

Purification and characterization of an extracellular agglutinin from *Tricophyton rubrum* with specificity towards sialic acid containing glycoconjugates

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An agglutinin, a monomeric glycoprotein with a molecular mass of about 6.5 kDa and containing 18% sugar has been purified to an apparent homogeneity from a 21 days old culture filtrate of an anthropophilic dermatophyte *Tricophyton rubrum*. It is a human blood group non-specific agglutinin which also agglutinates animal erythrocytes and Ehrlich ascites carcinoma and Sarcoma-180 cells. It is thermally stable and exhibits maximum activity at pH 8. Amino acid analysis shows a significant amount of glycine, with no cysteine. Glycoproteins inhibited the hemagglutination of the agglutinin, but not the simple sugars, including sialic acid. Fetuin is the most potent inhibitor among the glycoproteins tested. This inhibition gives a hint to binding with Gal β 1-3GalNAc or Gal β 1-4GlcNAc residue containing sialic acid at the terminal position with a α 2-6 or α 2-3 linkage.

Keywords: *Tricophyton rubrum*, dermatophyte, agglutinin, glycoproteins, hemagglutination

The omnipresence of lectins or agglutinins in all forms of living systems has been established by their capacity to agglutinate cells and precipitate glycosubstances in solution¹. Earlier, a sialic acid-specific lectin from the wood-rotting mushroom *Hericium erinaceum*² and sialic acid-binding agglutinins from a saprophyte *Aspergillus fumigatus*³, several isolates of dermatophytes⁴ and related keratinolytic fungi⁵ have been reported. The hemagglutinating activity of lectins isolated from *Pleurotus ostreatus*⁶ and *Psathyrella velutina*⁷ was inhibited by neuraminic acid. The lectin isolated from an edible fungus *Agrocybe cylindracea* showed weak affinity towards sialic acid and lactose, but had strong affinity towards trisaccharides containing Neu5Ac α 2-3Gal β 1-3-GlcNAc/GalNAc sequences⁸. The lectin purified from a mushroom *Polyporus squamosus* showed its specificity towards Neu5Ac α 2-6Gal- β 1-4-Glc/GlcNAc⁹, while an agglutinin isolated from a

phytopathogenic fungus *Macrophomina phaseolina* exhibited highest binding specificity towards Neu5Ac α 2-3Gal β 1-4GlcNAc¹⁰. Earlier, we reported the presence of an intracellular agglutinin (within the mycelia) and extracellular agglutinin (in the culture filtrate) from an anthropophilic dermatophyte, *Tricophyton rubrum* showing identical hemagglutination patterns against human and animal erythrocytes and specificity for complex carbohydrates¹¹. Here, we describe the purification and characterization of an extracellular agglutinin from 21 days culture filtrate from *T. rubrum*.

Materials and Methods

Materials

Gel electrophoresis chemicals, SDS, fetuin-Sepharose 4B, galactobiose, melibiose, lactose, raffinose, stachyose, N-acetylneuraminic acid, N-glycolylneuraminic acid, colominic acid, N-acetylneuraminyl- α 2-3/2-6-lactose (bovine colostrum and human milk), N-acetylglucosamine, simple D-sugars and their glycosides, fetuin, porcine thyroglobulin, bovine submaxillary mucin, porcine salivary mucin and α ₁-acid glycoprotein were purchased from Sigma, Chemical Co, St. Louis, Ms, U.S.A. Sephadex G-50 and G-200 and DEAE Sephadex A-50 were purchased from Amersham

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Abbreviations used: TBS, Tris-buffered saline; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TRA, *Tricophyton rubrum* agglutinin; Tag, D-tagatose; Tal, D-talose; EAC, Ehrlich ascites carcinoma; FPLC, fast protein liquid chromatography.

Biosciences. T-disaccharide (D-Gal β 1-3-D-GalNAc) was generously supplied by Prof N Roy of this department, edible bird's nest glycoprotein was provided by Prof J F G Vliegthart, Utrecht University, Utrecht, The Netherlands and human glycophorin was by Prof E Lisowska, Ludwik Hirsfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland. Methyl N-acetyl- α -D-galactosaminide was prepared as described¹². Ant (*Oecophylla smaragdina* Fabr.) egg glycoprotein was purified on immobilized jacalin^{13,14}. Human transferrin, ceruloplasmin, α_2 -macroglobulin and 9.5S glycoprotein were from Behringwerke AG, Marburg, Germany. All other reagents and chemicals used were of the highest analytical grade.

Fungal strain and growth condition

Tricophyton rubrum (MTCC 296) was obtained from the Institute of Microbial Technology, Chandigarh, India. The fungus was grown on Sabouraud's medium [1% bactopectone (Oxoid), 4% D-glucose, and 1.5% agar (Oxoid), pH 5.6, 30°C] under stationary condition as described earlier¹¹. A saline suspension (0.2 ml) of 15 days old *T. rubrum* mycelium was used as inoculum. After 21 days, the mycelia were separated by filtration, and the solid mass was washed several times with distilled water, then lyophilized. The culture filtrate and the mycelia were stored at -20°C till use.

Preparation of erythrocytes suspension

Red blood cells of human (A, B, AB and O groups), rat, rabbit, chicken, sheep and horse were washed in Tris-buffered saline (10 mM Tris-HCl, 150 mM NaCl, pH 8), and adjusted to 2% (v/v) suspensions.

Preparation of asialo derivatives of glycoproteins

Glycophorin (4 mg) and all glycoproteins (100 mg each) were desialylated by heating with 0.05 M H_2SO_4 at 80°C for 1 hr. The extent of desialylation was measured by the method of Aminoff¹⁵.

Purification of extracellular agglutinin

T. rubrum agglutinin was precipitated from the culture filtrate with $(\text{NH}_4)_2\text{SO}_4$ up to 80% saturation. The pellets collected after centrifugation (Sorvall RC 5B refrigerated centrifuge, 10,000 rpm for 30 min) were dissolved in a small volume of 100 mM Tris-HCl buffer (pH 8) and exhaustively dialyzed against the same buffer. The dialyzed material was

concentrated by ultrafiltration and applied to a Sephadex G-200 column (118 $\times$ 1.9 cm) previously equilibrated with 100 mM Tris-HCl buffer (pH 8) and eluted with the same buffer. The major fraction showing hemagglutinating activity was resolved into two subfractions on a Sephadex G-50 (119 $\times$ 1.9 cm) column using the same buffer as eluent. The fraction with strong hemagglutinating activity was then applied to fetuin-Sepharose affinity column (3 $\times$ 1.5 cm) at 4°C , previously equilibrated with 100 mM Tris-HCl buffer (pH 8). After washing the column with the same buffer, the bound protein was desorbed with 10 mM glycine-HCl buffer (pH 3.5). The eluted fractions were immediately neutralized with 0.1 M NaOH, dialyzed against 100 mM Tris buffer (pH 8), concentrated and stored at -20°C till use.

The homogeneity of the purified agglutinin was judged by electrophoresis in 12.5% polyacrylamide non-denaturing gel at pH 8.9¹⁶. The gel was stained with 0.05% Coomassie Brilliant blue R-250 in 7% acetic acid and destained in 7% acetic acid containing 5% methanol. The homogeneity of affinity-purified agglutinin was further tested by fast protein liquid chromatography (FPLC) using Superose 12 HR 10/30 column (Amersham Biosciences) with TBS (pH 8) as the eluent at a flow rate of 0.25 ml/min.

Assay procedures

Protein concentration of different fractions was determined by Lowry *et al*¹⁷, using bovine serum albumin as a standard. The hemagglutination and hemagglutination-inhibition assays were performed in 96 well U-bottomed microtiter plates (Flow laboratories, UK) in 10 mM TBS (pH 8), as described previously¹⁸. The hemagglutination titer was defined as the reciprocal of the highest dilution of lectin showing visible agglutination.

Molecular weight determination

The molecular weight of purified agglutinin was estimated by SDS-PAGE and gel filtration chromatography in an FPLC system. SDS-PAGE was carried out on 15% gel with an electrode buffer containing 0.1% SDS in Tris/glycine (pH 8.9), according to Laemmli¹⁹. Dissociation and reduction of agglutinin were performed by heating the sample at 100°C for 5 min in 0.1% SDS, with or without 2-mercaptoethanol (1%). Staining and destaining were performed as described above. Protein markers used were BSA (M_r 66,000), ovalbumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000),

carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,000), lactalbumin (14,200) and aprotinin (6,500).

The molecular weight of purified agglutinin was further confirmed by gel filtration chromatography in an FPLC system. Purified agglutinin (100 μ l) was applied to the column and eluted with 100 mM Tris-HCl buffer (pH 8) at a flow rate of 0.25 ml/min. The protein peaks were assayed by automated UV detection. Carbonic anhydrase (29,000), cytochrome *c* (12,400) and aprotinin (6,500) were used as protein markers for calibration in the FPLC system.

Carbohydrate and amino acid analysis

Total neutral carbohydrate content of the purified agglutinin was determined by the phenol-sulphuric acid method, using D-glucose as standard²⁰. The composition of individual sugars was quantitatively determined by GLC²¹ as alditol acetates after hydrolysis^{22,23} using inositol as the internal standard.

Amino acid analysis was performed by hydrolyzing agglutinin (0.5 mg) with 6 M HCl at 110°C for 24 hr, and analyzing the hydrolyzates by HPLC (Shimadzu) on a PICO TAG C₁₈ reverse phase column²⁴. Tryptophan was determined spectrophotometrically²⁵.

Effect of pH and temperature

Effect of pH on agglutinin (50 μ g/ml) was performed by incubating aliquots of agglutinin solutions for 1 hr with different buffers in pH range of 4-12 (citrate-phosphate, pH 4-6, phosphate pH 7, Tris-HCl buffer pH 8-10). The pH of solutions was raised further up to pH 12 by successive addition of saturated aqueous NaHCO₃. Before hemagglutinating activity was tested, the pH was adjusted to 7.0 by addition of 0.1 N HCl or 0.1 N NaOH. The thermal stability of purified agglutinin (50 μ g/ml) in Tris-HCl buffer (pH 8) was examined by hemagglutinating activity of the agglutinin after incubation in a water-bath from 10-100°C for 30 min. At each specified temperature, aliquots (100 μ l) were withdrawn, cooled and hemagglutination was performed as described before.

Ultraviolet difference spectroscopy

The intensities of UV difference spectra at 405 nm in a Hitachi model 100-60 of agglutinin (0.5 mg/ml in 100 mM Tris-HCl buffer, pH 8) vs. agglutinin-fetuin were recorded at 12°C as a function of increasing fetuin concentration²⁶ (5-50 μ l, 10 mg/ml). The association constant K_a of agglutinin-fetuin complex

was calculated²⁷ from the intercept on the ordinate of plot $[S]/\Delta A$ against $[S]$, where $[S]$ is the concentration of fetuin and ΔA is the difference of UV absorptions of agglutinin and agglutinin-fetuin complex.

Interaction with transformed cells

The interaction of agglutinin (50 μ g/ml) with Ehrlich ascites carcinoma (EAC) and Sarcoma (S-180) cells (1.2×10^6 cells/ml each) was performed by agglutination and the specificity of reaction was determined by agglutination-inhibition test, using the cells instead of erythrocytes²⁸.

Results

Partially purified *T. rubrum* agglutinin [0-80% (NH₄)₂SO₄ fraction] was purified successively by gel filtration chromatography on Sephadex G-200 and G-50 columns, followed by affinity chromatography on fetuin-Sepharose 4B (Fig. 1). Table 1 summarizes the purification scheme of agglutinin. The agglutinin was proved to be apparently homogeneous electrophoretically as it showed a single band in PAGE (Fig. 2a). The homogeneity was also confirmed by gel filtration on FPLC (Fig. 2b), as it gave a single symmetrical peak. The purified agglutinin (TRA) on SDS-PAGE with or without the presence of 2-mercaptoethanol revealed a single band (Fig. 3), corresponding to an apparent molecular mass of 6.5 kDa which is, similar to that obtained from FPLC gel filtration (6.6 kDa) (Fig. 4). Thus, TRA exists as a monomeric protein devoid of any

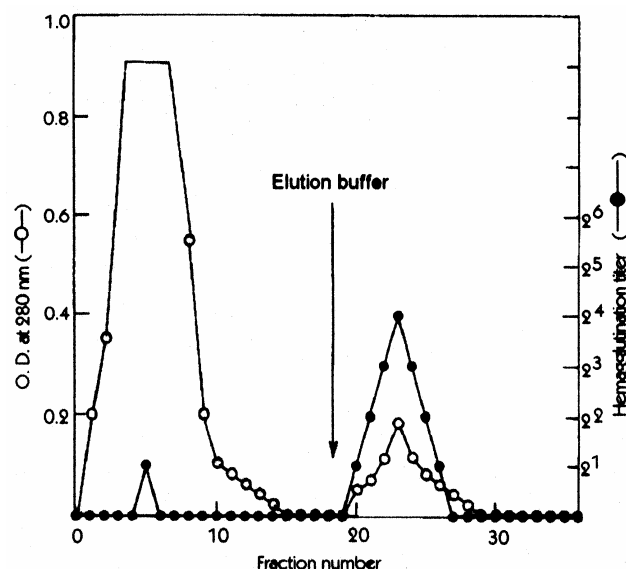


Fig. 1 —Affinity chromatography of *T. rubrum* agglutinin on a fetuin-Sepharose column

Table 1—Purification scheme of *Tricophyton rubrum* extracellular agglutinin

Fraction	Protein (mg/ml)	Titer ^a	Specific activity ^b	Purification fold
Crude culture filtrate	3.2	4	1.25	1
0-80% (NH ₄) ₂ SO ₄ precipitation	1.6	16	10	8
Sephadex G 200 eluent	1.4	32	22.8	18.24
Sephadex G 50 eluent	0.9	32	35.5	28.4
Fetuin-Sepharose adsorbed fraction	0.12	64	533.3	426.6

^aHemagglutination titer was determined with normal human B erythrocytes;

^bExpressed as titer per mg of protein per ml

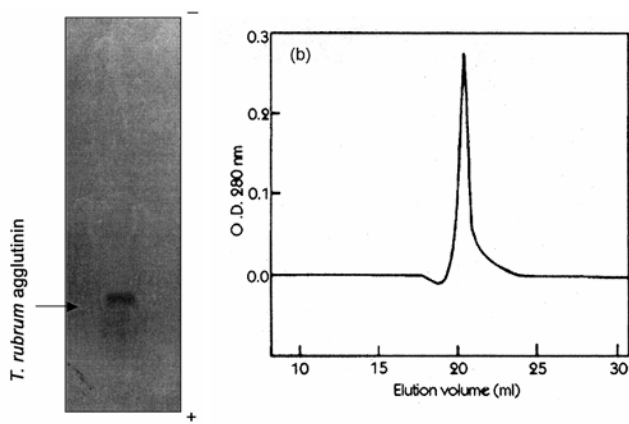


Fig. 2—(a) Polyacrylamide slab-gel electrophoresis of *T. rubrum* agglutinin [Electrophoresis was performed on 12.5% acrylamide gel (pH 8.9) with 100 µg of agglutinin at 35 mA for 3 hr. The migration of agglutinin was from the top]; (b) Gel filtration chromatography of *T. rubrum* agglutinin on Superose 12 HR 10/30 column in FPLC

disulphide linkage. TRA is active between pH 6 and 12; the maximum activity being at pH 8, and after which it declines with increasing pH. It is stable for 7 days at room temperature, and thermally stable up to 100°C. The hemagglutination titer remained unchanged between 20 and 90°C.

TRA agglutinated normal human erythrocytes of A, B, AB and O blood groups almost equally, thus indicating its blood group non-specificity. The minimum concentration of TRA to agglutinate normal human B erythrocytes was 0.78 µg/ml. It also agglutinated erythrocytes of rat, rabbit, chicken and sheep fairly well. Horse erythrocytes were weakly agglutinated.

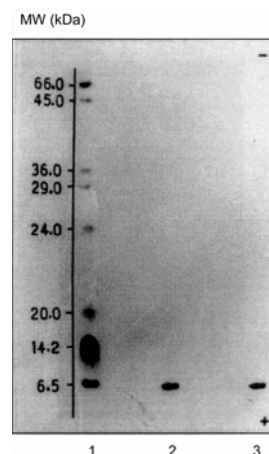


Fig. 3 —SDS-polyacrylamide slab-gel electrophoresis of *T. rubrum* agglutinin [Electrophoresis was performed on 15% acrylamide gel with 100 µg of TRA in presence of 0.1% SDS and 0.1% 2-mercaptoethanol at 35 mA for 3 hr. Lane 1, molecular weight marker proteins; lane 2, TRA without 2-mercaptoethanol; lane 3, TRA with mercaptoethanol. The migration of agglutinin was from the top]

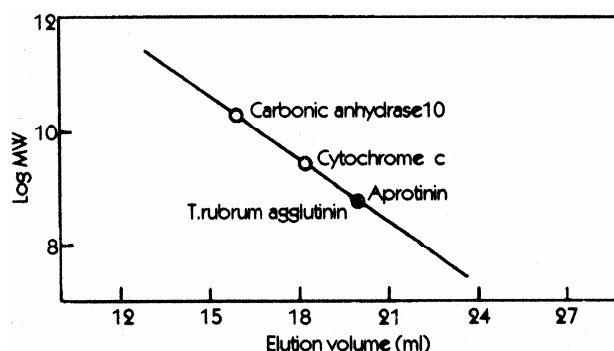


Fig. 4 —Determination of molecular weight of TRA by gel filtration on Superose 12 column in FPLC

The glycoprotein contained 18% neutral carbohydrate, a result in good agreement with the amount of neutral sugar (17.8%) and amino sugar (1.07%). The glycan part contained mannose (16.8%), arabinose (0.67%) and N-acetylglucosamine (1.07%) and a trace of glucose and galactose. Amino acid analysis revealed significant amounts of glycine, alanine, glutamic acid, aspartic acid, leucine and the absence of cysteine (Table 2).

The results of hemagglutination-inhibition assays of TRA with glycoproteins are shown on Table 3. Simple carbohydrates, including Neu5Ac, Neu5Gc were non-inhibitors. In addition, T-disaccharide, N-acetyllactosamine, N-acetylneuraminy- α 2-3/ α 2-6 lactose and colomimic acid also failed to inhibit

Table 2—Amino acid composition of *T. rubrum* agglutinin

Amino acid	g/100 g	mole/mole of agglutinin
Asp	8.5	4.15
Glu	10.0	4.42
Ser	6.5	4.02
Gly	19.5	16.9
Lys	4.1	1.82
Arg	4.0	1.48
His	2.4	1.0
Thr	4.2	2.29
Ala	13.1	9.56
Pro	5.0	2.82
Tyr	1.5	0.53
Val	6.6	3.66
Met	1.0	0.43
Cys	-	-
Leu	8.3	4.11
Ile	2.5	1.24
Phe	2.6	1.02
*Trp	2.5	0.8

*Tryptophan was determined separately by a spectrophotometric method²⁵

hemagglutination. However, several glycoproteins inhibited the hemagglutination; fetuin, in particular was the most potent inhibitor. Human glycophorin, α_2 -macroglobulin and α_1 -acid glycoprotein inhibited agglutination fairly well. Porcine thyroglobulin, ceruloplasmin, bird's nest glycoprotein, porcine salivary mucin glycoprotein, human transferrin and 9.5 S glycoprotein also inhibited the hemagglutination, but with lesser potency. In contrast, asialo derivatives of these glycoproteins did not inhibit hemagglutination reaction.

The association constant for TRA-fetuin interaction at 12°C was determined from the ordinate of Sctachard plot. The value of K_a for fetuin was $10.9 \times 10^5 \text{ M}^{-1}$, suggesting that the fetuin-TRA interaction was fairly strong.

TRA agglutinated EAC and S-180 cells almost equally well. The minimum concentrations of agglutinin required to agglutinate EAC and S-180 cells were 3.12 $\mu\text{g/ml}$ and 6.4 $\mu\text{g/ml}$, respectively. The respective values were 4 and 8 times higher than the value (0.78 $\mu\text{g/ml}$) for human erythrocytes. Agglutination of both EAC and S-180 cells was inhibited fairly by fetuin (1.25 $\mu\text{g/ml}$), porcine thyroglobulin (2.5 $\mu\text{g/ml}$) and porcine salivary mucin (2.5 $\mu\text{g/ml}$), suggesting that the interaction was carbohydrate-specific.

Table 3—Hemagglutination inhibition of *T. rubrum* agglutinin by various glycoproteins

[Tests were performed with normal human B-erythrocytes]

Glycoproteins	Minimum inhibitory concentration (mg/ml) ^a
α_1 -Acid glycoprotein	0.625
α_2 -Macroglobulin	0.625
Bird's nest glycoprotein	1.25
Ceruloplasmin	1.25
Fetuin	0.312
Glycophorin	0.625
Human transferrin	1.25
9.5S Glycoprotein	1.25
Porcine salivary mucin	1.25
Porcine thyroglobulin	1.25

^aRequired for complete inhibition of two hemagglutinating doses of TRA.

D-Glc, D-Man, D-Gal, D- and L-Fuc, D- and L-Ara, D-GlcNAc, D-GalNAc, D-GlcNH₂, D-GalNH₂, 2-deoxy-D-Gal, Me-1-thio-D-Gal, D-Tal, D-Tag, Me- α -D-Gal, Me- β -D-Gal, Me- α -D-Glc, Me- β -D-Glc, Me- α -D-Fuc, Me- β -D-Fuc, *o*- and *p*-NO₂-ph- α -D-Gal, *o*- and *p*-NO₂-ph- β -D-Gal, D-Gal- β -1-4-D-Gal, D-Gal- α -1-6-D-Glc, D-Gal- β -1-4-D-Glc, D-Gal- α -1-6-D-Glc- β -1-2-D-Fru, stachyose, Neu5Ac and Neu5Gc were non-inhibitors up to 200 mM concentration.

D-Gal- β -1-4-D-GlcNAc and D-Gal- β -1-3-D-GalNAc were non-inhibitors upto 100 mM.

Neu5Ac- α 2-6/2-3-D-Gal- β 1-4-D-Glc was non-inhibitor upto 50 mM.

Asialoglycophorin, asialoporcine thyroglobulin, asialoporcine salivary mucin, asialobird's nest glycoprotein, bovine submaxillary mucin, ant egg glycoprotein and colominic acid were also non-inhibitors up to 5 mg/ml.

Discussion

Trichophyton rubrum produced an intracellular agglutinin (within mycelia) and an extracellular agglutinin (in culture filtrate), which showed highest hemagglutinating activity at 15 days and 21 days, respectively¹¹. Similarly, the fungi *Kluyveromyces bulgaricus*²⁹ and *Conidiobolus lamprauges*³⁰ and *Neurospora sitophila*³¹ produced two agglutinins, one excreted in the culture medium and other present in the cell wall. The extracellular agglutinin may be due to the proteolytic digestion from the intracellular precursor. Such phenomenon is already evidenced for sexual agglutination factor in *Saccharomyces kluyveri*³².

A monomeric glycoprotein of molecular mass 6.5 kDa, TRA is a human blood group non-specific agglutinin, is most active at pH 8 and is thermally stable, similar to other fungal agglutinins, such as

those from *Saccharomyces cerevisiae*³³, *Agaricus blazei*³⁴, *Beauveria bassiana*³⁵ and *M. phaseolina*³⁶. Its thermostable nature may be due to high sugar content (18%), which could protect the protein moiety against heating action, as reported in phytopathogenic fungus *M. phaseolina*³⁶ and edible mushroom *Psilocybe barrerae*³⁷ and dermatophyte agglutinins⁴. TRA agglutinated EAC cells like *Ficus cunia*²⁷ and jack fruit²⁸ lectins. Besides, TRA could also have an antiproliferative effect against cancer cells, like jack fruit²⁸ lectin.

Hapten inhibition studies showed that no monosaccharides, including free Neu5Ac and Neu5Gc, and oligosaccharides, including Gal β 1-4GlcNAc and Gal β 1-3GalNAc, inhibited TRA-induced hemagglutination. Although neuraminic acid itself was non-inhibitory, several glycoproteins containing terminal neuraminic acid-linked oligosaccharide chains inhibited hemagglutination (Table 3), indicating the specificity of TRA towards the complex carbohydrate chains of glycoproteins. However, surprisingly Neu5Ac α 2-3/2-6Gal β 1-4Glc was non-inhibitory. The highest inhibitory potency of fetuin is suggested to be attributed to the glycoside cluster effect³⁸ generated by the three O-linked oligosaccharide chains of Neu5Ac α 2-3Gal β 1-3GalNAc α 1-O-Ser/Thr and three N-linked carbohydrate chains of Neu5Ac α 2-3/2-6Gal β 1-4GlcNAc β sequences to the protein core per molecule.

Although different sialooligosaccharide chains are present in glycophorin A³⁹, α_2 -macroglobulin⁴⁰ and α_1 -acid glycoprotein⁴¹ (Fig. 5), where the terminal sialic acid is either α 2-6 and α 2-3 linked to T-disaccharide (Gal β 1-3GalNAc) and N-acetylglucosamine (Gal β 1-4GlcNAc) units, yet they inhibited the hemagglutination with equal potency. Asialoglycoproteins, N-acetylglucosamine, and T-disaccharide have no inhibitory effect (Table 3). So, Neu5Ac is required in conjugation with the carbohydrate chain of glycoprotein for inhibitory activity. The non-inhibitory effect of neuraminyl lactose may allude to the importance of the acetamido group at C-2 of GlcNAc for binding, since neuraminylglucosamine unit present in the glycoprotein is responsible for binding.

It is surprising that human transferrin⁴² having analogous biantennary oligosaccharide structure to α_2 -macroglobulin (Fig. 5) showed a less inhibitory effect than the latter. However, ceruloplasmin⁴³

despite triantennary structure of same sugar-like chain (Fig. 5) of human transferrin showed equal inhibitory potency, indicating that one additional oligosaccharide chain did not enhance binding. It is interesting to note that porcine thyroglobulin⁴¹ (Fig. 5) with biantennary structure having one Neu5Ac α 2-6Gal β 1-4GlcNAc unit contributed equally in the binding (Table 3). The inhibitory potency of bird's nest glycoprotein⁴⁴ (Fig. 5), porcine salivary mucin glycoprotein⁴⁵ and 9.5S glycoprotein⁴⁶ is 4 times less than fetuin and could be referred to a single carbohydrate side chain consisting of Neu5Ac α 2-3Gal β 1-3GalNAc, α -O-glycosidically linked to Ser or Thr, which is common in fetuin and glycophorin. Non-inhibitory effect of bovine submaxillary mucin suggests that Neu5Ac α 2-6GalNAc α -sequence is not the strict requirement for the binding site of TRA. Colominic acid, a homopolymer of neuraminic acid linked in (2-8) fashion is not favourable for the binding site.

Our results clearly demonstrate that TRA does not bind free neuraminic acid, but recognizes terminal Neu5Ac having both α 2-6 and α 2-3 linkage with either Gal β 1-3GalNAc or Gal β 1-4GlcNAc units present in glycoproteins. Lectins isolated from the fruiting bodies of *A. cylindracea*⁸ and *P. squamosus*⁹ show weak or no binding for sialic acid, but have a strong affinity towards sialoglycoconjugates containing Neu5Ac α (2-3)Gal β (1-3)GlcNAc/GalNAc and Neu5Ac α (2-6)Gal β (1-4)Glc/GlcNAc sequences, respectively. A lectin from the filament of *Saraca indica* flower exhibits binding specificity towards Neu5Ac α (2-6)Gal β (1-4)Glc/GlcNAc⁴⁷. The extra cellular agglutinin from the phytopathogenic fungus *M. phaseolina* shows that among sialic acid-containing trisaccharides, Neu5Ac α 2-3Gal β 1-4GlcNAc possess the highest specificity¹⁰. However, the present study could not define the exact linkage specificity of neuraminyl oligosaccharides. Quantitative inhibition studies, using different neuraminyl oligosaccharides of specific linkage will be required to determine the preferred linkage specificity of Neu5Ac, α 2-3 or α 2-6, as well as the penultimate sugar of Neu5Ac α 2-3/2-6Gal β 1-3/4- for the highest binding.

Conclusion

It can be suggested from the foregoing results that TRA (having specificity for sialic acid-containing complex carbohydrate) may find as a valuable tool in

- 18 Ahmed H & Chatterjee B P (1986) in *Lectins, Biology, Biochemistry and Clinical Biochemistry* (Bøg-Hansen T C & Van Driessche E, eds.), Vol 5, pp. 125-133, W de Gruyter, Berlin
- 19 Laemmli U K (1970) *Nature* (London) 227, 680-685
- 20 Dubois M, Gilles K A, Hamilton J K, Rebers P A & Smith F (1956) *Anal Chem* 28, 350-356
- 21 Lindahl U (1970) *Biochem J* 116, 27-34
- 22 Sarkar N & Chatterjee B P (1983) *Carbohydr Res* 112, 113-121
- 23 Pal R, Ahmed H & Chatterjee B P (1987) *Biochem Arch* 3, 399-412
- 24 Heinrikson R L & Meredith S C (1984) *Anal Biochem* 136, 65-74
- 25 Goodwin T W & Morton R A (1946) *Biochem J* 40, 628-632
- 26 Matsumoto I, Jinbo A, Kitagaki H, Galovtchenko-Matsumoto A M & Seno N (1980) *J Biochem* (Tokyo) 88, 1093-1096
- 27 Ray S, Ahmed H, Basu S & Chatterjee B P (1992) *Carbohydr Res* 242, 247-263
- 28 Ahmed H, Chatterjee B P & Debnath A K (1988) *J Biosci* 13, 419-424
- 29 Al-Mahmood S, Colin S & Bonaly R (1991) *J Biol Chem* 266, 20882-20887
- 30 Ishikawa F, Oishi K & Aida K (1983) *Agric Biol Chem* 47, 587-592
- 31 Ishikawa F & Oishi K (1989) *Agric Biol Chem* 53, 1769-1776
- 32 Weinstock K & Ballous C E (1986) *J Biol Chem* 261, 16174-16179
- 33 Basu J, Kundu M, Mukherjee K & Chakrabarti P (1986) *Biochem Biophys Res Commun* 136, 596-602
- 34 Kawagishi H, Nomura A, Yumen T & Mizuno T (1988) *Carbohydr Res* 183, 150-154
- 35 Kossowska B, Lamer-Zarawska E, Olczak M & Katnik-Prastowska I (1999) *Comp Biochem Physiol B* 123, 23-31
- 36 Bhowal J (2002) *Studies on Agglutinin from Phytopathogenic Fungus Macrophomina phaseolina with respect to Biochemical and Physiological Properties*, Ph.D. thesis, Jadavpur University, Kolkata
- 37 Hernandez E, Ortiz R, Lopez F, Masso F, Montano LF, Martinage A & Zenteno E (1993) *Phytochemistry* 32, 1209-1211
- 38 Wu J H, Song S C, Chen Y Y, Tsai M C, Kabat E A & Wu A M (1998) *FEBS Lett* 427, 134-138
- 39 Lisowska E (2001) in *The Molecular Immunology of Complex Carbohydrates-2* Kluwer, (Wu A M, ed), pp.155-169, Academic Publishers
- 40 Hanaoka K, Pritchett T J, Takasaki S, Kochibe N, Sabesan S, Paulson J C & Kobata A (1989) *J Biol Chem* 264, 9842-9849
- 41 Heerze L D & Armstrong G D (1990) *Biochem Biophys Res Commun* 172, 1224-1229
- 42 Spik G, Bayard B, Fournet B, Strecker G, Bouquelet S & Montreuil J (1975) *FEBS Lett* 50, 296-299
- 43 Endo M, Suzuki K, Schmid K, Fournet B, Karamanos Y, Montreuil J, Dorland L, van Halveek H & Vliegenthart J F G (1982) *J Biol Chem* 257, 8755-8760
- 44 Wieruszkeski J M, Michalski J C, Montreuil J, Strecker G, Peter-Katalinic J P, Egge H, van Halbeek H, Mutsaers J H & Vliegenthart J F G (1987) *J Biol Chem* 262, 6650-6657
- 45 Herp A, Wu A M & Moschera J (1979) *Mol Cell Biochem* 23, 27-44
- 46 Chatterjee B P, Vaith P, Chatterjee S, Karduck D & Uhlenbruck G (1979) *Int J Biochem* 10, 321-327
- 47 Majumder M & Chatterjee B P (2001) *XVI Int Symp on Glycoconjugates*, The Hague, The Netherlands