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A lectin from the Asian horseshoe crab *Tachypleus tridentatus*: purification, specificity and interaction with tumour cells

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A lectin from the haemolymph of the Asian horseshoe crab *Tachypleus tridentatus* was purified to homogeneity by affinity chromatography on Sepharose 4B-bound *N*-acetylneuraminic acid. The specificity of this lectin was studied by haemagglutination inhibition with sialic acid analogues, *N*-acetylhexosamines and glycoproteins. For the interaction with the agglutinin the *N*-acetyl group and the glyceryl side chain of *N*-acetylneuraminic acid are important, while presence of an aglycon, specially an α-glycosidically linked lactose increases affinity to the lectin. The strongest glycoprotein inhibitors were ovine as well as bovine submaxillary mucin and *Collocalia* mucin, all being *O*-chain glycoproteins but carrying completely different carbohydrate chains. The majority of *N*-chain proteins were inactive. As the lectin agglutinates human erythrocytes, but not the murine lymphoma lines Eb and ESb or the human colon carcinoma HT 29, these cancer cells apparently lack the '*Tachypleus tridentatus* agglutinin-receptor' which is present on red cells and *O*-chain glycoproteins.

Keywords: *Tachypleus tridentatus* lectin, affinity chromatography, sialic acids, *N*-acetylhexosamines, glycoprotein inhibition, tumour cells

Abbreviations: TTA, *Tachypleus tridentatus* agglutinin; SDS, sodium dodecyl sulfate; BSM, bovine sub-maxillary mucin; VCS, *Vibrio cholerae* sialidase; OSM, ovine submaxillary mucin; WGA, Wheat germ agglutinin; NeuAc, *N*-acetylneuraminic acid.

Introduction

Lectins are proteins or glycoproteins from non-immunological origin interacting with carbohydrates [1]. They are known to play specific roles in a diversity of cell-cell interactions in health and disease [2]. In addition, they are valuable tools to study cell surface glycosylation [3, 4]. Among the increasing number of new lectins, those interacting with sialic acids are objects of growing interest (for review see [5, 6]), Sialic acid specific lectins have been purified from plants and several invertebrates. Immobilized on agarose they were used to purify glycoconjugates by affinity chromatography [7–10].

Several preparations of *Tachypleus* agglutinins have been reported which differ in molecular weight and number of subunits [11–14]. The rather large variation may be explained by differing proportions of isolectins. Here we report on the purification of a lectin from *Tachypleus tridentatus* haemolymph by affinity chromatography employing a novel material. Results of haemagglutination and haemagglutination inhibition by sialic acids, *N*-acetylhexosamines, and glycoproteins are discussed as well as the results of cell agglutination assays with the murine T-cell lymphoma lines Eb and ESb, differing in metastasizing capacity, and the human colon carcinoma cell line HT 29.

Materials and methods

Tachypleus tridentatus horseshoe crabs were collected at the Northern Pacific coast area (Gulf of Tonking, Vietnam). Sepharose 4B was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Sialic acids, *N*-acetylmannosamine, and *N*-acetylallosamine were prepared in the Institute of Biochemistry II, Heidelberg. All other sugars were from Serva (Heidelberg, Germany). Structures of sialic acid analogues are shown in Table 1 and Fig. 1. The origin of glycoproteins employed in this study and the preparation of asialoglycoproteins have been reported elsewhere [15]. Low and high range standards from Bio-Rad (Munich,

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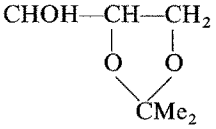
Table 1. Structures of sialic acid analogues used for haemagglutination inhibition studies. The positions of R_1 to R_5 are shown in Fig. 1.

Sialic acid analogue	R_1	R_2	R_3	R_4	R_5
NeuAc[a]	OH	COOH	OH	Ac	$(CHOH)_2$-CH_2OH
2-α-Me-NeuAc	COOH	OCH_3	OH	Ac	$(CHOH)_2$-CH_2OH
2-α-Bzl-NeuAc	COOH	OBzl	OH	Ac	$(CHOH)_2$-CH_2OH
2-β-Me-NeuAc	OCH_3	COOH	OH	Ac	$(CHOH)_2$-CH_2OH
NeuAc-Me-ester	OH	$COOCH_3$	OH	Ac	$(CHOH)_2$-CH_2OH
2-α-Me-NeuAc-Me-ester	$COOCH_3$	OCH_3	OH	Ac	$(CHOH)_2$-CH_2OH
2-α-Me-NeuAc-amide	$CONH_2$	OCH_3	OH	Ac	$(CHOH)_2$-CH_2OH
2,3-Dehydro-2-deoxy-NeuAc	COOH	–	OH	Ac	$(CHOH)_2$-CH_2OH
N-Glycoloyl-Neu	OH	COOH	OH	$COCH_2OH$	$(CHOH)_2$-CH_2OH
2-α-Bzl-5-NH_2-Neu[b]	COOH	OBzl	OH	H	$(CHOH)_2$-CH_2OH
2-α-Bzl-5-*N*-formyl-Neu	COOH	OBzl	OH	CHO	$(CHOH)_2$-CH_2OH
2-α-Me-5-*N*-thioacetyl-Neu	COOH	OCH_3	OH	$CSCH_3$	$(CHOH)_2$-CH_2OH
2-α-Bzl-5-*N*-fluoroacetyl-Neu	COOH	OBzl	OH	$COCH_2F$	$(CHOH)_2$-CH_2OH
2-α-Bzl-5-*N*-trifluoroacetyl-Neu	COOH	OBzl	OH	$COCF_3$	$(CHOH)_2$-CH_2OH
2-α-Me-9-*O*-Ac-NeuAc	COOH	OCH_3	OH	Ac	$(CHOH)_2$-CH_2OH
2-α-Bzl-8,9-*O*-isoprop-NeuAc	COOH	OBzl	OH	Ac	
2-α-Me-9-*O*-tosyl-NeuAc	COOH	OCH_3	OH	Ac	$(CHOH)_2$-CH_2O-Tosyl
2-α-Bzl-5-*N*-Ac-heptulosaminic acid	COOH	OBzl	OH	Ac	CH_2OH
2,4,7,8,9-Penta-*O*-Ac-NeuAc	OAc	COOH	OAc	Ac	CHOAc-CHOAc-CH_2OAc

[a] Abbreviations used in this table: Ac, acetyl; Bzl, benzyl; isoprop, isopropylidene: Me, methyl; Neu, neuraminic acid (with unsubstituted NH_2 group); [b] unsubstituted NH_2 group.

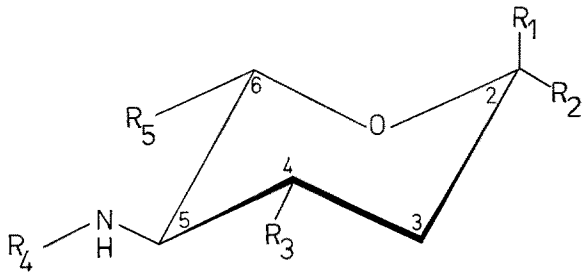


Figure 1. Structure of the sialic acid analogues applied for haemagglutination inhibition studies. Substituents R_1–R_5 are listed in Table 1.

Germany) were used as marker proteins for polyacrylamide gel electrophoresis. Sialidase (E.C. 3.2.1.18) from *Vibrio cholerae* (VCS) was obtained from Behringwerke (Marburg, Germany).

Purification of the lectin

Crude haemolymph (25 ml) was dialysed for 2 h against 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl and centrifuged for 30 min at 15000 × **g**. The supernatant was applied to a column of sialic acid immobilized on Sepharose 4B using divinylsulfone as the activating agent (6.5 ml gel, 4.79 μmol sialic acid per ml gel). A detailed procedure on the preparation and use of this affinity material will be published elsewhere (H. Watzlawick, U. Rose and R. Brossmer). The column was washed with 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl until the baseline was reached again. Subsequently, the lectin was eluted with the same buffer, but containing in addition 0.5 M *N*-acetylglycine, dialysed exhaustively against 10 mM $(NH_4)HCO_3$, and lyophilized. Stored at −20 °C, the lectin could be used for several months without loss of activity.

Protein estimation

Protein concentration was determined using the Bio-Rad assay (Munich, Germany) with bovine serum albumin as standard.

Polyacrylamide gel electrophoresis (PAGE)

PAGE was carried out according to the protocol of Smith [16] using a separating gel consisting of 12% of acrylamide, 0.1% SDS and 2-mercaptoethanol, and a gel with 8% acrylamide without SDS and without 2-mercaptoethanol. Gels were stained with Coomassie Brilliant Blue R-250.

Human erythrocytes and sialidase treatment

Citrated human blood of the groups A_1 Rh^+, B Rh^+ and O Rh^+ was obtained from the local blood bank. Erythrocytes were washed three times with 0.9% NaCl. A suspension of 100 μl of red cells in 390 μl 50 mM Tris-maleate pH 6.8

containing 6.8 mM $CaCl_2$ and adjusted to 300 mOsm with 0.9% NaCl, was incubated with 10 µl sialidase (10 mU) for 30 min at 37 °C. Erythrocytes were then washed three times with saline. Finally, native and enzyme-treated cells were suspended in saline to obtain a 10% suspension and directly used for the haemagglutination assay.

Tumour cells

Eb cells, the methylcholanthrene-induced T-cell lymphoma L 5178/Eb from a DBA/2 mouse with low metastatic capacity, and its spontaneous variant ESb with high metastatic properties were generous gifts from Dr V. Schirrmacher (Heidelberg, Germany). Cells were maintained in suspension culture in RPMI-Medium 1640 supplemented with 10% fetal calf serum, and L-glutamine (200 mM).

Human colon carcinoma cells HT 29 were from ATCC (Rockville, MD, USA) and maintained as monolayers in the same medium. Cells were detached by short treatment with a mixture of 0.05% trypsin and 0.02% EDTA in PBS, washed once with medium containing fetal calf serum, and then twice with 50 mM Tris-HCl pH 7.5 containing 125 mM NaCl. Cells were either treated with sialidase or used directly for the cell agglutination assay.

Treatment of tumour cells with sialidase

A suspension of 0.5×10^7 cells in 50 mM Tris-maleate pH 6.8 containing 6.8 mM $CaCl_2$ and adjusted to 300 mOsm with NaCl was incubated with 50 µl sialidase (50 mU) for 30 min at 37 °C, washed three times with 50 mM Tris-HCl pH 7.5 containing 125 mM NaCl, and suspended in this buffer at 0.5×10^7 cells per ml.

Fluorimetric determination of sialic acid

After sialidase-treatment cells were centrifuged at 800 rpm for 7.5 min. Sialic acid in the supernatant was determined by a modified version of the thiobarbituric acid assay according to [17] as described in [18]. Controls without enzyme were treated correspondingly.

Haemagglutination assay

Haemagglutination assays were performed in V-well microtitre plates (96 wells). Two-fold serial dilutions of the lectin were made in 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl (25 µl per well). To this was added 10 µl of a suspension of human erythrocytes. After careful shaking, the plates were incubated for 1 h at room temperature. The highest dilution of lectin showing macroscopic agglutination was taken as the haemagglutination titre. The minimum amount of lectin protein in the well agglutinating O Rh^+ erythrocytes was defined as one haemagglutination unit (HU), being 0.1 µg lectin protein per well.

Haemagglutination inhibition assay

Haemagglutination inhibition assays were performed as described in [15], with inhibitors diluted in 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl.

Cell agglutination assay

Agglutination assays with tumour cells were carried out in 96-well microtitre plates (F-plates). To two-fold serial dilutions of the lectin in 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl, 25 µl per well of a cell suspension (0.5×10^7 cells per ml) were added. After 1 h of incubation at room temperature plates were read under a phase contrast microscope (100× magnification). The highest dilution of the lectin showing agglutination was taken as agglutination titre.

Results

Chromatography of 25 ml crude haemolymph and elution with *N*-acetylglycine afforded 5.6 mg pure lectin with a specific activity of 20000 HU per mg protein determined with human O Rh^+ erythrocytes (Fig. 2 and Table 2). This preparation was named *Tachypleus tridentatus* agglutinin (TTA). Specific activity was increased about one hundred-fold. SDS-PAGE in the presence of 2-mercaptoethanol showed one major band with M_r 27000 and two bands with 30000 and 32000, respectively (Fig. 3). PAGE without SDS

Table 2. Purification of *Tachypleus tridentatus* agglutinin (TTA) by affinity chromatography on NeuAc-Sepharose 4B. Haemagglutination activity was determined with human group O Rh^+ erythrocytes.

Fraction	*Volume* (ml)	*Protein* (mg)	*Total activity* (HU)[a]	*Specific activity* (HU per mg protein)
Crude haemolymph	25	2500	510000	204
Break-through effluent	65	2470	10300	4.17
Eluted TTA	12	5.6	112000	20000

Recovery 22%.
[a] Abbreviation: HU, haemagglutination unit (see the Materials and methods section).

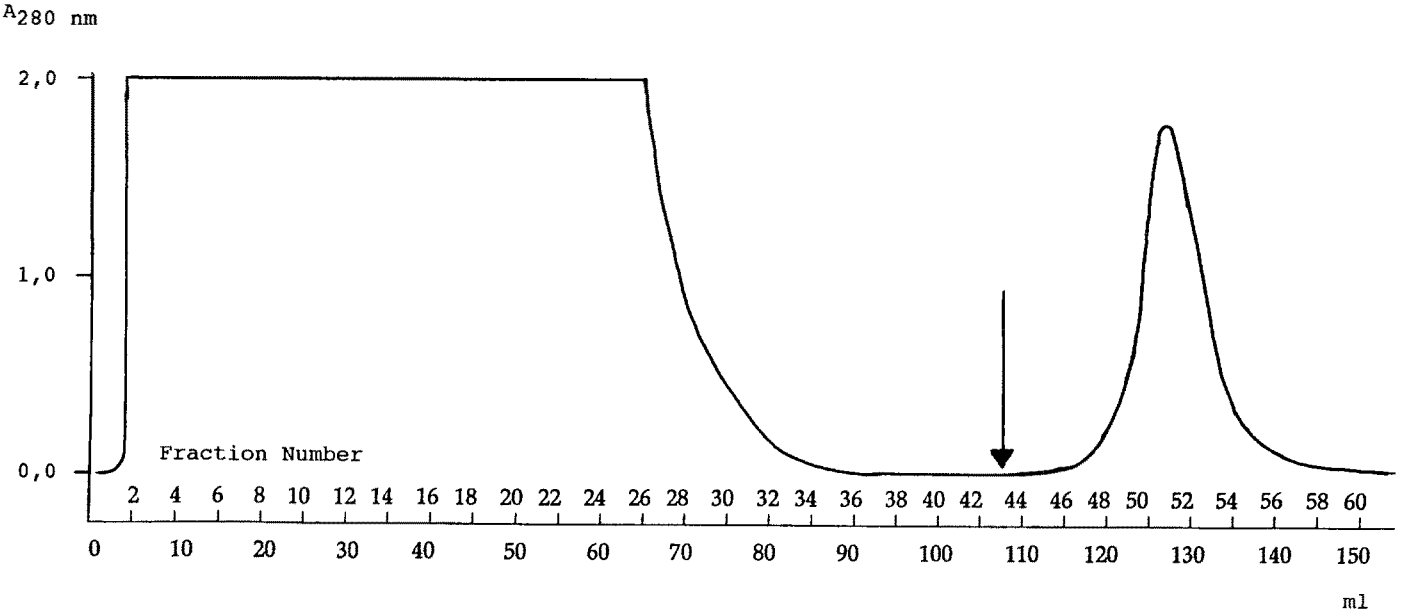


Figure 2. Affinity chromatography of *Tachypleus* haemolymph on NeuAc-Sepharose 4B. 25 ml of crude haemolymph was applied to the column which was washed with 50 mM Tris-HCl pH 8.5 containing 10 mM $CaCl_2$ and 110 mM NaCl. The lectin was eluted employing 0.5 M *N*-acetylglycine in the same buffer (arrow). Haemagglutination activity could be detected in fractions number 48–55, which were pooled, dialysed against 10 mM $(NH_4)_2HCO_3$ and lyophilized. Fractions pooled were active within the range of titre 16–256 which, however, do not represent the actual values due to the presence of inhibiting *N*-acetylglycine.

yielded one band of M_r 130 000 and a few weak bands with higher M_r (Fig. 4). Results of haemagglutination, haemagglutination inhibition, and cell agglutination are summarized in Tables 3, 4, 5, and 6. The amount of sialic acid removed from the cell lines Eb, ESb and HT29 by sialidase treatment was 12.5, 11.6, and 13.2 nmol NeuAc per mg of cell protein, respectively.

Discussion

Purification of TTA

In this paper we describe a one step purification procedure of a lectin from the haemolymph of the Asian horseshoe crab *Tachypleus tridentatus* on a novel *N*-acetylneuraminic acid containing affinity material using *N*-acetylglycine for elution. An electrophoretically homogeneous lectin was obtained with 22% of the haemolymph's haemagglutination activity. Earlier, agglutinins from *Tachypleus tridentatus* were purified by affinity chromatography on BSM coupled to Sepharose [11, 13, 14] or on GalNAc-Sepharose [12]. Our purification procedure yielded three bands, one major band (M_r 27 000) and two bands of M_r 30 000 and 32 000, respectively. PAGE without SDS showed one single band at about 130 000, indicating that the lectin is pure and may consist of four subunits. There is some material visible between M_r 130 000 and M_r 200 000 (Fig. 4) which may be considered as a result of lectin oligomerization. Previously, *Tachypleus* haemolymph has been fractionated into four groups of lectins which differed in subunit composition and biological properties [13]. Another preparation was reported with M_r 700 000, consisting of M_r 72 000 and M_r 31 000 peptide doublets [14]. By the time this manuscript had been completed, a study on a sialic acid-binding lectin from *Tachypleus* haemolymph had been published [19]. While the M_r of the subunits were similar to our results the undenatured lectin's M_r was much higher (533 000). This may be explained by the formation of lectin aggregates. In

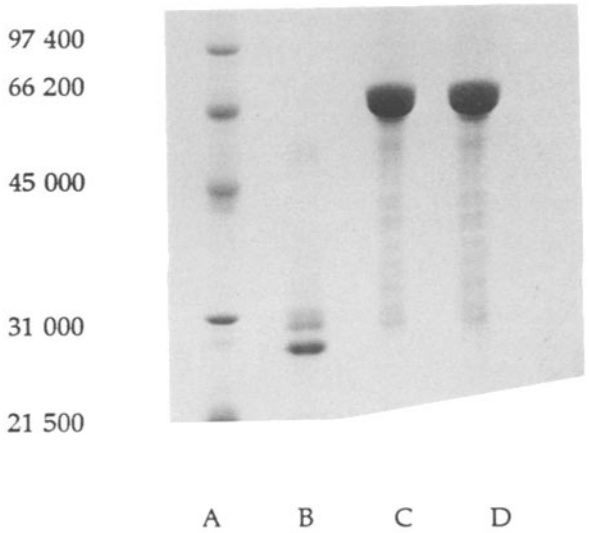


Figure 3. PAGE of TTA with 0.1% SDS and 2-mercaptoethanol. Lane A: marker proteins lysozyme 14 400 Da, soybean trypsin inhibitor 21 500 Da, carboanhydrase 31 000 Da, ovalbumin 45 000 Da, bovine serum albumin 66 200 Da, phosphorylase b 97 400 Da; lane B: purified TTA; lane C: crude haemolymph; lane D: column break-through.

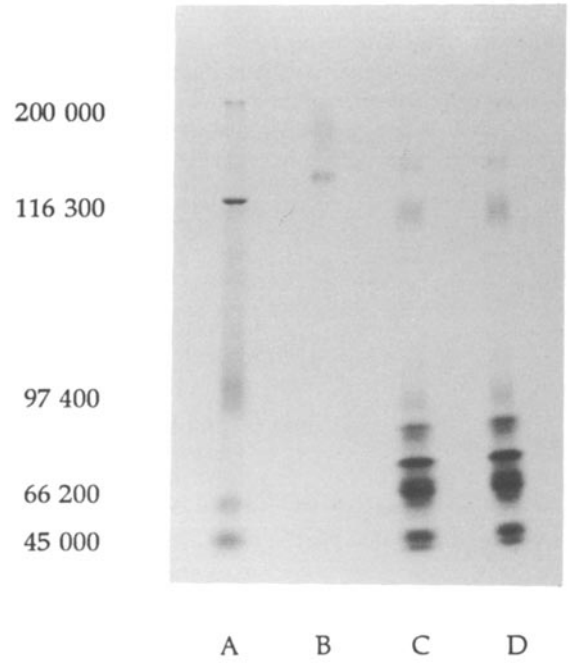


Figure 4. PAGE of TTA without SDS and without 2-mercaptoethanol. Lane A: marker proteins ovalbumin 45 000 Da, bovine serum albumin 66 200 Da, phosphorylase B 97 400 Da, β-galactosidase 116 300 Da, myosin 200 000 Da; lane B: purified TTA; lane C: crude haemolymph; lane D: column break-through.

addition, there are significant discrepancies in specificity, the reason for which are so far unknown.

Haemagglutination

Crude haemolymph agglutinated human erythrocytes of all three blood groups. After sialidase treatment, A_1 Rh^+ red cells, but not B Rh^+ or O Rh^+ cells were agglutinated. This finding suggests the presence of a GalNAc-specific lectin in the haemolymph, which might correspond to one of the lectins reported earlier [12]. Purified TTA reacted with native erythrocytes in a way which was not blood group specific, and was completely without activity towards sialidase-treated A_1 Rh^+, indicating that the GalNAc-specific activity was removed.

Inhibition by monosaccharides

Of all *N*-acetylhexosamines tested, *N*-acetylglucosamine (GlcNAc) and *N*-acetylallosamine (AllNAc), the C-3 epimer of *N*-acetylglucosamine, were the best inhibitors and were only slightly less active than *N*-acetylneuraminic acid. The *N*-acetylglucosamine molecule exhibits similarities to *N*-acetylneuraminic acid and both can be superimposed [20]. The acetamido group and its vicinal hydroxyl are then in the same position with reference to the ring oxygen. An axial hydroxyl at C-3 as in *N*-acetylallosamine apparently does not impair binding to the lectin, but an equatorially orientated bulky lactyl group in *N*-acetylmuramic acid does. The three times lower activity of *N*-acetylgalactosamine, compared to *N*-acetylglucosamine and *N*-acetylallosamine, emphasizes the importance of the equatorial hydroxyl group at C-4. It has to be noted that the acetamido group of *N*-acetylmannosamine is in an axial position, and this molecule is only two times less active than *N*-acetylglucosamine. Thus the orientation of the acetamido group seems to be less important for binding to the lectin. Finally, lack of inhibition of glucose, galactose and mannose points to the necessity of an acetamido group for lectin binding (Table 5.

Inhibition by sialic acids

2-α-Methyl-NeuAc, 2-α-benzyl-NeuAc and even 2-β-methyl-NeuAc were better inhibitors than *N*-acetylneuraminic acid itself (Table 4). Presence of a larger aglycon as lactose in NeuAcα(2-3)Galβ(1-4)Glc and NeuAcα(2-6)Galβ(1-4)Glc increased, compared to *N*-acetylneuraminic acid and 2-α-methyl-NeuAc, inhibition potency nine and four times, respectively. Interestingly, in contrast to other sialic acid

Table 3. Haemagglutination and cell agglutination assays with *Tachypleus tridentatus* agglutinin (TTA). The minimal concentrations of the lectin required for total agglutination are shown. For details see the Materials and methods section.

	R Rh⁺		*B Rh⁺*		*O Rh⁺*	
	Native	*Sialidase-treated*	*Native*	*Sialidase-treated*	*Native*	*Sialidase-treated*
Titre of crude haemolymph	1530	1210	960	< 30	1210	< 30
μg TTA purified per ml	3.9	> 500	3.9	> 500	4	> 500
	Eb		*ESb*		*HT 29*	
	Native	*Sialidase-treated*	*Native*	*Sialidase-treated*	*Native*	*Sialidase-treated*
Titre of crude haemolymph	108	9.5	76	13	23	16
μg TTA purified per ml	> 500	> 500	> 500	> 500	> 500	> 500

Table 4. Inhibition of *Tachypleus tridentatus* agglutinin (TTA) haemagglutination by sialic acid analogues. The minimal concentrations of the compounds required for total haemagglutination inhibition induced by four haemagglutination units are shown.

Sialic acid analogue	mM
NeuAc[a]	35
2-α-Me-NeuAc	17
2-α-Bzl-NeuAc	12.5
2-β-Me-NeuAc	12.5
NeuAc-Me-ester	12.5
2-α-Me-NeuAc-Me-ester	20
2-α-Me-NeuAc-amide	12.5
2,3-Dehydro-2-deoxy-NeuAc	25
N-Glycoloyl-Neu	>12.5
2-α-Bzl-5-amino-Neu[b]	>50
2-α-Bzl-5-*N*-formyl-Neu	>200
2-α-Me-5-*N*-thioacetyl-Neu	>100
2-α-Bzl-5-*N*-fluoroacetyl-Neu	50
2-α-Bzl-5-*N*-trifluoroacetyl-Neu	>100
2-α-Me-9-*O*-Ac-NeuAc	12.5
2-α-Bzl-8,9-*O*-isoprop-NeuAc	16
2-α-Me-9-*O*-tos-NeuAc	20
2-α-Bzl-5-*N*-Ac-heptulosaminic acid	>100
2,4,7,8,9-Penta-*O*-Ac-NeuAc	25
NeuAcα(2-3)Galβ(1-4)Glc	4
NeuAcα(2-6)Galβ(1-4)Glc	4
NeuAcα(2-8)$_{n=10-12}$ (colominic acid)	2[c]

[a] Abbreviations used in this table: Ac, acetyl; Bzl, benzyl; isoprop, isopropylidene; Me, methyl; Neu, neuraminic acid (with unsubstituted NH_2 group); tos, tosyl. [b] unsubstituted NH_2 group. [c] denotes concentration in terms of colominic acid.

Table 5. Inhibition of *Tachypleus tridentatus* agglutinin (TTA) haemagglutination by simple sugars, *N*-acetylhexosamines and MurNAc. The minimal concentration of the compounds required for total haemagglutination inhibition induced by four haemaglutination units of TTA are shown.

	mM
Glc	>200
GlcNAc	50
Gal	>200
GalNAc	160
Man	>200
ManNAc	100
AllNAc[a]	63
MurNAc[b]	160
Lactose	>200

[a] AllNAc, *N*-acetyl-D-allosamine (the C-3 epimer of *N*-acetylglucosamine); [b] MurNAc, *N*-acetylmuramic acid.

specific lectins, for TTA a free carboxyl group at C-2 is not a prerequisite for binding. *N*-Acetylneuraminic acid and its methylester possessed similar activities which indicates that ionic interactions between the carboxyl and the lectin do not significantly contribute to binding.

N-Deacetylated *N*-acetylneuraminic acid, as 2-α-benzyl-5-NH_2-Neu, was devoid of any activity. Substitution of hydrogen in the *N*-acetyl group by the electronegative fluorine decreased inhibition potency four times, substitution of all three hydrogens at least 16 times. Likewise, when the amide oxygen at C-5 of NeuAc α-glycoside is replaced by sulfur, producing a thioamide group, the affinity to the lectin was lowered by at least 12 times. 2-α-Methyl-9-*O*-acetyl-NeuAc, 2-α-benzyl-8,9-*O*-isopropylidene-NeuAc and 2-α-methyl-9-*O*-tosyl-NeuAc were as good inhibitors as the corresponding unsubstituted α-glycosides, showing that even bulky substituents at the glyceryl side chain are well accepted by the lectin. In contrast, shortening of the carbon chain by two carbons leading to 2-α-benzyl-5-*N*-acetyl-heptulosaminic acid caused complete loss of inhibition activity which demonstrates the importance of an intact glyceryl side chain.

Based on these results, important parts for the interaction with the lectin are the intact acetamido group at C-5 and the glyceryl side chain of sialic acid. For the acetamido group a specific pocket on the lectin must exist, since even small structural changes abolish biological activity.

NeuAcα(2-3)Galβ(1-4)Glc and NeuAcα(2-6)Galβ(1-4)Glc were inhibitory at equal concentrations which proves that the lectin does not distinguish between α(2-3)- and α(2-6)-linked *N*-acetylneuraminic acid. Lactose itself was inactive indicating the lectin's specificity for *N*-acetylneuraminic acid. Presence of a subterminal sugar in both trisaccharides caused a stronger sugar-lectin interaction in comparison to 2-α-methyl-NeuAc. Inhibition by colominic acid supports this conclusion and shows that the NeuAcα(2-8)-linkage is equally well accepted by the *Tachypleus* lectin.

Inhibition by glycoproteins

All potent glycoprotein inhibitors were *O*-chain proteins with Tamm-Horsfall protein as an exception. Ovine submaxillary mucin was the strongest inhibitor, being active at 1.3 μg ml^{-1} which corresponds to 0.8 μM *N*-acetylneuraminic acid. Thus, sialic acid linked to glycoprotein oligosaccharides is about 15 000 times more inhibitory than free sialic acid. This finding may be explained by the avidity effect [21]. OSM contains multiple NeuAcα2-6GalNAc(1-0)Ser/Thr-sequences, while in Collocalia mucin B (CM B), almost as potent as OSM, *N*-acetylneuraminic acid occurs in α(2-3)-glycosidic linkage to galactose [22]. Tamm-Horsfall protein is the only glycoprotein carrying *N*-chains only but showing nevertheless a high inhibition activity. This finding was discussed recently for the sialic acid-specific lectin from *Cepaea hortensis* (CHA I) and explained by the avidity effect towards high molecular weight aggregates of Tamm-Horsfall protein [15].

Table 6. Inhibition of *Tachypleus tridentatus* agglutinin (TTA) haemagglutination by glycoproteins. The minimal concentration of glycoproteins required for total haemagglutination inhibition induced by four haemagglutination units of TTA are shown. Values are expressed in μg protein per ml and in corresponding μM NeuAc.

Glycoprotein	μg *protein per ml*	*NeuAc* (μM)	*O/N-chains*
BSM	4	1.2	only O
Asialo-BSM	>1000		
OSM	1.3	0.8	800 O
Asialo-OSM	>1000		
Fetuin	>1000	>130	3 O + 3 N
Asialo-fetuin	>1000		
Collocalia mucin B	5	2.1	O ≫ N
Glycophorin A	36	38	15 O + 1 N
Galactoglycoprotein	10	8	43 O + 3 N
α_1-Acid-glycoprotein	>2000	>540	5 N
Zn-α_2-Glycoprotein	>2000	>220	3 N
Transferrin	>1000	>48	2 N
Fibronectin	>1000		only N
β_2-Glycoprotein I	>2000	>270	5 N
Tamm-Horsfall-Protein	50	6.4	only N
Antithrombin III	>2000	>250	4 N

Cell agglutination

TTA did not agglutinate the tumour cell lines studied in the present report, either native or sialidase-treated, while it was very active towards erythrocytes, even at a 125 times higher dilution. This is surprising because the presence of sialic acid on the tumour cell surface is well documented [4, 23]. In addition, we have determined sialic acid after treating the cells with sialidase (see the Results section). We therefore conclude that Eb, ESb and HT 29 cells lack the receptor structures complementary for TTA, which, however, are present on red blood cells and *O*-chain glycoproteins, but absent on *N*-chain glycoproteins. This phenomenon is to be noted although it remains unexplained.

Comparison with other lectins interacting with sialic acid

The importance of different functional groups of *N*-acetylneuraminic acid for binding the sialic acid-specific lectin from *Cepaea hortensis* (CHA I) was discussed recently [15, 24]. CHA I was compared with other sialic acid-specific lectins as *Limulin*, K99 fimbriae from E. coli, WGA, *Cancer antennarius*, *Sambucus nigra* and *Maackia amurensis* [15]. In this context it should be noted that the acetamido group and glyceryl side chain are important for both TTA and CHA I while the acetamido group is essential for WGA [20]. The axially orientated carboxyl of α-glycosidically bound *N*-acetylneuraminic acid is a prerequisite for CHA I [15] and not for TTA. Inhibition by 2,4,7,8,9-penta-*O*-acetyl-NeuAc is a common attribute of TTA and *Limulin* [25], but not of CHA I. While TTA interacts with *N*-acetylhexosamines and *N*-acetylmuramic acid, CHA I is more specific and reacts with sialic acid only.

The *Tachypleus* lectin adds to the list of sialic acid specific agglutinins that are well characterized and therefore valuable tools for various applications. In addition, precise knowledge about the regions of the sialic acid molecule that are important for binding to the lectin will allow the tailored synthesis of analogues suitable for photoaffinity-labelling and structural studies of the lectin's complementary binding site.

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