. Chem. Biochem. **37,** 225–281 (1980).
[73] H.-J. Gabius, P. L. Debbage, N. Lang, and W. Lange, Histochemistry **92,** 283–287 (1989).
[74] S. Basu, C. Mandal, and K. Allen, Biochem. J. **254,** 195–202 (1988).
[75] I. J. Goldstein and R. D. Poretz, in: Lectins: Properties, Functions and Applications in Biology and Medicine (I. E. Liener, N. Sharon, and I. J. Goldstein, eds.), pp. 35–244, Academic Press Inc., New York 1986.
[76] F. Shimishikura and K. Sekiguchi, J. Biochem. **93,** 1539–1546 (1983).
[77] J. Vanderwall, P. A. Canapbel, and C. A. Abel, Dev. Comp. Immun. **5,** 679–684 (1981).
[78] P. H. Jiang, F. Chany-Fournier, M. Sarragne, A. Gregoire, and C. Chany, Int. J. Cancer **34,** 625–632 (1984).
[79] K. Titani, K. Takio, M. Kuwada, K. Nitta, F. Sakakibara, H. Kawauchi, G. Takayanagi, and S. Hakomori, Biochemistry **26,** 2189–2194 (1987).
[80] B. Calabretta, R. Battini, L. Kaczmarek, J. K. de Riel, and R. Baserga, J. Biol. Chem. **261,** 12628–12632 (1986).
[81] G. S. Kansas, O. Spertini, L. M. Stoolman, and T. F. Tedder, J. Cell Biol. **114,** 351–358 (1991).
[82] F.-Y. Zeng and H.-J. Gabius, Biochem. Int. **26,** 17–24 (1992).
[83] C. Mandal, S. Basu, and C. Mandal, Biochem. J. **257,** 65–71 (1988).
[84] A. C. Roche, J. Maurizot, and M. Monsigny, FEBS Lett. **91,** 233–236 (1978).
[85] M. W. Thomas, E. F. Walborg, Jr., and B. Jirgensons, Arch. Biochem. Biophys. **178,** 625–630 (1977).
[86] C. S. Wright, J. Mol. Biol. **178,** 91–104 (1984).
[87] W. Weis, J. H. Brown, S. Cusack, J. C. Paulson, J. J. Skehel, and D. C. Wiley, Nature **333,** 426–431 (1988).
[88] M. Lindahl, R. Brossmer, and T. Wadström, in: Lectins: Biology, Biochemistry, Clinical Biochemistry (T. C. Bog-Hansen and J. Breborowicz, eds.), **Vol. 4,** pp. 425–433, de Gruyter Verlag, Berlin 1985.

[89] L. D. Heerze and G. D. Armstrong, Biochem. Biophys. Res. Commun. **172,** 1224–1229 (1990).
[90] D. Roelcke, Immunol. Immunopath. **2,** 266–280 (1974).
[91] E. L. Berg, M. K. Robinson, D. Mansson, E. C. Butcher, and J. L. Magnani, J. Biol. Chem. **266,** 14869–14872 (1991).
[92] S. H. Barondes, Annu. Rev. Biochem. **50,** 207–231 (1981).
[93] K. A. Karlsson, Trends Pharmacol. Sci. **12,** 265–272 (1991).
[94] G. Uhlenbruck, Funkt. Biol. Med. **2,** 40–48 (1983).
[95] M. Monsigny, C. Kieda, and A. C. Roche, Biol. Cell. **47,** 95–110 (1983).
[96] H.-J. Gabius, R. Engelhardt, and F. Cramer, Anticancer Res. **6,** 573–578 (1986).
[97] K. Drickamer, J. Biol. Chem. **263,** 9557–9560 (1988).
[98] A. Raz and R. Lotan, Cancer Metastasis Rev. **6,** 433–452 (1987).
[99] V. Kery, Int. J. Biochem. **23,** 631–640 (1991).
[100] R. Schauer, Methods Enzymol. **50,** 66–85 (1987).
[101] F. Sakakibara, G. Takayanagi, H. Ise, and H. Kawauchi, Yakugaku Zasshi **97,** 855–862 (1977).
[102] G. R. Vasta, G. H. U. Ilodi, E. Cohen, and Z. Brahmi, Dev. Comp. Immun. **6,** 625–639 (1982).
[103] M. Kurisu, M. Yamazaki, and D. Mizuno, Cancer Res. **40,** 3798–3803 (1980).
[104] M. Esumi-Kurisu, N. Iwata-Dahi, D. Mizuno, and M. Yamazaki, GANN **74,** 398–404 (1983).
[105] A. Novogrodsky and E. Katchalski, Proc. Natl. Acad. Sci. U.S.A. **69,** 3207–3210 (1972).
[106] A. C. Roche and M. Monsigny, in: Biological Application of the Horseshoe Crab (Limulidae) (E. Cohen, ed.), pp. 603–616, Alan R. Liss Inc., New York 1979.
[107] C. Mandal and M. Chowdhury, Immunopharmacology **20,** 63–72 (1990).
[108] G. R. Vasta and J. J. Marchalonis, in: Development and Comparative Immunology, Progress in Clinical and Biological Research (E. Cooper, C. Langlet, and J. Bierne, eds.), **Vol. 233,** pp. 23–32, Alan R. Liss Inc., New York 1987.
[109] R. Gallily, B. Vray, I. Stain, and N. Sharon, Immunology **52,** 679–686 (1984).
[110] S. H. Barondes, Trends Biochem. Sci. **13,** 480–482 (1988).
[111] R. G. MacDonald, S. R. Pfeffer, L. Coussens, M. A. Tepper, C. M. Brocklebank, J. E. Mole, J. K. Anderson, E. Chen, M. P. Czech, and A. Ullrich, Science **239,** 1134–1137 (1988).
[112] P. Lobel, N. M. Dahms, J. Breitmeyer, J. M. Chirgwin, and S. Kornfeld, Proc. Natl. Acad. Sci. U.S.A. **84,** 2233–2237 (1987).
[113] A. P. Sherblom, N. Sathyamoorthy, J. M. Decker, and A. V. Muchmore, J. Immunol. **143,** 939–944 (1989).
[114] H. Lee, S. Kelm, J. C. Michalski, and R. Schauer, Biol. Chem. Hoppe-Seyler **371,** 307–316 (1990).
[115] N. Shibuya, I. J. Goldstein, W. F. Broekaert, M. Nsimba-Lubaki, B. Peeters, and W. J. Peumans, Arch. Biochem. Biophys. **254,** 1–8 (1987).
[116] D. T. Dorai, B. K. Bachhawat, and S. Bishayee, Anal. Biochem. **115,** 130–137 (1981).
[117] S. Mohan, S. Bishayee, and B. K. Bachhawat, Indian J. Biochem. Biophys. **18,** 177–181 (1981).
[118] S. Itai, J. Nishikata, T. Yoneda, K. Ohmori, H. Yamabe, S. Arii, T. Tobe, and R. Kannagi, Cancer **67,** 1576–1587 (1991).
[119] D. F. Smith and V. Ginsburg, J. Biol. Chem. **255,** 55–59 (1980).
[120] M. Mazzuca, A. C. Roche, M. Lhermitte, and P. Roussel, J. Histochem. Cytochem. **25,** 470–473 (1977).
[121] K. Yamada and S. Shimizu, Histochem. J. **11,** 457–471 (1979).
[122] H. Holthöfer, R. A. Hennigar, and B. A. Schulte, Cell Differentiation **24,** 215–222 (1988).
[123] D. J. Taatjes, J. Roth, W. Peumans, and I. J. Goldstein, Histochem. J. **20,** 478–490 (1988).
[124] K. Hedman, I. Pastan, and M. C. Willingham, J. Histochem. Cytochem. **34,** 1069–1077 (1986).
[125] R. Schauer, G. Reuter, H. Muhlpfordt, A. F. Andrade, and M. E. Pereira, Hoppe-Seyler's Z. Physiol. Chem. **364,** 1053–1057 (1983).
[126] S. Schenkman, M. S. Jiang, G. W. Hart, and V. Nussenzweig, Cell **65,** 1117–1125 (1991).