Purification and characterization of a sialic acid-specific lectin from *Tritrichomonas mobilensis*

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New sialic acid-specific lectin has been isolated from culture supernatant of the protozoan *Tritrichomonas mobilensis*. It was purified by adsorption by erythrocytes or bovine submaxillary gland mucin (BSM)–Sepharose affinity chromatography. The *T. mobilenis* lectin (TML) does not require bivalent cations for activity and agglutinates all human erythrocytes. The lectin forms multimeric complexes with molecular mass 556 and 491 kDa as determined by size-exclusion chromatography. SDS/PAGE under reducing conditions disclosed a large band of 343 kDa and three bands of 246, 265 and 286 kDa which, after denaturation with urea, were split into three subunits of 56, 61 and 66 kDa; under non-reducing conditions there were two bands, of 360 and 260 kDa. Western blots performed with anti-TML monoclonal antibodies revealed bands identical with those in the silver-stained gels, suggesting homogeneity of the BSM –Sepharose-purified lectin. TML is a highly glycosylated protein with approx. 8% of N-linked glycosides found by protein–*N*-glycanase F treatment; the total amount of saccharides revealed by chemical deglycosylation was 20%. Haemagglutination-inhibition studies documented exclusive specificity for sialic acid (NeuAc). Both (α2→6)- and (α2→3)-linked and free NeuAc were eight times more potent inhibitors than *N*-glycolylneuraminic acid. The lectin does not require *O*-acetyl groups on NeuAc for recognition. A spectrum of mono- and oligo-saccharides other than sialic acid had no inhibitory effect at 200 mM. Anti-TML monoclonal antibodies strongly inhibited the lectin activity. TML was stable at temperatures below 4 °C and lyophilized with 3% (w/w) glycerol.

INTRODUCTION

Lectins originating from plants and higher animals have been widely described, and, on the basis of their selective carbohydrate binding, used for detection, isolation and study of glycoconjugates. Only a few lectins produced by protozoa have been described. Best characterized is a glucose- and mannose-specific surface lectin of *Giardia lamblia* [1,2] which requires trypsin digestion and bivalent cations for activation [3]. *Entamoeba histolytica* produces a lectin with specificity for galactose [4,5] and *N*-acetylgalactosamine [6]. Haemagglutinins were found in cultures of some members of the *Tritrichomonas* genus [7,8]. Further studies with *Tritrichomonas mobilensis* indicated that the agglutinating activity of this colonic parasite of squirrel monkeys could be inhibited by sialic acid [9].

Almost 40 analogues of sialic acid [10] linked to proteins in a variety of sialylated glycoconjugates have been reported [11]. Among well-characterized sialic acid-binding lectins are those isolated from the haemolymph of sea crabs [12,13], from the Indian scorpion [14], frog eggs [15,16], slug [17] and snails [18,19]. A sialic acid-specific lectin has also been isolated from human placenta [20]. Of the known plant lectins, few bind to sialic acid [21–24]. Only the animal lectins from *Limax flavus* (LFA) and *Limulus polyphemus* (LPA) [25,26], and a few plant lectins, of wheat germ (WGA) [20], *Sambucus nigra* (SNA-I) [23] and *Maackia amurensis* (MAL-II) [21], have been found to be useful in histochemical localization [4,26–29] or lectin-affinity-chromatography separation of sialylated glycoproteins [21,25, 30,31]. With its specificity for sialic acid, excellent results in histochemistry [32] and simple production at high purity, the *T. mobilensis* lectin (TML) described in the present paper represents a useful reagent for studies of sialylated glycoconjugates.

EXPERIMENTAL

T. mobilensis culture

ATCC 40597 strain was cultured under previously described conditions [33]. Cultures, maintained for up to 3 weeks with daily adjustment to pH 7, were used immediately or stored at −70 °C. After centrifugation for 20 min at 2000 *g* and 4 °C, haemagglutination titre was verified and the material was concentrated 20-fold with a 10 kDa cut-off filter apparatus (Amicon, Beverly, MA, U.S.A.).

Purification of TML by erythrocyte adsorption

Human erythrocytes were washed three times in PBS and saturated with the lectin in culture supernatant at 4 °C until a compact erythrocyte agglutinate was formed. Agglutinated erythrocytes were washed twice with cold PBS, and the lectin was eluted from erythrocytes with an equal volume of 12.5 mM sialic acid in PBS.

Bovine submaxillary mucin (BSM)–Sepharose affinity purification of TML

Mucin–Sepharose was prepared with Sepharose 4B and BSM type I (Sigma, St. Louis, MO, U.S.A.) by the method of Ahmed and Gabius [20]. BSM–Sepharose was then fixed for 2 h in 10% (v/v) formalin to prevent loss of mucin during elution of the lectin, washed with 20 vol. of PBS and stored at 4 °C in PBS

Abbreviations used: TML, *Tritrichomonas mobilensis* lectin; NeuGc, *N*-glycoloylneuraminic acid; WGA, wheat germ agglutinin; SNA-I, *Sambucus nigra* agglutinin; MAL-II, *Maackia amurensis* leucoagglutinin; LPA, *Limulus polyphemus* agglutinin; LFA, *Limax flavus* agglutinin; BSM, bovine submaxillary gland mucin; NeuAc-1Me, NeuAc methyl ester; NeuAc-β2Me, NeuAc acid β-methyl glycoside; NeuAc-1Me-β2Me, NeuAc acid methyl ester β-methyl glycoside.

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containing 0.02 % NaN_3. All steps were performed at 4 °C. A 200 ml sample of 20-fold concentrated culture supernatant was passed through 50 ml of BSM–Sepharose in an Econo-Column (2.5 cm × 20 cm) (Bio-Rad Laboratories, Richmond, CA, U.S.A.) equilibrated with PBS. After the column had been washed with 15 vol. of PBS, TML was eluted with 30 ml of 20 mM sialic acid in PBS. The elution was monitored at 280 nm. TML-containing fractions, detected by haemagglutination assay, were pooled and dialysed against PBS. Alternatively the sialic acid was washed out with 3 vol. of PBS on a YM-10 or YM-100 (10 and 100 kDa cut off respectively) membrane filters. Membrane-retained material was recovered into PSB, and haemagglutination titre was verified. Protein was determined with the bicinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.).

De-*O*-acetylation of sialic acid in BSM

This was performed on a 2 ml BSM–Sepharose column which was washed with ice-cold 0.1 M NaOH and incubated on wet ice for 2 h [34] followed by a thorough wash with PBS. A duplicate 2 ml column containing untreated BSM–Sepharose was used as a control.

Amino acid and monosaccharide composition of TML

The purified TML was hydrolysed with 6 M HCl vapour at 100 °C for 1 h, and amino acids were separated on a Waters Pico-Tag amino acid analysis system. The monosaccharide composition was analysed by preparing alditol acetate derivatives of the glycosyl components [35]. Gas chromatography and combined gas chromatography/mass spectrometry analysis was performed using a Hewlett–Packard 5890 g.c. coupled to a 5970 m.s. detector. For alditol acetates of the neutral sugars, the column was a Supelco 2330, and for amino sugars a Supelco DB1-fused silica capillary column was used.

Haemagglutination assay

The haemagglutination assay was routinely performed with human erythrocytes group O Rh^+ (erythrocytes of other groups were equally suitable). The cells were washed three times with saline and adjusted to a concentration of 3 % (v/v). TML samples (25 μl) were serially diluted in PBS (pH 7.3) and mixed with 25 μl of 3 % erythrocyte suspension in 96-well Falcon U-shaped microtitre plates (Becton-Dickinson, Oxnard, CA, U.S.A.). Haemagglutination titre was evaluated as the highest dilution giving a homogeneous suspension of agglutinated erythrocytes in the well bottom after 1 h at room temperature. One unit of TML was defined as the smallest amount of lectin in the well causing agglutination.

Haemagglutination-inhibition assay

This was performed in microtitre plates as described above. To each well was added 25 μl of inhibitor serially diluted in PBS and 25 μl containing 2 units of TML. After 30 min incubation at room temperature, 25 μl of 3 % erythrocyte suspension was added. The haemagglutination inhibition was defined as the lowest concentration of inhibitor with no haemagglutination after 1 h at room temperature. Basic types of gangliosides, mucins and fetuin were applied with concentrations expressed in terms of sialic acid content. Mouse ascitic fluid from the B-VI-10C4 hybridoma, purified on a protein A membrane (Amicon), was also tested for haemagglutination inhibition, with mouse ascitic fluid (HyClone, Logan, UT, U.S.A.) as a control.

Sugar inhibitors

NeuAc, poly[(α2→8)NeuAc], α-L-fucose, D-galactose, *N*-acetyl-D-glucosamine, *NN'*-diacetylchitobiose, *N*-acetylgalactosamine, α-D-glucose, α-lactose, D-mannose, sucrose, mattose, xylose, arabinose, fructose, *N*-acetyl-β-mannosamine, *N*-acetylmuramic acid, D-galacturonic acid, mannose 1-phosphate, glucuronic acid, 3′-sialyl-lactose [NeuAc(α2→3)Gal(β1→4)Glc], 6′-sialyl-lactose [NeuAc(α2→6)Gal(β1→4)Glc], fetuin and gangliosides type II, III, IV and V were from Sigma. 2,3-Dehydro-2-deoxyNeuAc was obtained from Boehringer (Mannheim, Germany). *N*-Glycoloylneuraminic acid (NeuGc), NeuAc methyl ester (NeuAc-1Me), NeuAc acid β-methyl glycoside (NeuAc-β2Me), NeuAc acid methyl ester β-methyl glycoside (NeuAc-1Me-β2Me), ovine submaxillary mucin and BSM were from Accurate Chemical & Scientific Corp. (Westbury, NY, U.S.A.). NeuAc(α2→8) dimer was from EY Laboratories (San Mateo, CA, U.S.A.).

Anti-TML monoclonal antibodies

These were produced by immunization of Balb/c mice with three intraperitoneal inoculations of about 100 μg of BSM-affinity-purified TML [36] using the P3-X63 mouse myeloma cell line. Clone B-VI-10C4 was used in this study.

SDS/PAGE and Western blotting

Samples were electrophoresed by the procedure of Laemmli [37] with or without reduction by 2 % (v/v) 2-mercaptoethanol or 10 mM dithiothreitol. Some samples were electrophoresed under reducing conditions and with 4 % (w/v) urea to study fragmentation of the high-molecular-mass TML molecule. Polyacrylamide gels were silver stained or electroblotted to nitrocellulose filters in a wet blotting apparatus (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). Transferred proteins were immunochemically detected using B-VI-10C4 hybridoma culture supernatant and goat anti-(mouse IgG) peroxidase-labelled antibody (HyClone). Colour reaction was developed with 3-amino-9-ethylcarbazole (Biomeda, Foster City, CA, U.S.A.).

Deglycosylation of TML

The amount of N-linked saccharides in the TML molecule was evaluated by enzymic digestion with protein–*N*-glycanase F for 18 h at 37 °C using the Protein De-*N*-glycosylation Kit (Oxford Glycosystems, Abingdon, Oxfordshire, U.K.). Total lectin content of sugar was determined by chemical deglycosylation, incubating the lectin for 5 h at 0 °C with trifluoromethanesulphonic acid using the Glyco-Free Chemical Deglycosylation Kit (Oxford Glycosystems).

Molecular mass determination of native TML

Molecular mass was measured by molecular size-exclusion chromatography on Sephadex-300HR (Pharmacia, Uppsala, Sweden) packed in an Econo-Column (1.0 cm × 50 cm) (Bio-Rad Laboratories). The column was calibrated with a set of proteins of specified molecular mass (Sigma). When using SDS/PAGE, the molecular mass was calculated by comparing the TML mobility with that of known proteins in the MW-SDS-200 and P-8906 kits (Sigma).

Neuraminidase digestion of TML

In order to assess the possible role of lectin-associated terminal sialic acid in complexing of TML, 200 μg of TML in 1 ml of acetate buffer (pH 5.5) was mixed with 50 μg of *Clostridium perfringens* neuraminidase (Sigma) or with PBS only. After 18 h incubation at 37 °C, the samples were subjected to SDS/PAGE.

Stability and storage of TML

Purified lectin (100 μg/ml) in PBS was supplemented with 0.02 % NaN_3 or was filter-sterilized with a 0.45 μm-pore filter (Amicon). Matching samples were stored at room temperature, 4 °C and at −70 °C. Samples of TML were lyophilized in water, PBS or PBS plus glycerol (Gibco-BRL, Gaithersburg, MD, U.S.A.).

RESULTS

Purification of TML

The lectin was purified from the *T. mobilensis* culture supernatant. Centrifugation and concentration on a YM-10 Amicon filter did not lead to loss of haemagglutination activity. This preparation was used as the starting material for subsequent purification steps.

Human erythrocytes of different ABO blood groups adsorbed the lectin equally, and there was no difference in SDS/PAGE pattern of the eluted TML samples. Recovery of purified TML obtained by erythrocyte adsorption and elution was 80 % according to haemagglutination activity (Table 1). Traces of haemoglobin and other undetermined proteins were noted.

BSM–Sepharose affinity chromatography results in over 90 % recovery of TML haemagglutination activity (Table 1). Until the BSM–Sepharose column became saturated with lectin, no haemagglutination was detected in the flow-through (Figure 1). Adsorption as well as elution of the lectin was optimal at 4 °C. The final yield of TML from 4 litres of crude *T. mobilensis* culture was approx. 6 mg (Pierce protein assay). De-acetylation of *O*-acetyl-NeuAc in BSM–Sepharose by incubation with 0.1 M NaOH did not result in a changed yield of purified TML.

Characterization of TML

SDS/PAGE of TML under non-reducing conditions showed two high-molecular-mass bands of 360 and 260 kDa (Figure 2, lane a). In the presence of 2-mercaptoethanol or dithiothreitol, the lectin was detected as a large band of 343 kDa and three bands of 286, 265 and 246 kDa, representing multimeric complexes of TML subunits apparent as bands of 66, 61 and 56 kDa (Figure 2, lane b). Several weak bands identified besides those mentioned probably represented fragments of TML. Under reducing conditions and in the presence of urea, TML high-molecular-mass complexes were dissociated into subunits (Figure 2, lane c).

The YM-100 membrane filter flow-through of purified TML was twice repassed through the filter. After reconcentration on YM-10 filter, on SDS/PAGE, this material showed the subunits and bands of TML above 200 kDa (Figure 2, lane d), apparently formed by aggregation of the subunits into larger complexes. The presence of sialic acid (used for elution) before dialysis did not alter TML mobility during SDS/PAGE.

Western blotting with B-VI-10C4 anti-TML monoclonal antibody revealed bands identical with those obtained on silver-stained SDS/PAGE (Figure 3, lane a), indicating homogeneity of the TML purified by BSM–Sepharose affinity chromatography.

Table 1 Purification of sialic acid-specific lectin from *T. mobilensis*

Haemagglutination unit (HAU) is defined as the smallest amount of TML in the microtitre well causing haemagglutination.

	Fraction	Volume (ml)	Protein (mg)	Total activity (HAU)	Specific activity (HAU/mg)	Recovery (%)
Erythrocyte	Crude	400	1360	1.024×10^6	753	100
	Purified	10	8	0.82×10^6	102400	80
BSM	Crude	4000	13600	10.24×10^6	753	100
	Purified	60	6	9.83×10^6	1638404	96

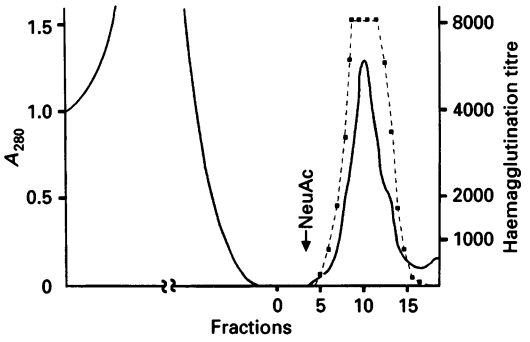

Figure 1 BSM–Sepharose affinity-chromatography purification of TML

Concentrated *T. mobilensis* culture supernatant (200 ml) was pumped at a flow rate of 100 ml/h through a 50 ml BSM–Sepharose column equilibrated with PBS, pH 7.3. After the column had been washed with PBS, the lectin was eluted with 30 ml of 20 mM NeuAc in PBS. ——, Absorbance profile; ■, haemagglutination profile.

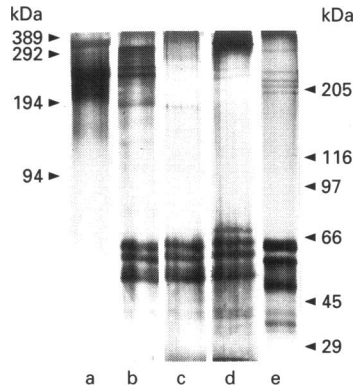

Figure 2 SDS/PAGE of BSM–Sepharose affinity-purified *T. mobilensis* lectin

Samples containing approx. 10 μg of TML run under non-reducing conditions (lane a), reduced with 2% 2-mercaptoethanol (lane b), 100 kDa cut-off membrane filter flow-through of purified TML concentrated on 10 kDa filter (lane c), reducing conditions with 4 M urea (lane d) and with trifluoromethanesulphonic acid-deglycosylated TML (lane e). Silver-stained proteins were compared with phosphorylase *b* cross-linked molecular-mass standards (left) and proteins of MW-SDS-200 kit (right) (Sigma).

Neuraminidase digestion of TML did not alter the band pattern obtained on SDS/PAGE compared with those of controls (TML/neuraminidase mixture before incubation and TML incu-

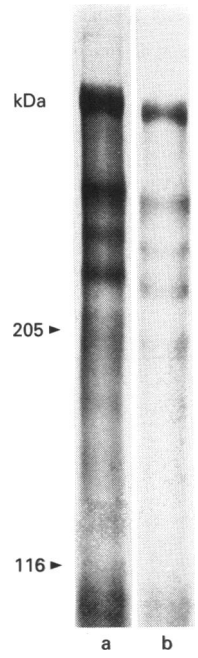

Figure 3 Western blot of TML stained with B-VI-10C4 anti-TML monoclonal antibody

Electroblotted SDS/PAGE (5% gels) run under reducing conditions are shown: TML incubated with PBS only (lane a) and after digestion by protein–*N*-glycanase F (lane b).

Table 2 TML haemagglutination inhibition with mono- and oligosaccharides

Inhibitor	Lowest effective concentration (mM)
6′-Sialyl-lactose	1.56
3′-Sialyl-lactose	3.125
NeuAc	3.125
NeuGc	25.0
NeuAc(α2→8) dimer	6.25
[NeuAc(α2→8)]$_n$	> 200.0*
2,3-Dehydro-2-deoxy-NeuAc	> 200.0
NeuAc-1Me	25.0
NeuAc-β2Me	> 100.0
NeuAc-1Me-β2Me	> 100.0

* Concentration denoted in terms of sialic acid monomers.

bated with PBS). This indicated that NeuAc was not present in the TML molecule, and that the multimeric complex-formation could not result from self-affinity aggregation of the lectin subunits.

Size-exclusion chromatography of *T. mobilensis* culture supernatant concentrated on a YM-100 membrane filter revealed haemagglutination activity in the first three fractions after the void volume (results not shown). The chromatography profile of BSM–Sepharose affinity-purified TML showed two peaks, at 556 and 491 kDa. Haemagglutination titre in separated fractions correlated with the size-exclusion chromatography profile of TML (results not shown).

Table 3 TML haemagglutination inhibition with sialylated glycoconjugates

Concentrations are denoted in terms of sialic acid contents.

Inhibitor	Lowest effective concentration (mM)
Ganglioside type II	1.56
Ganglioside type III	50.0
Ganglioside type IV	> 20.0
Ganglioside type V	> 20.0
Fetuin	0.0081 (39 μg/ml)
BSM	0.0006 (0.2 μg/ml)
Ovine submaxillary mucin	0.0026 (4.9 μg/ml)
Bovine submandibular gland mucin	0.0026 (4.9 μg/ml)

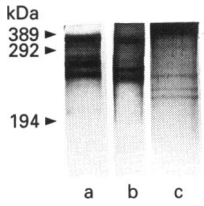

Figure 4 Molecular-mass changes in SDS/PAGE of TML after deglycosylation

SDS/PAGE (6% gels) run under reducing conditions are shown: lane a, untreated TML; lane b, TML digested by protein–*N*-glycanase F; lane c, TML deglycosylated with trifluoromethanesulphonic acid.

There were no differences in haemagglutination of TML when serial dilutions were prepared in saline, PBS and Hepes/saline with 0.1 M $CaCl_2$ or 0.01 M EDTA. These results show that the lectin did not require the presence of bivalent cations for activity. The results of haemagglutination inhibition by carbohydrates demonstrate exclusive specificity of the lectin for sialic acid and its derivates (Table 2). With other saccharides, no inhibition was obtained at concentrations up to 200 mM (results not shown). The oligosaccharide 6′-sialyl-lactose proved to be the most potent inhibitor followed closely by free NeuAc and 3′-sialyl-lactose. NeuAc(α2→8) dimer was one-half and NeuGc and NeuAc-1Me were one-eighth as potent as free NeuAc. In contrast, colominic acid {poly[NeuAc(α2→8)]} and the sialic acid analogues 2,3-dehydro-2-deoxy-NeuAc, NeuAc-β2Me and NeuAc-1Me-β2Me did not inhibit lectin activity. Among the glycoconjugates tested, BSM proved to be the most potent inhibitor of TML (Table 3). B-VI-10C4 hybridoma-purified ascites inhibited TML haemagglutination activity at a protein concentration of 0.0297 mg/ml compared with the control mouse ascites at a concentration of 1.2 mg/ml.

Deglycosylation of TML with peptide–*N*-glycanase F led to a decrease of approx. 8% in molecular mass as shown by SDS/PAGE (Figure 4). Chemical deglycosylation with trifluoromethanesulphonic acid resulted in an approx. 20% decrease in molecular mass of bands above 200 kDa (Figure 4), and splitting of TML into subunits was apparent (Figure 2, lane e). Western-blot analysis of deglycosylated proteins showed preserved anti-

Table 4 Amino acid composition of TML

Amino acid	Content (mol%)
Asp	0.37
Glu	0.15
Ser	2.67
Gly	2.90
His	4.94
Arg	0.00
Thr	7.57
Ala	0.00
Pro	6.48
Tyr	12.10
Val	7.53
Met	8.10
Cys	18.31
Ile	0.00
Leu	3.34
Phe	10.57
Lys	14.97

Table 5 Glycosyl composition of TML

Glycosyl residue	Percentage of carbohydrate mass
Fucose	26.8
Mannose	16.2
Rhamnose	15.6
Glucose	10.6
N-Acetylglucosamine	9.6
Galactose	8.9
Deoxyhexose	6.0
Xylose	5.3
Arabinose	1.0

genic activity after enzymic N-deglycosylation (Figure 3b), but no activity after the chemical removal of saccharides linked to the lectin.

The amino acid composition of TML (Table 4) was characterized by a high content of cysteine, tyrosine, lysine and phenylalanine. Analysis of the monosaccharide composition disclosed a high content of fucose (Table 5), representing more than one-quarter of all the carbohydrate.

Haemagglutination activity was unchanged after 0.45 μm-pore filter-sterilization, addition of 0.02% NaN_3 or storage at +4 °C and −70 °C for a period of 1 year. Storage at room temperature led to a 2–3-fold decrease in the original titre over the same period of time. Lyophilization of TML in water or PBS only led to formation of an insoluble protein sample in both water alone and water containing detergents (Triton X-100, CHAPS, dithiothreitol). Addition of glycerol to 3% (w/w) of the protein mass preserved solubility without affecting the activity of the lyophilized TML.

DISCUSSION

Only a modest amount of information exists on haemagglutinins produced by trichomonads [8]. *T. mobilensis* haemagglutinin was suggested to have lectin-like properties [7]. As implied by cell-adhesion properties of *T. mobilensis* [9], detailed haemagglutination-inhibition studies demonstrated exclusive specificity of TML for sialic acid and its conjugates.

The importance of an intact *N*-acetyl group on sialic acid was evident from the 8-fold lower affinity of TML for NeuGc than for NeuAc. BSM contains a high amount of 9-*O*-acetyl-NeuAc [38,39]. De-*O*-acetylation of BSM–sepharose [34] did not change the binding of TML, thus the 9-*O*-acetyl group on NeuAc does not play a role in TML binding. In contrast, some other sialic acid-specific lectins have shown high specificity for *O*-acetylated sialic acid residues [13,20]. This binding property of TML correlated with previous data showing strong agglutination of human and monkey erythrocytes [7], which contain only *N*-acetylsialic acid [19,20], and mouse erythrocytes (P. Babál, unpublished work), having predominantly *O*-acetylsialic acid on their surface [19,34]. As horse erythrocytes containing high amounts of 4-*O*-acetylated NeuAc [13,40] were strongly agglutinated by the lectin [7], 4-*O*-acetylation cannot be important either. Lowered inhibition of TML activity with NeuAc-1Me indicated the importance of a free carboxy group for lectin binding. The TML haemagglutination-inhibition potential of NeuAc(α2→8) dimer was twice as low as that of monomeric NeuAc and no inhibition was found with colominic acid. This indicated that the unlinked C8 hydroxy group was essential for binding to the lectin. Modifications of NeuAc at the C2 and/or C3 position, as was shown with the NeuAc-β2Me and 2,3-dehydro-2-deoxy-NeuAc respectively resulted in no inhibition of TML haemagglutination. A tentative model of the interaction between the TML-binding site and sialic acid was constructed on the basis of these facts (Figure 5).

Unlike lectins MAL-II, SNA-I and *Trichoxantes japonica* agglutinin, which can differentiate between (α2→3)- [21,31] and (α2→6)-[23,24,30] linked terminal NeuAc respectively, TML showed nearly equal affinity for both linkages of NeuAc. Although sharing with other sialic acid-specific lectins [14,17–19] broader specificity for free or terminal sialic acid in both linkages, TML haemagglutination activity could not be inhibited with other saccharides (even at 200 mM concentration), other than sialic acid and its conjugates.

Similarly to some lectins [12,18], TML is a high-molecular-mass glycoprotein. Its complex was found to be composed of three different subunits. These subunits are held together by intramolecular thiol groups which become fully accessible to 2-mercaptoethanol action only after denaturation with urea [41]. The multimeric form of TML could not be disrupted at low pH

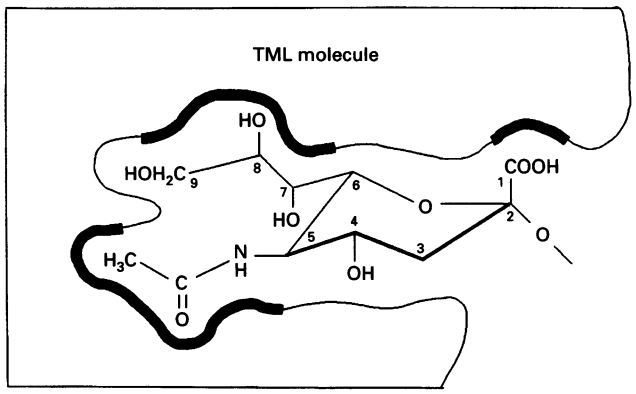

Figure 5 Schematic construction of TML-binding site interaction with its ligand NeuAc

(results not shown), unlike multimeric complexes of other lectins [17,42]. Small amounts of TML, however, existed dissociated in the form of free subunits which were filterable through the 100 kDa cut-off membrane filter; these subunits, after being concentrated, readily associated into multimers.

The high cysteine/cystine content detected on amino acid analysis indicated the involvement of disulphide bonds in the association of TML subunits.

Chemical deglycosylation by trifluoromethanesulphonic acid was reported to cleave over 80% of carbohydrates from glycoproteins while preserving their biological activity in most instances [43]. This method leads to dissociation of multimeric glycoproteins into subunits which can reassociate after the deglycosylation [43]. The same was observed with deglycosylated subunits of TML which partly reassociated into multimers analogous to those of untreated glycoprotein, but of approx. 20% lower molecular mass. Other lectins have been reported to be glycoproteins with similar high contents of saccharides [5,44]. After removal of N-linked carbohydrates, TML maintained its antigenicity for B-VI-10C4 monoclonal antibody. Loss of TML reactivity towards this antibody after chemical deglycosylation may indicate that the removed O-linked sugars were part of the antigenic site, or that the lectin molecule was denaturated thereby resulting in loss of antigenicity.

TML is a stable glycoprotein that is readily available at high purity and has exclusive specificity for sialic acid and its conjugates. Histochemical studies revealed that it shows membrane positivity on a number of normal and neoplastic cells [32] and offers complementary application to the use of SNA-I [27,28] and MAL-II [27] lectins. These characteristics should allow TML to be a useful tool in histochemical diagnostics and characterization and purification of sialylated glycoproteins. With the growing knowledge on the significance of cellular-adhesion-molecule recognition of carbohydrate structures as their ligands [45,46], TML with its high specificity could be of value in studies of adhesion molecules that have affinity for ligands with terminally linked sialic acid. The non-toxicity of high TML concentrations to different cell lines (results not shown) and the availability of antibodies to block its activity make TML a promising tool in the study of biological systems as well.

This work was supported by the South Alabama Medical Sciences Foundation. We are indebted to Dr Margarita Mora de Pindak for the generous supply of *T. mobilensis* cultures, Dr. Jane D. Funkhouser and Dr. Peter Bozner for reviewing the manuscript, Jeannie Napper for technical assistance, Dr. C. S. Sikes, Department of Biological Sciences, for amino acid analysis and Dr. Roberta Merkle, University of Georgia, Athens, U.S.A. for glycosyl composition analysis.

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Received 1 June 1993/18 October 1993; accepted 2 November 1993