Reviews

Sialic acid binding lectins

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Summary. The literature contains several reviews on lectins in general, covering mainly those from plants and invertebrates. However, the sialic acid binding lectins have not been reviewed so far. Considering the importance of sialic acids in cell sociology, lectins which specifically recognize terminal sialic acid residues are potentially useful as analytical tools in studying the biological functions of sialoglycoconjugates. These lectins, along with monoclonal antibodies raised against sialoglycoconjugates, have been used in the detection, affinity purification, cytochemical localization and quantitation of such glycoconjugates. In this review the main emphasis has been placed on the occurrence, general purification procedures, macromolecular properties, sugar specificities and applications of these lectins.

Key words. Sialic acid; lectin; sialoglycoconjugate; cell surface; antibody; invertebrate lectin.

Introduction

Sialic acids are a family of about 30 derivatives of N-acetyl or N-glycolyl neuraminic acids[74]. Neuraminic acid does not exist as such in nature; the amino group is acylated by an acetyl or a glycolyl group, and one (or more) of the hydroxyl groups is methylated or esterified with an acetyl, lactyl, sulfate or phosphate group. Neuraminic acid is the most diversely substituted natural sugar. Sialic acid residues occur in the membranes of almost all members of the animal kingdom phylogenically above the echinoderms[75]. Various sialic acid derivatives exhibit interesting species and tissue specific distribution[15,83].

Sialic acids play a pivotal role in receptors for viruses, peptide hormones and toxins[15,72], and also in the social behaviour of cells[49]. Extensive studies on influenza viruses established that the agglutinin responsible for their attachment to the cell surface binds to the sialic acid moieties of the receptors[15,103,105]. In contrast to their receptor functions, sialic acids act as masking agents on antigens, receptors and other recognition sites of the cell surface[75,76,109]. O-acetylation of sialic acid residues changes with transformation or other alterations in the cellular environment. O-acetylation influences enzymatic reactions in the catabolism of glycoconjugates by slowing down or inhibiting bacterial and viral sialidases and thus altering their immunopotency[77]. Sialoglycoproteins and sialoglycolipids play very important roles in the physiology of normal and transformed cells. The extent of acetylation is also known to change during differentiation[78]. The lectins which specifically bind to sialic acids are potentially useful in the detection, quantitation, localization, purification and characterization of many biomolecules containing sialic acids, e.g. glycoconjugates, gangliosides and polysaccharides. These lectins can be used as specific probes for certain derivatives of sialic acid which serve as molecular markers in some physiological and pathological developments.

Of the more than 100 lectins purified from plant and animal sources, many of which are commercially available, only a few bind to sialic acids. These lectins have been detected in a variety of invertebrates[9,19,29,108,109] mainly in the hemolymph, but only a few have been purified and characterized. Among the plant lectins, wheat germ agglutinin is known to bind sialic acids[28] although it binds preferentially to N-acetyl-D-glucosamine and its (β1–4) linked oligomers. Recently two plant lectins with high specificity for complex oligosaccharides have been reported[79,80,104]. Sialic acid specific lectins have been detected in mammals[18,64] and exceptionally in an abnormal human serum[89]. Some hybridoma cell lines produce monoclonal antibodies against specific sialoglycoconjugates[17,31,86].

Several reviews on plant[7,26,29,48] and invertebrate[9,19,29,108,109] lectins are available, but there is none which specifically deals with sialic acid binding lectins. This review is an up-to-date summary of the known lectins which bind sialic acids including a systematic presentation of their occurrence, methods of purification, macromolecular properties, sugar specificity and scope of application. Such information may be useful in providing a basis for understanding the mechanism of cell-cell recognition. The use of various purified lectins with defined narrow specificity may provide new tools for the study of cell surface architecture. Analysis of the binding specificity may pave the way for the understanding of the origin of the diversity of invertebrate lectins, which are popularly believed to function as the immune system of these organisms.

Occurrence

Invertebrate lectins

Sialic acid binding lectins are ubiquitous among invertebrates. Arthropoda, Mollusca and Urocordata, a group of lower vertebrates, are abundant sources of these

Table 1. A summary of sialic acid binding invertebrate lectins

Phylum Subphylum Class Species (common name)	Sample (number of lectins)	Sugar specificity	References
Arthropoda			
Chelicerata			
Merostomata			
Limulus polyphemus (American horseshoe crab)	h (3)	NeuNAc, GalNAc, GlcA, PC	19, 51, 57, 58, 61, 66–68
Carcinoscorpius rotunda (Indian horseshoe crab)	h (1)	NeuNAc, GalNAc, GlcA, PC, KDO	1, 9, 20–23, 27, 54
Tachypleus tridentatus (Japanese horseshoe crab)	h (4)	NeuNAc, GalNAc, GlcNAc	78, 79
Cancer antennarius (marine crab)	h	NeuNAc	63, 64
Birgus latro (Coconut crab)	–	NeuNAc	19
Arachnida			
Androctonus australis (Saharan scorpion)	h (2)	NeuNAc, Hexuronic acid	18, 93
Centruroides sculpturatus (Arizona scorpion)	h	NeuNAc, KDO	92
Masticoproctus giganteus (whip scorpion)	h	NeuNAc, GalNAc, GlcNAc	97
Heterometrus granulomanus (Indian scorpion)	h	NeuNAc, GalNAc	3
Hadrurus arizonensis (hairy scorpion)	h	NeuNAc	98
Paruroctonus mesaensis (scorpion)	h	NeuNAc	96
Aphonopelma cepaeahortensis (American spider)	h	NeuNAc	94
Mandibulata			
Insecta			
Teleogryllus commodus (walker insect)	h	NeuNAc, Gal	32
Hyalophora cecropia (caterpillar)	h	NeuNAc	7
Allomyrina dichotoma (beetle)	–	NeuNAc	88
Crustacea			
Homarus americanus (lobster)	h (2)	NeuNAc, GalNAc, ManNAc, GlcNAc, Glc	30, 31, 36, 89
Macrobrachium rosenbergii (fresh water prawn)	h	NeuNAc	95
Mollusca			
Gastropoda			
Achatina fulica (African land snail)	h (2)	NeuNAc, Gal	4, 5, 39, 48–50
Achatina granulata (land snail)	–	NeuNAc, GalNAc, GlcNAc	10
Cepaea hortensis (land snail)	–	NeuNAc	37
Biomphalaria glabarata (fresh water snail)	–	NeuNAc	11
Dolabella (sea snail)	–	NeuNAc	40
Limax flavus (sea slug)	–	NeuNAc	52
Pelecypoda			
Mytilus edulis (sea mussel)	–	NeuNAc	12, 34
Crassostrea gigas (Pacific oyster)	h (2)	NeuNAc	2, 33, 35, 91
Echinodermata			
Echinozoa			
Echinoidea			
Psammechinus miliaris	h	NeuNAc	86

Abbreviations: h = hemolymph; NeuNAc = N-acetylnuraminic acid; Gal = D-galactose; GalNAc = N-acetyl-D-galactosamine; Glc = D-glucose; GlcN = D-glucosamine; GlcNAc = N-acetyl-D-glucosamine; ManNAc = N-acetyl-D-mannosamine; GlcA = Glucuronic acid; KDO = 2-keto-3-deoxyoctonate; PC = phosphorylcholine.

lectins[108] (table 1). Many species in these phyla contain sialic acid specific lectins, more than one in some species, of which only a few have been purified and characterized. Extensive studies have been carried out in Chelicerata, a subphylum of Arthropoda. Though the study of lectins from these species was pioneered by Noguchi[60] at the beginning of this century, the characterization of limulin, the hemagglutinin from *Limulus polyphemus* (American horseshoe crab) and the demonstration of its specificity for sialic acids were completed by later workers[21,53,70,71]. Limulin is one of the few commercially available lectins specific for sialic acids. *Limulus polyphemus* belongs to the class of Merostomata, and in this class, sialic acid binding lectins are found in the hemolymph of Indian[10] and Japanese[81,82] horseshoe crabs, a marine crab[65,66] and the coconut crab[21] (table 1). Within the class of Arachnida lectins specific for sialic acids have been found in the hemolymph of the Saharan scorpion[20,97], the Arizona scorpion[96], the whip scorpion[101], the Indian scorpion[3], an American spider[98] and in some other species of scorpion[100,102].

Other Arthropods of the subphylum Mandibulata contain sialic acid specific lectins e.g. in the class Insecta, the walker insect[34], caterpillars[8] and beetles[92] are known to contain these lectins. In this subphylum of Mandibulata many sialic acid specific lectins have also been found in the crustaceans prawn[99] and lobster[32,33,38,93]. The sugar specificities of lectins in the hemolymph of most invertebrates and lower cordata groups do not bear any relationship to taxonomy, differing even within a particular genus[98]. However, the Chelicerata form a relatively homogeneous group; all the members studied contain sialic acid binding lectins in their hemolymph.

Sialic acid binding lectins have been reported in many species of Mollusca, e.g. snails[5,6,11,12,39,41–43,50–52] and sea slug[54] of the class Gastropoda, and sea mussel[14,36] and Pacific oyster[2,35,37,95] of the class Pelecypoda. *Psammechinus miliaris*, an echinordean of the phylum Echinodermata, also contains a sialic acid binding lectin[90].

Plant lectins

Only a few plant lectins bind sialic acids. It is not clear whether this is because plants do not contain sialic acids. For many years wheat germ agglutinin was the only plant lectin known to have specificity for sialic acids[28,45,57,87,106]. This specificity is due to the structural similarity of sialic acid to D-GlcNAc which is the preferred ligand of this lectin. Recently, a lectin isolated from elderberry (*Sambucus nigra* L.) bark has been shown to bind the sequence NeuNAc (α 2–6)-D-Gal/D-GalNAc with high specificity[79,80]. This lectin has a weak affinity for D-galactose or D-GalNAc but does not bind to either NeuNAc or NeuNGc. Another important characteristic of this lectin is that it has a very high affinity for terminal sialic acid linked (α 2–6) to D-galactose compared to the (α 2–3) linked isomer. On the other hand a leukoagglutinin from the seeds of the leguminous plant *Maackia amurensis* binds preferentially to terminal sialic acid linked (α 2–3) to penultimate D-galactose residues but neither to the corresponding (α 2–6) linked oligosaccharide nor to the component sugars (NeuNAc or lactose)[104]. A lectin isolated from prickly lettuce (*Lactanea seariale*) was also reported to bind sialic acids[110].

Other lectins

The virus family Orthomyxoviridae, comprising the influenza viruses, and others such as the Paramyxoviridae, Piconaviridae, Papoviridae, Reoviridae and Adenoviridae, contain agglutinins that bind to the sialic acid residues of the receptors situated on their host cells[15]. Several lectins have been found on the cell surfaces of bacteria, e.g. *Escherichia coli*[84], *Streptococcus sanguis*[62] and *Pseudomonas aeruginosa*[67]. Mammalian sialic acid specific lectins have been found in the uterus[18] and brain[64] of rat. Frog egg lectin is a rare example from an amphibian source[86]. Tunicates are the lower vertebrates in which sialic acid specific lectins have been found[94,107].

Monoclonal antibodies

Monoclonal antibodies raised against sialic acids can supplement the analytical, preparative and diagnostic uses of these lectins. Hybridoma cell lines have been identified which produce monoclonal antibodies specific for sialic acids which have (α 2–3)D-Gal[86] and (α 2–6)D-Gal[31] linkages. Based on the autoimmune NZB mouse strain[27], a system has been developed for the production of monoclonal antibodies specific for weakly immunogenic sialoconjugates of *Escherichia coli* KI and group B *Meningococci.*

Purification

As these lectins exhibit high binding affinity for sialic acids most of the reported lectins were purified using sialoglycoproteins as affinity absorbents; namely, bovine, sheep or equine submaxillary mucin or fetuin coupled to cyanogen bromide-activated sepharose (table 2). For absorption and elution advantage is taken of the divalent cation (Ca^{2+}) requirement for lectin binding. The affinity columns are usually equilibrated with a buffer containing Ca^{2+} at their optimum pH. After washing of the unbound proteins, lectins are eluted with a buffer devoid of Ca^{2+}[3,5,10,18,29,65]. In addition, in certain cases the temperature is raised and/or the pH is shifted from the range of optimum binding. Besides the withdrawal of Ca^{2+} or shifting the pH a few lectins have been eluted with sialic acids[54]. Even D-GlcNAc has been used for eluting Japanese horseshoe crab agglutinin[82].

Recently, a lectin from a lobster was purified with colominic acid-sepharose as an affinity matrix and eluted with D-ManNAc[93]. The sialic acid binding lectins from

plant sources have also been purified by affinity chromatography[79,104]. Of the lectins listed in table 1 only a few have been purified and the rest have been detected in crude hemolymph.

Macromolecular property

Most of the sialic acid specific lectins are of high molecular weight and contain more than one subunit of molecular mass from 15 to 24 kDa (table 2). An interesting feature is that in their native state some of these lectins are composed of between 10 and 20 subunits. Very little is known about the nature of these subunits and their mutual interactions in forming the native protein. A high content of acidic amino acids seems to be a general characteristic of these lectins. Aspartic and glutamic acids together with their amides account for about 20% of the amino acids (table 2). Therefore the acidic pI values of limulin(4.8)[29] and achatinin$_H$(6.2)[6] are to be expected. However, the lectins from the sea slug(9–9.5)[54], the Indian scorpion(9–9.5)[3], wheat germ(8.7)[68] and frog eggs(12.2)[88] show alkaline pI values probably because of extensive amidation of the acidic residues. The optimum pH for stability and biological activity of most of these lectins lies between 7 and 9. All are stable at room temperature and can be stored for 6–12 months at 4 °C. No enzymatic activity has been detected.

Almost all the sialic acid binding lectins characterized so far are glycoproteins except those from frog egg[86] and wheat germ[4]. Indian horseshoe crab lectin, carcinoscorpin, contains 5.8% neutral sugar mainly as mannose[22], whereas limulin contains 4% neutral sugar mainly as GlcNAc[61,70]. Indian scorpion lectin contains mannose and GlcNAc as neutral sugars (2.8%)[3]. The snail lectin, achatinin$_H$, contains a large amount of carbohydrate (21%), with xylose (6%) as the most abundant sugar[5]. Xylose, as a constituent of N-glycosidic carbohydrate chains, has been found in the α-hemocyanin of *Helix pomatia*[46] and in trace amounts (0.6%) in the cold agglutinin of *Achatina fulica*[55]. Xylose may possibly be a common constituent of molluscan glycoproteins. In some lectins the hemagglutination activity is completely lost when the carbohydrate groups are removed by β-elimination[5], which indicates their importance in the biological activity.

Some of these lectins have been found to be mitogenic. The lobster lectin stimulates B lymphocytes but not T lymphocytes[16] whereas wheat germ agglutinin stimulates both[91]. Limulin stimulates mouse and human lymphocytes[29] and achatinin$_H$ stimulates human and rat lymphocytes[52].

The secondary structures of only a few of these lectins have been examined by circular dichroism measurements[51,69,87]. Three-dimensional structural studies on wheat germ agglutinin[106] and influenza virus agglutinin[105] as well as on their complexes with sialic acids by X-ray crystallography at atomic resolution have revealed interesting features of such binding. Three-dimensional structural studies on the invertebrate lectins have not been reported so far.

Sugar specificity

These lectins bind to a variety of sialic acid derivatives as well as to some other sugars (table 1). The affinity for

Table 2. A summary of macromolecular properties and methods of purification of a few lectins which bind sialic acids

Source	Molecular weight (in kDalton) Native	Subunit	Amino acid content	Carbohydrate content (%)	pI	Optimum pH	Methods of purification
American horseshoe crab	335	18	22% Glu & Asp	4	4.8	8.5	Starch gel electrophoresis & BSM-sepharose
Japanese horseshoe crab	420	42	High content of Glu & Asp	nd	nd	8.0–8.5	BSM-sepharose, Fractogel H.W. 75 & Cellulofine GC 700
Indian horseshoe crab	420	27 & 28	21% Glu & Asp	5.8	nd	7.5–8.5	BSM-agarose and BSM-sepharose
Marine crab	70	36	nd	nd	nd	7.5	BSM-agarose & BSM-sepharose
Lobster 1	19S	55	nd	nd	nd	7.8	Ammonium sulphate, gel filtration & electrophoresis
Lobster 2	70	70	nd	nd	nd	nd	Fetuin-sepharose & colominic acid
Sea slug	44	22	20% Glu & Asp	nd	9.0–9.5	7.5	Ammonium sulphate & BSM-sepharose
African land snail	242	15	22% Glu & Asp	21	6.2	8.0–9.0	SSM-sepharose
Indian scorpion	500	15	nd	2.8	9.0–9.5	6.5–7.5	ESGG-sepharose
Rat uterus	28 & 30	28 & 30	nd	nd	4.0 & 4.1	8.0	Fetuin-sepharose
Wheat germ agglutinin	35–48	17–24	high Gly & Cys, 18% Glu & Asp	0	8.7	–	Fetuin-sepharose
Escherichia coli lectin	nd	16.5	22% Glu & Asp	nd	nd	7.4–8.0	Sepharose-4B
Frog egg lectin	12.5	12.5	18% Glu & Asp	0	12.2	7.0–8.0	Sephadex G75, DEAE-cellulose hydroxyapatite and carboxymethyl cellulose

Abbreviations: nd = not done, BSM = bovine submaxillary mucin, SSM = sheep submaxillary mucin, ESGG = equine submandibular gland glycoprotein.

sugars is usually measured by determining the ability of a sugar to inhibit the hemagglutination caused by a lectin or to inhibit the precipitation of the lectin by polysaccharides or glycoproteins. The higher the affinity for the sugar the less is needed for inhibition. Precise values of binding affinities are obtained from measurement of the association constants by physico-chemical techniques. The association constants of sugar binding of only a few lectins, e.g. carcinoscorpin[29], achatinin$_H$[51], wheat germ agglutinin[45] and elderberry bark lectin[79] have been determined. Nonetheless, the relative binding affinities of many lectins for a number of sialic acid derivatives have been worked out from inhibition studies.

The affinity of carcinoscorpin does not change with the extent of O-acetylation of sialic acids but it decreases when the N-acetyl group is replaced by the N-glycolyl group[29]. Some lectins show higher affinities for O-acetyl derivatives, e.g. the marine crab lectin shows higher affinities for 9-O-(33-fold) and 4-O-acetyl(11-fold) derivatives compared to free sialic acid[65]. Achatinin$_H$ does not bind to 4-OAcNeuNAc, but binds very strongly to the 9-O-acetyl derivative compared to free sialic acid[51]. The orders of binding strength of a number of mono- and oligosaccharides with some lectins are presented in table 3.

Several bacterial surface lectins show interesting sugar specificity. A lectin from *Escherichia coli* shows higher affinity for 4-OAcNeuNAc but this is markedly reduced with 7-O- or 9-O-acetylation[47]. This kind of specificity may play a role in the choice of the host and tissue in infection and colonization. Similarly the frog egg lectin has been postulated to be involved in fertilization and in the development of the embryo[88]. It has recently been found that some plant lectins are highly specific for oligosaccharides which have a certain type of linkage of the sialic acid moiety with other sugars; e.g. the elderberry bark lectin is highly specific for oligosaccharides with a terminal NeuNAc(α2–6)Gal sequence which is a 1,600–10,000-fold stronger inhibitor than D-galactose alone[79]. On the other hand, oligosaccharides containing a terminal NeuNAc (α2–3) linkage are only 30–80 times more inhibitory than D-galactose. Monosaccharides of various sialic acid derivatives are also non-inhibitors. On the basis of inhibition with simple sugars the elderberry bark lectin was demonstrated to be specific for D-galactose[13]. The lectin from the seeds of *Maackia amurensis* shows a very high affinity for NeuNAc(α2–3)Gal(β1–4)Glc but is not inhibited by either NeuNAc(α2–6)Gal(β1–4)Glc, Gal(β1–4)Glc or NeuNAc[104] (table 3). These findings indicate that the specificity of a lectin for a monosaccharide may not reflect its specificity for a natural oligomer; the type of linkage and the position of the sugar may greatly influence the strength of such binding.

Sialoglycoconjugates like glycoproteins and glycolipids are better inhibitors than simple mono- or disaccharides. Submaxillary mucins from different sources contain various sialic acid derivatives and were found to be better inhibitors of many lectins. Bovine submaxillary mucin containing 9-OAc- and 8,9-diOAcNeuNAc has been found to be the best inhibitor of the marine crab[65], the American[29] and Japanese[78] horseshoe crabs, lobster[32] and slug[29] lectins and achatinin$_H$[50] but not of the Indian scorpion[3] lectin. Equine submaxillary mucin containing predominantly 4-OAcNeuNAc is also a good inhibitor of limulin[29], carcinoscorpin[29] and marine crab lectin[65]. Sheep submaxillary mucin contains only NeuNAc and is an inhibitor of achatinin$_H$[6] and horseshoe crab lectins[29, 78] but not of marine crab lectin[65]. Fetuin, human chorionic gonadotropin, α_1-acid glycoprotein, serotransferrin, lactotransferrin, etc. are sialic acid containing glycoproteins which inhibit the hemagglutination of sialic acid binding lectins. Besides these sialoglycoproteins, several glycosphingolipids have been found to be good inhibitors[3, 29, 50].

Erythrocytes from different species contain different kinds of sialic acid derivatives in varying amounts. Hence their agglutination by sialic acid specific lectins exhibit different hemagglutination patterns. The involvement of sialic acid in such agglutination has been proved by treatment of the erythrocytes with a sialidase, which completely inhibits agglutination. Achatinin$_H$ agglutinates only rabbit, guinea pig, hamster and rat erythrocytes which contain mainly 9-O-AcNeuNAc but not human and horse erythrocytes which lack 9-O-acetyl derivatives[50, 75]. The marine crab lectin, which binds both 4-O-Ac- and 9-O-AcNeuNAc, agglutinates horse, rabbit, rat and mouse erythrocytes[65]. Limulin[29] and lectins from

Table 3. The order of sugar specificity of some lectins

Lectin	Order of specificity	References
Carcinoscorpin	NeuNAc(α2–6)Gal·OH ≫ NeuNAc(α2–6)Gal(β1–4)Glc > NeuNGc > NeuNAc	27
Limulin	NeuNGc(α2–3)GalNAc > NeuNAc(α2–6)GalNAc > NeuNAc = NeuNGc	27
Achatinin$_H$	NeuNAc(α2–3)Gal(β1–4)Glc > NeuNAc(α2–6)Gal·OH > 9 – OAcNeuNAc > NeuNAc > NeuNGc	48
Slug lectin	NeuNAc > NeuNGc	52
	NeuNAc(α2–3)Gal = NeuNAc(α2–6)Gal > 9 – OAcNeuNAc(α2–6)Gal > NeuNGc(α2–6)Gal	63
Indian scorpion lectin	NeuNAc(α2–3)Gal(β1–4)Glc = diNeuNAc(α2–3)Gal(β1–4)Glc > NeuNAc > NeuNGc	3
Marine crab lectin	9 – OAcNeuNAc > 4 – OAcNeuNAc ≫ NeuNAc > NeuNGc	63
Lobster lectin	NeuNAc > NeuNGc > ManNAc > GlcN > GlcNAc	30
Elderberry bark lectin	NeuNAc(α2–6)Gal(β1–4)GlcNAc(β1–3)Gal(β1–4)Glc > NeuNAc(α2–6)Gal(β1–4)Glc > NeuNAc(α2–6)Gal(β1–4)Glc·OH ≫ NeuNAc(α2–3)Gal(β1–4)Glc > NeuNAc(α2–3)Gal(β1–3)GlcNAc(β1–3)Gal(β1–4)Glc	76
Escherichia coli lectin	NeuNAc(α2–3)Gal(β1–4)Glc > NeuNGc > NeuNAc	81

the Indian scorpion[3], the Japanese horseshoe crab[81,82], lobster[32,33] and sea slug[29] agglutinate erythrocytes from many animals; they differ only in hemagglutination titers, probably because of their broad specificity for sialic acid derivatives.

A few sialic acid binding lectins bind to other sugars in addition to sialic acids. Multispecificity of limulin and carcinoscorpin is well documented[29]. Carcinoscorpin binds to β-glycerophosphate[23], 2-keto-3-deoxy-octonate and lipopolysaccharides[24]. Both limulin and carcinoscorpin bind phosphorylcholine at a site different from the sialic acid binding site[29]. Lectins from the Indian horseshoe crab[29], the Indian scorpion[3] and rat uterus[18] bind to glucuronic acid. Wheat germ agglutinin binds to GlcNAc and its (β1–4) linked oligomer[28,87]. Elderberry bark lectin binds to D-galactose and its derivatives[13].

Application

Lectins have been widely used for the detection, isolation and characterization of glycoconjugates using their characteristic carbohydrate binding properties. The lectins which bind specifically to sialic acids or oligosaccharide units which include sialic acid derivatives would provide useful tools for biochemical studies of sialoglycoconjugates, especially because of the importance of sialylation and desialylation in the regulation of glycoconjugate metabolism and cell-cell interaction[49,76]. Monoclonal antibodies raised against sialic acid containing oligosaccharides would also find similar applications[27,31,86].

The structural diversity and very limited availability of specific sialoglycoproteins on the cell membrane or in the interior have made the isolation of these molecules a formidable task. Immobilized lectins have been used successfully in affinity chromatographic techniques for the isolation of sialoglycoconjugates. Wheat germ agglutinin has been used to fractionate brain glycoproteins into several populations differing in glucosamine:mannose:sialic acid content[30]. Immobilized elderberry bark lectin has been used for the fractionation of sialylated oligosaccharides, glycopeptides and glycoproteins[80]. *Maackia amurensis* lectin, immobilized on sepharose, has been used to separate a mixture of the complex tri- and tetra-antennary Asn-linked sialoglycopeptides[104]. Immobilized carcinoscorpin has been reported to resolve the isoenzymes of alkaline phosphatase from sheep[25] and rat[56] brains.

Sialic acid binding lectins or monoclonal antibodies in conjugation with enzymes, biotin or heavy metals can be used in the cytochemical localization of sialoglycoconjugates by light or by electron microscopy[59,73]. Limulin in conjugation with peroxidase or rhodamine has been used in the detection of sialoglycoconjugates in various cell types in rat pancreas[59]. Recently the elderberry bark lectin-gold technique has been used for the detection of NeuNAc(α2–6)Gal/GalNAc sequences[85]. *Limax flavus* lectin was used to examine the distribution of sialic acid residues in the pancreas, liver and distal colon of rat by light and electron microscopy[73].

These lectins and antibodies are potentially useful in assaying various sialoglycoconjugates in biological fluids. A very sensitive lectin binding assay has been reported using carcinoscorpin and polyethylene glycol[1]. Recently, influenza C virus agglutinin has been used in the quantitative determination of 9-O-acetylation in sialoglycoconjugates after the inactivation of its esterase activity[58]. The marine crab lectin has been successfully used to identify an O-acetylsialylated ganglioside, a human melanoma antigen, which may be an important tumor marker for the detection and treatment of human melanoma[66]. The narrow specificity of achatinin$_H$ might make it useful in such analyses. Knowledge of the interaction and specificity of binding of the viral agglutinins to sialic acid residues on their receptors, obtained from crystallographic studies on the influenza virus, may aid in the design of drugs against those viruses[105].

Conclusion

This article reviews a special class of lectins which bind sialic acids either in the free state or as carbohydrate moieties in biologically important cellular constituents. Apart from the extensively studied viral agglutinins, these lectins have been isolated from many invertebrate and a few plant sources. Such lectins may also be found in many of the higher vertebrates, if properly searched for. Of special interest are those having a narrow specificity for certain derivatives of sialic acids. These are of potential utility in probing the expression of specific sialic acid derivatives in cellular constituents during a number of important biological events such as differentiation, transformation and immunosuppression.

Though the structure of many sialoglycoconjugates has begun to be revealed, the biological roles of these lectins still remain largely speculative. Detailed studies would pave the way for an understanding of their function. We hope that this review will stimulate the search for new sialic acid binding lectins with more precise specificity from diverse sources. These might provide tools for the study of important biological events and could become very useful in the diagnosis of many diseases.

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Research Articles

Mauthner cells in the medulla of the weakly electric fish *Gymnotus carapo*

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Summary. The medulla of the gymnotoid fish *Gymnotus carapo* contains two large neurons exhibiting all the characteristics of Mauthner cells (M-cells). Their presence was demonstrated by means of Golgi-like labeling with horseradish peroxidase. This is the first description of M-cells in a fish belonging to the order Gymnotiformes.
Key words. Mauthner neurons; electric fish; HRP-labeling.

One of the most typical characteristics of the central nervous system (CNS) of teleosts and certain amphibians is the presence in the medulla of two large nerve cells whose thick axons extend all the way along the spinal cord. These two peculiar nerve fibers were discovered by Mauthner in 1859[1]. Approximately thirty years later, Goronvitsch[2] found in the medulla of the sturgeon the cell bodies from which the pair of axons discovered by Mauthner originate. Physiological studies have demonstrated that excitation of a single Mauthner cell (M-cell) elicits the so-called Mauthner-reflex. The reflex consists of: a) a forceful contraction of the contralateral muscles of the trunk and tail, b) sudden movements of both eyeballs, c) contraction of both opercular muscles and d) synchronous movement of the lower jaw. As pointed out by Diamond[3], this response resembles the startle reaction of a free-swimming fish, caused by mechanical or photic stimuli.

The morphology of the M-cell has been the subject of numerous investigations covering a variety of species[4–7]. However, investigations on the presence of M-cells in the so-called 'weakly electric fish' seem to be very scarce. According to the lists provided by Zottoli[7] in his review chapter, M-cells do occur in the African Mormyriformes. On the other hand, the important group of the South-American Gymnotiformes has hardly been investigated, with the exception of gymnotoid eels, which were included by Zottoli[7] in the list of fish in which these peculiar neurons have not been found.

This report is concerned with the location and morphology of the M-cells in the weakly electric gymnotoid *Gymnotus carapo*. Interest in the identification of M-cells in weakly electric fish goes beyond the goal of adding new members to the list of species in which these peculiar neurons have been found. In all fish investigated, the dorso-lateral dendrites receive afferents from vestibular