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Specific Interaction of Human Tamm–Horsfall Glycoprotein with Leucoagglutinin, a Lectin from *Phaseolus vulgaris* (Red Kidney Bean)

By Franca SERAFINI-CESSI, Claudio FRANCESCHI and Simonetta SPERTI
Istituto di Patologia Generale, Via San Giacomo 14, 40126 *Bologna, Italy*

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Human Tamm–Horsfall glycoprotein inhibits lymphocyte transformation induced by leucoagglutinin and haemagglutinin from *Phaseolus vulgaris* (red kidney bean). The glycoprotein interacts with the two lectins, giving insoluble precipitates. The interaction with leucoagglutinin is highly specific, and the shape of the precipitin curve is that of an antigen–antibody reaction; precipitation is specifically inhibited by *N*-acetyl-D-galactosamine. Results are discussed, and it is suggested that inhibition of lymphocyte transformation is due to competition between human Tamm–Horsfall glycoprotein and carbohydrate receptors on lymphocytes for the two lectins. The interaction between human Tamm–Horsfall glycoprotein and *Phaseolus vulgaris* lectins has been used to develop a one-step procedure for the separation of the two lectins by affinity chromatography on (human Tamm–Horsfall-glycoprotein)–Sepharose.

Phytohaemagglutinin from *Phaseolus vulgaris* (red kidney bean) contains several lectin-like proteins. One of them, devoid of haemagglutinating power but with high lymphocyte-stimulating activity, has been purified to homogeneity and is named leucoagglutinin because of its strong leucoagglutinating activity (Allen *et al.*, 1969; Weber *et al.*, 1972). The haemagglutinating power of phytohaemagglutinin is concentrated in a second protein fraction, named haemagglutinin, which is also lymphocyte-stimulating. This fraction shows some heterogeneity on isoelectric focusing (Weber *et al.*, 1972), possibly because of varying proportions of the different subunits in the tetrameric structure of the molecule (Miller *et al.*, 1973).

Yachnin (1972, 1975) reported that some glycoproteins that inhibit lymphocyte transformation induced *in vitro* by phytohaemagglutinin were able to interact with haemagglutinin, but not with leucoagglutinin. Previously we found that human Tamm–Horsfall glycoprotein was a strong inhibitor of lymphocyte transformation induced by commercial phytohaemagglutinin, and it was suggested that inhibition depended on the interaction of the glycoprotein with the mitogens (Serafini-Cessi *et al.*, 1977).

Tamm–Horsfall glycoproteins have been isolated from the urine of several mammals and are produced by the distal tubules of the kidney (Tamm & Horsfall, 1952; Cornelius *et al.*, 1965; Marr *et al.*, 1971). Significant differences have been found in the carbohydrate composition of Tamm–Horsfall glycoproteins from different species (Dunstan *et al.*, 1974).

We chose to study the interaction of human Tamm–Horsfall glycoprotein with the lectins of *Phaseolus vulgaris* both to obtain more information on the glyco moiety of human Tamm–Horsfall glycoprotein and to investigate the carbohydrate specificity of leucoagglutinin. Experiments were designed to correlate the inhibitory power of human Tamm–Horsfall glycoprotein on lymphocyte transformation with its ability to interact with the two lectins. The carbohydrate specificity of leucoagglutinin was studied by the monosaccharide-inhibition test of the precipitin reaction with ^{14}C-labelled human Tamm–Horsfall glycoprotein.

In the present paper a rapid procedure is also described for the purification of leucoagglutinin from commercial phytohaemagglutinin by affinity chromatography on (human Tamm–Horsfall glycoprotein)–Sepharose. The purification of leucoagglutinin from other mitogenic proteins by a one-step procedure rests on the observation that the interaction between leucoagglutinin and human Tamm–Horsfall glycoprotein is specifically inhibited by *N*-acetyl-D-galactosamine.

Experimental

Materials

Leucoagglutinin and haemagglutinin were purified from Bacto-Phytohaemagglutinin P (Difco Laboratories, Detroit, MI, U.S.A.) by the chromatographic technique described by Allen *et al.* (1969). Human Tamm–Horsfall glycoprotein was purified from human urine by the technique of Tamm & Horsfall (1950, 1952). Desialylated glycoproteins were ob-

tained by mild acid hydrolysis or by neuraminidase treatment as previously described (Serafini-Cessi, 1977). Iodoacetamide-alkylated human Tamm–Horsfall glycoprotein was prepared as described by Fletcher *et al.* (1970). ^{14}C-labelled human Tamm–Horsfall glycoprotein was prepared by a procedure based on the method of Means & Feeney (1968), in which reductive alkylation of the amino groups of the protein was obtained by treatment with [^{14}C]-formaldehyde. The reaction was performed at 0°C; 10mg of glycoprotein was stirred with 1ml of 0.2M-borate buffer, pH9, containing 0.5mg of sodium borohydride. Five portions (10μCi) of aqueous [^{14}C]formaldehyde solution (14.1mCi/mmol) were added over a period of 30min. The solution was exhaustively dialysed against water, sonicated (3min) and filtered through a glass-fibre disc with a yield of soluble compound of about 50%. The specific radioactivity was 1800d.p.m./μg. Rabbit and hamster Tamm–Horsfall glycoproteins were a gift of Dr. R. D. Marshall, St. Mary's Hospital, London. Antiserum to human Tamm–Horsfall glycoprotein was raised in rabbits as described by Bloomfield *et al.* (1977). Pure immunoglobulin G from control and immunized rabbits was obtained as previously described (Bloomfield *et al.*, 1977). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Concanavalin A was obtained from Miles Laboratories, Elkhart, IN, U.S.A., and pokeweed mitogen from Gibco Bio-Cult Ltd., Hounslow, Middx., U.K. Other glycoproteins and monosaccharides were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. CNBr-activated Sepharose 4B was from Pharmacia, Uppsala, Sweden. Ampholines were supplied by LKB-Produkter A.B., Bromma, Sweden. The Radiochemical Centre, Amersham, Bucks., U.K., supplied [^{14}C]formaldehyde (14.1mCi/mmol), and [^{3}H]-thymidine (5Ci/mmol).

Methods

Culture of lymphocytes. Peripheral-blood human lymphocytes were isolated by the Ficoll/Hypaque technique (Böyum, 1968). Cells (1×10^5 viable lymphocytes) in 0.1ml of complete medium (Franceschi *et al.*, 1978) were distributed in quadruplicate in the wells of Microtest plates and stimulated by addition of mitogen in 0.1ml of complete medium. Human Tamm–Horsfall glycoprotein or other proteins, dissolved in 0.05ml of complete medium, were added at the start of the culture, just before the mitogen. The cultures were incubated in a humified atmosphere of CO_2/air (1:19) at 37°C for 66h, followed by a 6h pulse of 0.5μCi of [^{3}H]thymidine. The cells were harvested and the radioactivity measured as fully detailed elsewhere (Franceschi *et al.*, 1978).

Agglutination tests. Haemagglutination was tested as described by Falasca *et al.* (1979) and leucoagglutination by the method of Allan & Crumpton (1970).

Precipitation studies. Double diffusion in gel was done in Ouchterlony plates with 1% agarose containing 0.02M-phosphate buffer, pH7.0, 0.05M-NaCl and 0.001% thiomersal. All glycoproteins (1mg/ml) were dissolved in 0.1% sodium dodecyl sulphate and were incubated before being added to the plate for 1h at room temperature.

For the quantitative determination of the precipitin reaction, ^{14}C-labelled human Tamm–Horsfall glycoprotein in 0.02M-phosphate buffer, pH7.0, was mixed in glass tubes with lectins dissolved in the same buffer; the final volume was 1ml. The tubes, including a sample without lectins, were incubated for 1h at room temperature. At this time the mixtures were filtered on glass-fibre filters and washed twice with 1ml of phosphate buffer. The filters and portions of the filtrates were put in separate counting vials containing 5ml of methoxyethanol and 10ml of scintillation fluid {0.05% POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene and 0.4% PPO (2,5-diphenyloxazole) in toluene} and counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer. When the monosaccharide-inhibition test was performed, the monosaccharides were added just before ^{14}C-labelled human Tamm–Horsfall glycoprotein.

Affinity chromatography. Human Tamm–Horsfall glycoprotein was attached to CNBr-activated Sepharose 4B under conditions described by Sela *et al.* (1975). The protein was coupled at a concentration of 4–5mg/g of gel. The conjugated gel was equilibrated with 0.02M-sodium phosphate buffer, pH7.4, containing 0.15M-NaCl. In a typical fractionation, 50mg of commercial phytohaemagglutinin dissolved in 20ml of equlibrating buffer was loaded on a column (1cm×15cm). After extensive washing (80ml) with the same buffer, at a flow rate of 10ml/h, the column was eluted with 0.2M-*N*-acetyl-D-galactosamine dissolved in the buffer (40ml) and then with 0.05M-glycine/HCl buffer, pH3.0, containing 0.5M-NaCl. Chromatography was performed at room temperature. The A_{280} of the eluate was measured and the fractions of each peak were pooled and dialysed for 72h against 500vol. of 0.02M-sodium phosphate buffer, pH7.0, with three changes. The affinity gel was usable for several times and was stored at 4°C equilibrated with the buffer at pH7.4.

Polyacrylamide-gel disc electrophoresis and isoelectric focusing. Electrophoresis was performed at pH4.5 as described by Reisfeld *et al.* (1962) with an acrylamide concentration of 7%. Micro isoelectric focusing was carried out in polyacrylamide-gel columns (5mm×100mm) as described by Catsimpoolas (1968) with 5% (w/v) acrylamide and 2% ampholytes (pH3.5–10).

Table 1. *Effect of human Tamm–Horsfall glycoprotein on lymphocyte transformation*

Each mitogen was present at the concentration giving maximal stimulation: leucoagglutinin, 1 μg/ml; haemagglutinin, 5 μg/ml; concanavalin A, 2 μg/ml; and pokeweed mitogen, 1 μg/ml. Results are expressed as radioactivity (d.p.m.) of [^{3}H]thymidine incorporated per culture and are the means ± S.E.M. for four determinations.

Amount of human Tamm–Horsfall glycoprotein added (μg/ml)	Mitogen... Leucoagglutinin [^{3}H]Thymidine incorporated (d.p.m.)	Inhibition (%)	Haemagglutinin [^{3}H]Thymidine incorporated (d.p.m.)	Inhibition (%)	Concanavalin A [^{3}H]Thymidine incorporated (d.p.m.)	Inhibition (%)	Pokeweed mitogen [^{3}H]Thymidine incorporated (d.p.m.)	Inhibition (%)
None	74292 ± 4304	—	58227 ± 8237	—	71781 ± 2908	—	53082 ± 2342	—
0.5	34981 ± 1920	53	58976 ± 3811	0	71142 ± 3500	0	54004 ± 2914	0
1	14803 ± 1321	80	55203 ± 3921	5	72244 ± 411	0	53184 ± 2472	0
10	796 ± 465	99	672 ± 263	99	64042 ± 3446	11	48146 ± 3592	9
50	199 ± 83	100	695 ± 14	99	47885 ± 2496	33	43862 ± 691	14

Results

Inhibition of lymphocyte transformation

Lymphocyte transformation was induced by leucoagglutinin, haemagglutinin, concanavalin A and pokeweed mitogen. The dose–response inhibition by human Tamm–Horsfall glycoprotein is illustrated in Table 1. In the cultures stimulated by leucoagglutinin, the dose of 0.5 μg/ml gave an inhibition of about 50%, which increased with the concentration

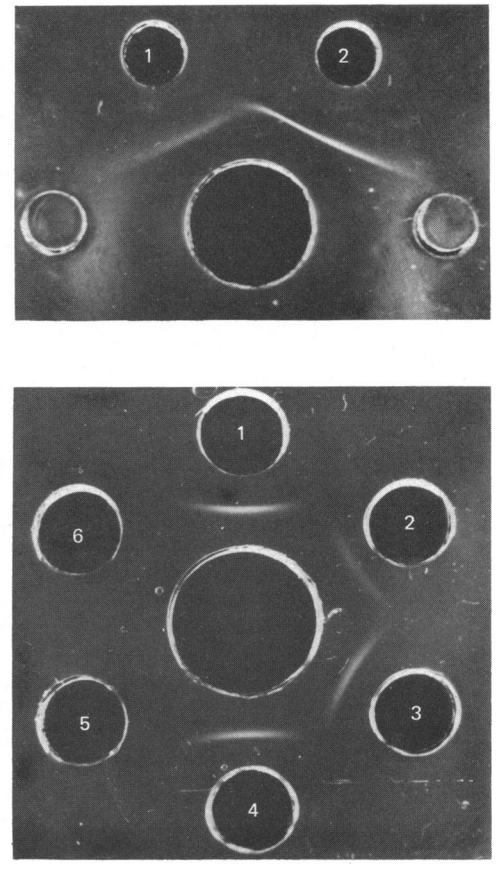

Fig. 1. *Ouchterlony double-diffusion analysis of human Tamm–Horsfall glycoprotein*

Diffusion was carried out for 48 h at room temperature in a moist atmosphere as described under 'Methods'. (*a*) Leucoagglutinin (well 1) and haemagglutinin (well 2) were diffused against human Tamm–Horsfall glycoprotein (centre well). (*b*) The centre well contained leucoagglutinin; wells 1 and 4, human Tamm–Horsfall glycoprotein, well 2, iodoacetamide-alkylated human Tamm–Horsfall glycoprotein; well 3, neuraminidase-treated human Tamm–Horsfall glycoprotein; well 6, acid-treated human Tamm–Horsfall glycoprotein; well 5 was empty.

of glycoprotein. In the set stimulated by haemagglutinin, inhibition occurred only at higher doses. Much lower, if any, inhibition was observed in the cultures stimulated by concanavalin A and pokeweed mitogen. A non-specific effect of the addition of protein was ruled out by running parallel experiments with human serum albumin and fetuin. No inhibition was observed.

Precipitation studies

The formation of insoluble complexes between lectins of *Phaseolus vulgaris* and human Tamm–Horsfall glycoprotein was tested by double diffusion in agarose gel. Precipitation bands were obtained when both leucoagglutinin and haemagglutinin were diffused against human Tamm–Horsfall glycoprotein (Fig. 1*a*). The spur pointing to the well containing leucoagglutinin indicates that besides binding sites common to the two lectins, other specific binding sites are present in the haemagglutinin fraction. This observation is in agreement with the proposed subunit composition of the two lectins. According to Weber *et al.* (1972) and Miller *et al.* (1973, 1975) the lectins from *Phaseolus vulgaris* are isomeric tetramers composed of varying proportions of two different subunits (L and R). Leucoagglutinin is formed by four identical subunits (L_4), whereas the haemagglutinin fraction is a mixture of the four tetramers R_1L_3, R_2L_2, R_1L_3 and R_4. The spur in Fig. 1(*a*) may be due to specific binding sites on the R-subunit of haemagglutinin.

The ability of the two lectins to form insoluble complexes with other glycoproteins was also investigated. Haemagglutinin precipitated with many glycoproteins, including rabbit and hamster Tamm–Horsfall glycoproteins. Leucoagglutinin gave precipitation bands with rabbit Tamm–Horsfall glycoprotein but failed to precipitate hamster Tamm–Horsfall glycoprotein and all other glycoproteins tested. The effect of different treatments of human Tamm–Horsfall glycoprotein on its ability to precipitate with leucoagglutinin is illustrated in Fig. 1(*b*). Desialylation by mild acid hydrolysis strongly decreased the formation of the precipitate, whereas desialylation with neuraminidase did not, although both procedures remove 95% of the sialic acid of Tamm–Horsfall glycoprotein (Serafini-Cessi, 1977). Alkylation with iodoacetamide only slightly decreased the formation of precipitate. Table 2 summarizes the data obtained by double-diffusion analysis.

The quantitative determination of the precipitin reaction was studied with a sample of ^{14}C-labelled human Tamm–Horsfall glycoprotein. Fig. 2 shows the percentage of labelled glycoprotein precipitated as a function of the concentration of the two lectins, and compares the results with those obtained with concanavalin A, with immunoglobulin G from normal rabbits, and with immunoglobulin G from rabbits immunized with human Tamm–Horsfall

Table 2. *Precipitation of leucoagglutinin and haemagglutinin by glycoproteins in Ouchterlony plates*

Glycoproteins (1 mg/ml) were dissolved in 0.1% sodium dodecyl sulphate and incubated for 1 h at room temperature before being added to the plates; leucoagglutinin and haemagglutinin were present at 1 mg/ml.

Glycoprotein	Leuco-agglutinin	Haem-agglutinin
Human Tamm–Horsfall	++	++
Neuraminidase-treated human Tamm–Horsfall	++	++
Acid-treated human Tamm–Horsfall	±	++
Iodoacetamide-alkylated human Tamm–Horsfall	+	++
Rabbit Tamm–Horsfall	++	++
Hamster Tamm–Horsfall	−	+
Fetuin	−	+
Neuraminidase-treated fetuin	−	++
Porcine thyroglobulin	−	+
α_1-Acid glycoprotein	−	++
Bovine submaxillary mucin	−	+
Acid-treated bovine submaxillary mucin	−	+
Porcine submaxillary mucin	−	+
Acid-treated porcine submaxillary mucin	−	+

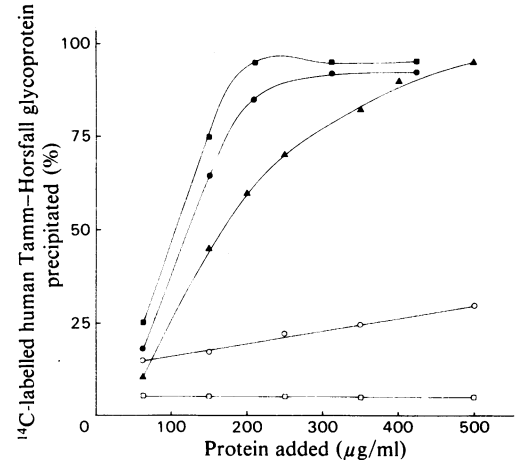

Fig. 2. *Precipitation of ^{14}C-labelled human Tamm–Horsfall glycoprotein*

The concentration of ^{14}C-labelled human Tamm–Horsfall glycoprotein in each reaction tube was 70 μg/ml. ■, Haemagglutinin; ●, leucoagglutinin; ▲, anti(human Tamm–Horsfall glycoprotein) immunoglobulin G; ○, concanavalin A; □, rabbit immunoglobulin G.

glycoprotein. Over 90% of the labelled glycoprotein was precipitated by specific antibodies and by leucoagglutin and haemagglutinin in the lectin-excess region, whereas only 30% was by a large amount of concanavalin A. There was no difference in the formation of precipitate when the pH of the medium was varied between 6.5 and 7.5. The influence of ionic strength was not investigated. The molarity of the phosphate buffer never exceeded 0.02 and NaCl was not added because of its non-specific ability to precipitate human Tamm–Horsfall glycoprotein. The precipitin curve obtained with a constant concentration of leucoagglutinin and increasing amounts of ^{14}C-labelled human Tamm–Horsfall glycoprotein is shown in Fig. 3. The profile is similar to that of an antigen–antibody reaction. The zone of equivalence (maximum precipitate) occurred when the molar ratio between leucoagglutinin and human Tamm–Horsfall glycoprotein reached the value of 4:3, calculated assuming a mol.wt. of 126000 for leucoagglutinin and of 80000 for human Tamm–Horsfall glycoprotein.

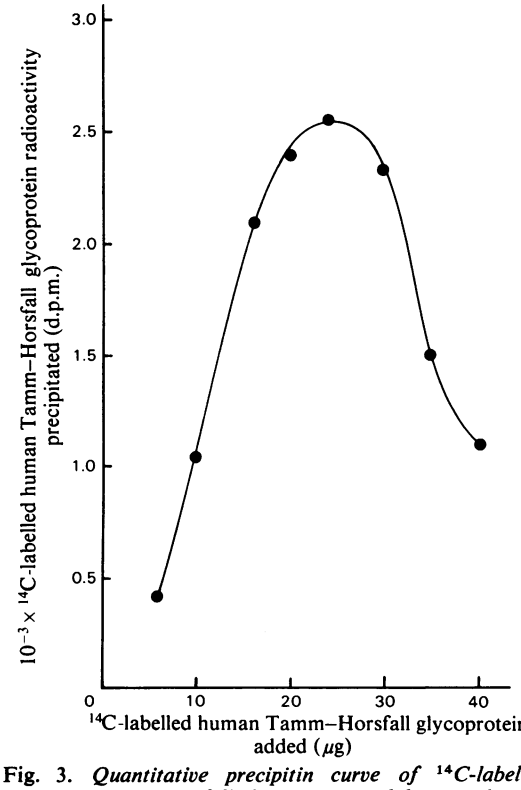

Fig. 3. *Quantitative precipitin curve of ^{14}C-labelled human Tamm–Horsfall glycoprotein with leucoagglutinin*
Each sample contained 48μg of leucoagglutinin in a final volume of 1 ml.

Table 3. *Effect of monosaccharides on the precipitin reaction between human Tamm–Horsfall glycoprotein and leucoagglutinin*
Each sample contained, in a final volume of 1 ml of 0.02M-phosphate buffer, pH 7.0, 210μg of leucoagglutinin and 70μg of ^{14}C-labelled human Tamm–Horsfall glycoprotein. Monosaccharides were added before the glycoprotein. Results are expressed as percentage of ^{14}C-labelled human Tamm–Horsfall glycoprotein precipitated in the absence of monosaccharides.

Monosaccharide (50mM)	^{14}C-labelled human Tamm–Horsfall glycoprotein precipitated (% of control)
N-Acetyl-D-galactosamine	25
N-Acetyl-D-glucosamine	97
N-Acetyl-D-mannosamine	95
D-Galactose	99
D-Glucose	98
D-Mannose	92
1-*O*-Methyl α-D-galactoside	93
1-*O*-Methyl β-D-galactoside	100
1-*O*-Methyl α-D-glucoside	90
1-*O*-Methyl β-D-glucoside	100
1-*O*-Methyl α-D-mannoside	100

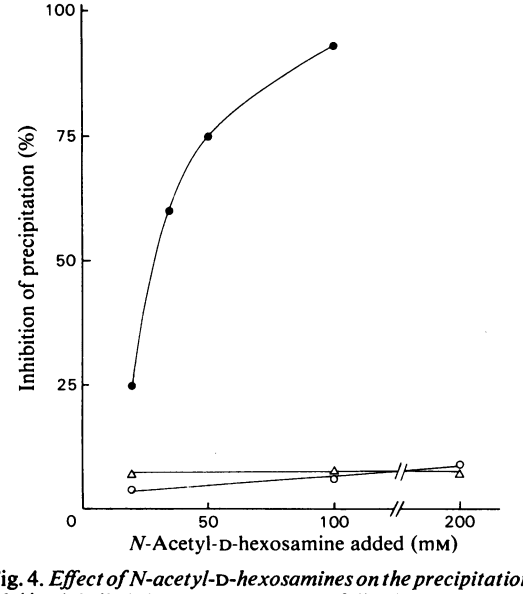

Fig. 4. *Effect of N-acetyl-D-hexosamines on the precipitation of ^{14}C-labelled human Tamm–Horsfall glycoprotein by leucoagglutinin*
^{14}C-labelled human Tamm–Horsfall glycoprotein (70μg), and leucoagglutinin (210μg) were present in a total volume of 1 ml of 0.02M-phosphate buffer, pH 7. ●, *N*-Acetyl-D-galactosamine; ○, *N*-acetyl-D-glucosamine; △, *N*-acetyl D-mannosamine.

Effect of monosaccharides on the precipitin reaction

The results of the addition of monosaccharides on the precipitin reaction between human Tamm–Horsfall glycoprotein and leucoagglutinin are reported in Table 3. Only *N*-acetyl-D-galactosamine proved inhibitory. The curves of inhibition as a function of the molarity of *N*-acetyl-D-aminohexoses are shown in Fig. 4. None of the monosaccharides tested was able to inhibit the precipitation between haemagglutinin and human Tamm–Horsfall glycoprotein.

Purification of leucoagglutinin by affinity chromatography on (human Tamm–Horsfall glycoprotein)–Sepharose

When commercial phytohaemagglutinin was applied to the column, 70% of the total protein (as A_{280}) passed unimpeded through the gel, 10% was eluted by *N*-acetyl-D-galactosamine and 15% by the glycine buffer, pH3. No agglutinating or mitogenic activity was present in the first peak. The second peak showed the lectin properties of pure leucoagglutinin, namely it was devoid of haemagglutinating activity and possessed powerful leucoagglutinating and mitogenic properties. The third peak showed all three activities as the haemagglutinin prepared as described by Allen *et al.* (1969)

On polyacrylamide-gel electrophoresis at pH4.5 (Fig. 5), the second peak gave a single band with the same mobility of leucoagglutinin prepared as described by Allen *et al.* (1969); the third peak moved as fast as the haemagglutinin fraction of Allen *et al.* (1969).

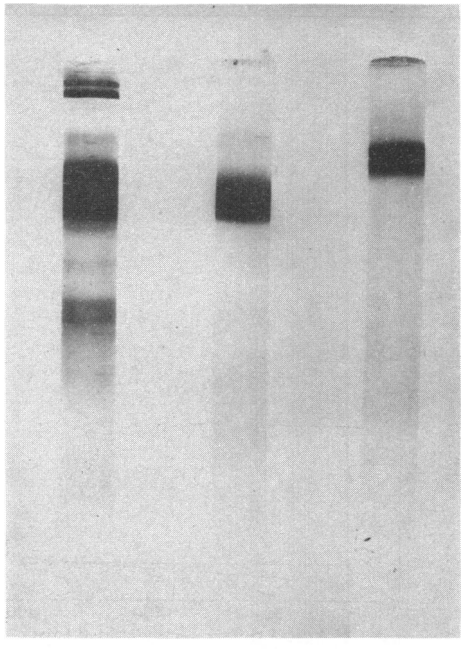

Fig. 5. *Polyacrylamide-gel electrophoresis at pH4.5 of commercial phytohaemagglutinin and of leucoagglutinin and haemagglutinin purified by affinity chromatography on (human Tamm–Horsfall glycoprotein)–Sepharose*

(*a*) Commercial phytohaemagglutinin, 75 μg/gel; (*b*) haemagglutinin, 50 μg/gel; (*c*) leucoagglutinin, 50 μg/gel. The gels were stained with Coomassie Blue.

Isoelectric focusing

Isoelectric focusing of leucoagglutinin and haemagglutinin purified by affinity chromatography was performed on polyacrylamide gels. Leucoagglutinin focused at pH5.1 as a single band precipitable by trichloroacetic acid. In contrast, haemagglutinin gave two distinct bands, which appeared as lines of precipitate also before fixation of the gels in trichloroacetic acid, indicating insolubility of the proteins at their isoelectric point (Catsimpoolas, 1968). The bands focused at pH6.1 and pH6.5 and appeared to be of similar intensity. These results indicate that the described single-step method of purification gives pure leucoagglutinin (L_4), but, as with other methods, does not separate the isomeric molecular species of the haemagglutinin fraction.

Discussion

The results reported show that: (i) human Tamm–Horsfall glycoprotein inhibits lymphocyte transformation induced by leucoagglutinin and haemagglutinin from *Phaseolus vulgaris*, but not by other mitogens such as concanavalin A and pokeweed mitogen; (ii) the glycoprotein reacts with the two lectins of *Phaseolus vulgaris* giving insoluble complexes; (iii) unlike haemagglutinin, leucoagglutinin does not form insoluble complexes with other glycoproteins; (iv) the precipitin reaction between human Tamm–Horsfall glycoprotein and leucoagglutinin is similar to a typical antigen–antibody reaction and is specifically inhibited by *N*-acetyl-D-galactosamine.

It is well known that cell agglutination and lymphocyte transformation induced by lectins depend on their binding to specific carbohydrate receptors on the cell surface. The receptor of erythrocytes for haemagglutinin from *Phaseolus vulgaris* has been extensively studied and the sequence galactose → *N*-acetylglucosamine → mannose has been suggested (Kornfeld & Kornfeld, 1970; Kornfeld *et al.*, 1971). This trisaccharide is present in several glycoproteins that bind haemagglutinins, such as fetuin (Spiro, 1964), α_1-acid glycoprotein (Schwarzmann *et al.*, 1974) and thyroglobulin (Toyoshima *et al.*, 1973). The fact that human Tamm–Horsfall glycoprotein

shares with these proteins the property of precipitating haemagglutinin may indicate the presence, within the molecule, of the same oligosaccharide structure.

The inability of leucoagglutinin to agglutinate erythrocytes and to precipitate serum proteins such as fetuin suggests that the lectin recognizes different carbohydrate receptors, but the carbohydrate specificity of leucoagglutinin has not yet been worked out. Its interaction with human Tamm–Horsfall glycoprotein suggests that specific binding sites are present on the human Tamm–Horsfall glycoprotein molecule; moreover, the shape of the precipitin curve indicates that, in this interaction, leucoagglutinin must be engaged with at least two valencies and that a bivalency of the glycoprotein must also be involved. According to Weber (1973), 2×10^6 receptors for leucoagglutinin are present on each lymphocyte, and the mitogenic activity of the lectin is related to its bivalency and attachment to multiple sites on the cell membrane. In our experiments, a 50% inhibition of lymphocyte transformation occurred when human Tamm–Horsfall glycoprotein was present in only 2-fold molar excess with respect to lymphocyte receptors. Assuming that inhibition occurs because of a competition between the glyco moiety of human Tamm–Horsfall glycoprotein and the carbohydrate receptors on lymphocytes for leucoagglutinin, this indicates a similar affinity of the lectin for the two ligands, and thus probably a similar or identical carbohydrate structure. The hypothesis is supported by the fact that *N*-acetyl-D-galactosamine, which inhibits lymphocyte transformation induced by commercial phytohaemagglutinin (Borberg *et al.*, 1968), also specifically inhibits the interaction between leucoagglutinin and human Tamm–Horsfall glycoprotein (see Table 3). The concentrations of *N*-acetyl-D-galactosamine required for the inhibition of the two reactions are of the same order of magnitude, but unusually high if compared with the concentration of specific monosaccharides that inhibits the precipitin reaction between other lectins and polysaccharides (Goldstein *et al.*, 1974; Baldo *et al.*, 1978). This may reflect the complexity of the carbohydrate specificity of leucoagglutinin (Hellström *et al.*, 1976), which probably requires a specific position of *N*-acetyl-D-galactosamine in the oligosaccharide structure. Moreover, the integrity of the protein backbone bearing the oligosaccharide-binding groups is also important. Thus, whereas desialylation by neuraminidase is ineffective, mild acid hydrolysis, which gives, besides desialylation, also a decrease in the molecular weight of human Tamm–Horsfall glycoprotein (Serafini-Cessi, 1977), inhibits its precipitation by leucoagglutinin.

The interaction between leucoagglutinin and human Tamm–Horsfall glycoprotein deserves further study, since its comprehension may be of great importance for the understanding of the mechanism triggering leucoagglutinin-induced lymphocyte transformation. Meanwhile, the specific binding properties of leucoagglutinin have been used to describe a one-step procedure for purification of the two lectins of *Phaseolus vulgaris* by affinity chromatography on (human Tamm–Horsfall glycoprotein)–Sepharose.

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