Isolation and Partial Characterization of the Major Sialoglycoprotein of Human T-Lymphoblastoid Cells of a MOLT-4B Cell Line

By MARIKO SAITO, SATOSHI TOYOSHIMA and TOSHIAKI OSAWA

Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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A sialoglycoprotein with an approx. mol.wt. of 95000 was isolated from human lymphoblastoid cells of a MOLT-4B cell line, which was of human T-lymphocyte origin, by ion-exchange chromatography, affinity chromatography on a column of wheat-germ agglutinin–Sepharose and preparative slab-gel electrophoresis. The localization of this glycoprotein on the cell surface was indicated by surface labelling by the periodate/ NaB^3H_4 and lactoperoxidase-catalysed iodination methods. Carbohydrate analyses of this glycoprotein revealed that its total carbohydrate content is 28% (w/w), and it contains fucose, galactose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid in molar proportions 1.0:4.0:3.7:3.5:1.2:2.5, suggesting that it has two types of sugar chain, i.e. sugar chains like those of serum glycoproteins and sugar chains of the type found in mucins. Actually, alkaline borohydride treatment of this glycoprotein yielded tri- and tetra-saccharide, the latter containing 1 molecule of fucose in addition to each molecule of galactose, *N*-acetylgalactosamine and sialic acid. This glycoprotein bound to *Ricinus communis* agglutinin and concanavalin A as well as to wheat-germ agglutinin.

Surface glycoproteins of mammalian cells are presumed to take part in many important biological processes, for example cellular interactions and malignant cell transformation (Cook & Stoddart, 1973). However, mainly because of their presence only in low concentrations and the limited availability of cells, only a few cell-surface glycoproteins other than the major sialoglycoprotein of human erythrocyte membranes have been chemically studied in detail. As reported in a previous paper (Saito *et al.*, 1977), we have isolated two types of glycopeptide, one of the type found in mucins (*O*-glycosidically linked glycopeptide) and one of the type found in serum glycoproteins (*N*-glycosidically linked glycopeptide), from lectin-stimulated human peripheral blood lymphocytes. These glycopeptides had similar carbohydrate composition to the two types of glycopeptide isolated from major sialoglycoprotein of human erythrocyte membranes.

In the present paper, we describe the isolation of a major sialoglycoprotein containing two types of sugar chain, *O*-glycosidically linked and *N*-glycosidically linked, from the human lymphoblastoid cells of a MOLT-4B cell line, which has been established and classified as T-lymphocytes by Minowada *et al.* (1972).

Abbreviation used: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

Materials and Methods

Human lymphoid cell lines

The MOLT-4B cell line was kindly given by Dr. Y. Hinuma, Kumamoto University Medical School, Kumamoto, Japan. The cells were grown in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY, U.S.A.) supplemented with 10% (v/v) heat-inactivated (56°C, 30 min) foetal calf serum (Grand Island Biological Co.) containing 60 µg of kanamycin (Meiji Seika Co., Tokyo, Japan)/ml. They were harvested in the exponential phase, washed twice with 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.5, and kept at –20°C.

Preparation of the plasma-membrane fraction from MOLT-4B cells

The plasma-membrane fraction from MOLT-4B cells was prepared by the method described by Kornfeld & Siemers (1974). The protein yield was 300 µg from 10^9 cells.

Labelling of cells

(a) The labelling of sialic acid on the cell surface was performed by the method of Gahmberg & Andersson (1977). Briefly, 10^8 cells were incubated in

1 ml of 0.01 M-sodium phosphate buffer/0.15 M-NaCl, pH 7.2, containing 0.001 M-sodium periodate and 2 mM-phenylmethanesulphonyl fluoride (Sigma Chemical Co., St. Louis, MO, U.S.A.) for 10 min at 0°C. Then glycerol equimolar to sodium periodate was added, and the cells were washed twice with 0.01 M-sodium phosphate buffer/0.15 M-NaCl, pH 7.2, by centrifugation, and reduced with 0.4 mCi of NaB^3H_4 (specific radioactivity 276 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) for 30 min at room temperature in 1 ml of 0.01 M-sodium phosphate buffer/0.15 M-NaCl, pH 7.2, containing 2 mM-phenylmethanesulphonyl fluoride. The cells were then washed four times with 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.5. It is assumed that cellular sialic acid is specifically labelled by this method. Actually, most radioactivity (70–80%) was recovered in 5-acetamido-3,5-dideoxy-L-arabino-2-heptulonic acid when it was determined by the method of Gahmberg & Andersson (1977).

(b) ^{125}I -labelling of the cell surface was performed by the method of Hubbard & Cohn (1972).

Lectin affinity columns

Wheat-germ agglutinin-Sepharose 6MB was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Ricinus communis agglutinin-Sepharose 4B and concanavalin A-Sepharose 4B affinity adsorbents were prepared by the method of Matsumoto & Osawa (1972). Ricinus communis agglutinin was purified by the method described by Tomita *et al.* (1972). Concanavalin A was purified from jack-bean meal (Sigma) by the method of Agrawal & Goldstein (1967).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Samples to be analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis were solubilized in 0.06 M-Tris/HCl buffer, pH 6.8, containing 2% sodium dodecyl sulphate, 5% 2-mercaptoethanol, 10% glycerol and 0.00015% Bromophenol Blue for 5 min at 100°C. The discontinuous sodium dodecyl sulphate/polyacrylamide-gel electrophoresis system was used as described by Laemmli (1970). The gel was stained with Coomassie Brilliant Blue for proteins and with periodate/Schiff reagent for carbohydrates. The location of radioactive components was determined by measuring the radioactivity after incubation of 1 mm slices of the gel in 0.5 ml of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, IL, U.S.A.) at 65°C for 2 h. To determine molecular weights of the components, the following proteins were used as standards (mol.wts. shown in parentheses): human γ -globulin (160 000), bovine serum albumin (68 000),

ovalbumin (45 000), α -chymotrypsinogen A (25 000). These standard proteins were purchased from Schwarz-Mann Co. (New York, NY, U.S.A.). For radioactive proteins, they were mixed with unlabelled standard proteins before electrophoresis.

Isolation of major sialoglycoprotein from MOLT-4B cells

Packed frozen cells (20 g) were thawed at 37°C, washed twice with 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.5, and suspended in 120 ml of 10 mM-Tris/HCl buffer, pH 7.5, containing 0.2% Triton X-100 and 2 mM-phenylmethanesulphonyl fluoride. After homogenization briefly with a glass Potter-Elvehjem homogenizer, the homogenate was incubated for 4 h at 15°C with stirring and centrifuged at 12 000 g for 30 min. The supernatant was dialysed against 10 mM-sodium phosphate buffer, pH 6.0, and freeze-dried. The material was then solubilized with 0.02 M-sodium phosphate buffer/0.05 M-NaCl, pH 6.0, containing 0.2% Triton X-100 and 1 mM-EDTA and applied to a DEAE-Sephadex A-50 column (2.5 cm $\times$ 20 cm) equilibrated with the same buffer. Stepwise elution was then carried out with the same buffer containing 0.3 M-, 0.6 M- and 1.0 M-NaCl. The fractions indicated in Fig. 1 by a double-headed arrow were pooled, dialysed against water and freeze-dried. The freeze-dried fraction was then solubilized with 5 ml of 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.5, containing 0.2% Triton X-100 and applied to a wheat-germ agglutinin-Sepharose-6MB column (1 cm $\times$ 9 cm). The proteins, which were not bound to the column, were eluted with 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.5, containing 0.2% Triton X-100 before the elution of bound glycoproteins with the same eluent containing 0.2 M-N-acetyl-D-glucosamine (Fig. 2). The glycoprotein specifically eluted from the wheat-germ agglutinin affinity column was dialysed against 50% (v/v) ethanol, and then freeze-dried. This material was subjected to sodium dodecyl sulphate/polyacrylamide slab-gel electrophoresis in 7.5% polyacrylamide gel. The gel was stained with Coomassie Brilliant Blue for protein and periodate/Schiff reagent for carbohydrate (Fig. 3). The section of the unstained gel corresponding to the major positive band with the periodate/Schiff reagent was cut out and eluted with 0.05 M-Tris/HCl buffer, pH 7.5, containing 0.1% sodium dodecyl sulphate and 2 mM-phenylmethanesulphonyl fluoride at 37°C overnight. The eluate was dialysed overnight against water and freeze-dried.

Chemical determinations

Protein was determined by the method of Lowry *et al.* (1951). The N-terminal amino acid was determined with 20 μg of the purified dansylated

Fig. 1. DEAE-Sephadex A-50 column chromatography of the lysates of MOLT-4B cells

Experimental details are described in the text. Fractions (4ml) were collected at a flow rate of 5ml/h at 4°C. Fractions were analysed for protein (○, A_{750}) and sialic acids (●, A_{630}), and pooled as indicated by a double-headed arrow.

Fig. 2. Affinity chromatography of the fraction obtained from DEAE-Sephadex column chromatography on a wheat-germ agglutinin-Sepharose 6MB column. Experimental details are described in the text. Fractions (2ml) were collected at a flow rate of 5ml/h at 4°C and analysed for sialic acids.

glycoprotein by the method described by Kinoshita *et al.* (1974). Hexose was determined by the phenol/sulphuric acid method of Dubois *et al.* (1956) and sialic acid by the method of Jourdian *et al.* (1971). The carbohydrate composition of glycoproteins was determined by the modified method of Takasaki & Kobata (1974) as described previously (Saito *et al.*, 1977). However, in this case, before separation of each sugar by high-voltage paper electrophoresis, the constituent sugars were separated into three groups, amino sugars, hexoses and pentoses, by paper chromatography by the method of Conrad *et al.* (1973). For this assay, the glycoprotein was hydrolysed in 2M-HCl for 4h at 100°C for determination of neutral sugars, and in 4M-HCl for 8h at 100°C for amino sugars, and in 0.05M-HCl for 10min at 100°C for sialic acids.

Alkaline borohydride treatment

The major glycoprotein containing 5–10 µg of the protein moiety was dissolved in 100 µl of 0.05M-

NaOH and mixed with 20 µl of NaB^3H_4 . The mixture was incubated for 48h at room temperature under N_2 in the dark, and then excess borohydride was destroyed by adjusting the pH to 6.0 with acetic acid. The acidic mixture was evaporated to dryness *in vacuo* and the residue was further evaporated *in vacuo* several times with water.

Results

Isolation of major glycoprotein of MOLT-4B cell surface

MOLT-4B plasma membranes were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as described by Laemmli (1970) in 7.5% polyacrylamide gels (Fig. 4). Although many bands were detectable with Coomassie Brilliant Blue, a strongly positive band was seen with periodic acid/Schiff reagent, and we called it the major glycoprotein of the MOLT-4B cell surface. The apparent molecular weight of the major glycoprotein estimated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was 95000. This band was not strongly stained with Coomassie Brilliant Blue.

Because of the low yield of the plasma-membrane fraction (the recovery of ^3H -labelled sialic acid from the cell labelled by the periodate/ NaB^3H_4 method

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the fraction specifically eluted from a wheat-germ agglutinin-Sepharose 6MB column

Experimental details are described in the text. (a) Coomassie Brilliant Blue staining; (b) periodate/Schiff staining. The glycoprotein eluted from the gel was re-electrophoresed and stained with Coomassie Brilliant Blue (c) or periodate/Schiff reagent (d).

was less than 10%), the purification of the major glycoprotein was attempted directly from intact cells by the method described in the Materials and Methods section. The yield of the purified glycoprotein was 300 µg of protein/20g of packed frozen cells or 18% of the total content of the glycoprotein estimated from the sialic acid content of the cells. The glycoprotein thus obtained appeared to be located on the plasma membrane and constitutes a major glycoprotein of the plasma membrane on the basis of the following observations.

(1) The glycoprotein was electrophoretically homogeneous, as revealed by staining with both Coomassie Brilliant Blue and periodate/Schiff reagent (Figs. 3c and 3d) and had the same apparent molecular weight (sodium dodecyl sulphate/polyacrylamide-gel electrophoresis) as that of the major glycoprotein of the isolated plasma-membrane fraction shown in Fig. 4(b).

(2) In general, membrane glycoproteins that are

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of plasma membranes of MOLT-4B cells (a) Coomassie Brilliant Blue staining; (b) periodate/Schiff staining. IgG, BSA and OA refer to positions of standard human immunoglobulin G, bovine serum albumin and ovalbumin respectively.

positive on periodate/Schiff staining, contain sialic acids and can be labelled by the periodate/ NaB^3H_4 method, which is known to specifically label sialic acids (Gahmberg & Andersson, 1977). When the MOLT-4B cells labelled by this method were solubilized and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the most strongly labelled band was seen at the position of an apparent mol.wt. of 95000 (Fig. 5). This ^3H -labelled major glycoprotein was found to be adsorbed on a wheat-germ agglutinin-Sepharose column by the following experiments. When MOLT-4B cells were labelled by the periodate/ NaB^3H_4 method, solubilized in 0.2% Triton X-100 containing 0.15M-NaCl and 2mM-phenylmethanesulphonyl fluoride and subjected to wheat-germ agglutinin-Sepharose 6MB affinity column chromatography, the adsorbed fraction showed a radioactive major glycoprotein band of apparent mol.wt. 95000 on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 6), but the fraction that was not adsorbed on the wheat-germ agglutinin column revealed no significant radioactive bands, except the one near the tracking dye used (Fig. 6). As we isolated the major glycoprotein by a similar method,

Fig. 5. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of solubilized MOLT-4B cells labelled with periodate/ NaB^3H_4 .

Experimental details are described in the text. The estimated mol.wt. of the protein peak is indicated. The abbreviations are as for Fig. 4.

the isolated glycoprotein must correspond to the major glycoprotein of MOLT-4B plasma membranes.

Furthermore, the major glycoprotein of the plasma membranes could also be adsorbed on the wheat-germ agglutinin-Sepharose column. That is, when the plasma membranes of cells labelled by the periodate/ NaB^3H_4 method were solubilized in 0.2% Triton X-100 containing 0.15M-NaCl and 2mM-phenylmethanesulphonyl fluoride and subjected to wheat-germ agglutinin-Sepharose column chromatography, the major glycoprotein having an approx. mol.wt. of 95000 was recovered in the wheat-germ agglutinin column adsorbed fraction, as shown in Fig. 6.

(3) When intact MOLT-4B cells were labelled with ^{125}I by lactoperoxidase-catalysed iodination, solubilized in 0.2% Triton X-100 containing 0.15M-NaCl and 2mM-phenylmethanesulphonyl fluoride and applied to a wheat-germ agglutinin-Sepharose column, the adsorbed fraction gave the pattern shown in Fig. 7 on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Thus the periodate/Schiff-positive major glycoprotein indicated by an arrow in Fig. 7 was shown to be labelled by this surface-labelling technique, though it did not correspond to the most strongly labelled bands.

(4) After labelling of MOLT-4B cells by the periodate/ NaB^3H_4 method, the ^3H -labelled cells (3×10^7) were suspended in 1 ml of 0.01M-sodium phosphate buffer/0.15M-NaCl, pH 7.2, containing 0.05% trypsin (266 units/mg; Worthington Bio-

Fig. 6. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the wheat-germ agglutinin-Sepharose-purified glycoproteins of MOLT-4B cells labelled with periodate/ NaB^3H_4 .

Experimental details are described in the text. (a) ○, Wheat-germ agglutinin-Sepharose-purified glycoproteins obtained from MOLT-4B whole cells; ●, the fraction not adsorbed on wheat-germ agglutinin-Sepharose; (b) wheat-germ agglutinin-Sepharose-purified glycoproteins obtained from the plasma membranes of MOLT-4B cells. The abbreviations are as for Fig. 4.

chemical Corp., Freehold, NJ, U.S.A.), 0.001% deoxyribonuclease I (Boehringer, Mannheim, Germany) and 1mM- CaCl_2 , and incubated at 37°C for 30 min. The reaction was terminated by the addition of 100 μg of 1% soya-bean trypsin inhibitor (Sigma). As a control experiment, the same number of ^3H -labelled cells were treated in the same fashion without addition of trypsin. Then these cells were solubilized in 10mM-Tris/HCl buffer, pH 7.5, containing 0.2% Triton X-100 and 2mM-phenylmethanesulphonyl fluoride and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Fig. 7. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the wheat-germ agglutinin-Sepharose-purified glycoproteins of MOLT-4B cells labelled with ^{125}I by lactoperoxidase-catalysed iodination

Experimental details are described in the text. The abbreviations are used as for Fig. 4.

As shown in Fig. 8, the labelled band corresponding to the major glycoprotein with a mol.wt. of 95000 is located on the cell surface.

Properties and carbohydrate composition of the major glycoprotein

The major glycoprotein was shown to have an apparent mol.wt. of 95000, regardless of the presence or absence of 2-mercaptoethanol during sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. However, when the glycoprotein was solubilized at 60°C for 10 min, a faint periodate/Schiff-positive band appeared at a higher-molecular-weight region on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Since this band could not be seen when the glycoprotein was solubilized at 100°C for 5 min, it was most probably the aggregated form of the major glycoprotein.

The *N*-terminal amino acid residue of the dansylated major glycoprotein was determined by the method described in the Materials and Methods section, and dansylaspartic acid was found on t.l.c. of the hydrolysate, suggesting that the *N*-terminal

Fig. 8. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of solubilized MOLT-4B cells with or without trypsin-treatment

Experimental details are described in the text. ○, Without trypsin treatment; ●, after trypsin treatment. The abbreviations are used as for Fig. 4.

residue is aspartic acid or asparagine. The carbohydrate composition of the major glycoprotein was determined by the method described in the Materials and Methods section and expressed as molar ratios to fucose in Table 1. Since the total hexose content of the glycoprotein was 13% (w/w) as revealed by the phenol/sulphuric acid method with D-mannose as a standard, the total carbohydrate content was estimated to be 28% (w/w) from the ratios in Table 1.

Alkaline borohydride treatment of the major glycoprotein

As this glycoprotein was revealed to contain galactosamine (Table 1), it appeared that this glycoprotein has sugar chains linked by alkali-labile *O*-glycosidic linkages to the peptide moiety. To clarify this point, the glycoprotein was treated with

Table 1. Carbohydrate composition of the major sialoglycoprotein of MOLT-4B cells

Sugar	Molar ratio to fucose
Fucose	1.0
Galactose	4.0
Mannose	3.7
<i>N</i> -Acetylglucosamine	3.5
<i>N</i> -Acetylgalactosamine	1.2
Sialic acid	2.5

NaB³H₄ under alkaline conditions. The alkali-labile ³H-labelled oligosaccharides released were subjected to high-voltage paper electrophoresis on Whatman 3MM paper in pyridine/acetic acid/water (1:10:89, by vol.; pH 3.5) at 2000V for 2h. The location of radioactive oligosaccharides was determined by using a guide strip. The areas containing the oligosaccharides were cut out and extracted with 1ml of water, and the radioactivity of the extracts was counted in a Beckman liquid-scintillation counter after addition of Bray's (1960) solution. Two fractions shown in Fig. 9, peak I and peak II, were eluted from the paper with water, and each fraction was applied to a column of Bio-Gel P-4 (2cm × 50cm; Bio-Rad Laboratories, Richmond, CA, U.S.A.) and eluted with water. To identify the reduced ³H-labelled terminal residue of these fraction, they were hydrolysed in 4M-HCl for 4h at 100°C. From both fractions labelled galactosaminitol was detected by high-voltage paper electrophoresis. The molecular weights of these fractions were estimated to be approx. 1000 by gel filtration on a column of Bio-Gel P-4. Carbohydrate compositions of these fractions are shown in Table 2. From these results, the major glycoprotein was confirmed to have alkali-labile sugar chains linked with *N*-acetylgalactosaminyl linkages to the peptide moiety, like those of mucins.

Table 2. Carbohydrate compositions of the oligosaccharides released from the major sialoglycoprotein of MOLT-4B cells by alkaline treatment
The fractions are as shown in Fig. 9.

Sugar	Molar ratio to galactose	
	Peak I	Peak II
Galactose	1.0	1.0
Fucose	0.9	—
<i>N</i> -Acetylgalactosamine	0.8	0.8
Sialic acid	0.9	1.0

Fig. 9. High-voltage paper electrophoresis of the oligosaccharides released by alkaline NaB³H₄ treatment from the major sialoglycoprotein of MOLT-4B cells
Experimental details are described in the text.

Affinity chromatography on various lectin-Sephrose columns

MOLT-4B cells labelled by the periodate/NaB³H₄ method were solubilized in 0.2% Triton X-100 and applied to a column of Sepharose 4B coupled with *R. communis* agglutinin or concanavalin A. The adsorbed glycoproteins were eluted with 0.2M-methyl α-D-mannopyranoside in the case of concanavalin A-Sepharose 4B. The eluted glycoproteins were then analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The band with the same electrophoretic mobility as that of the major glycoprotein obtained by using wheat-germ agglutinin-Sepharose 6MB was also detected in both cases (Fig. 10). When the materials not adsorbed on the concanavalin A or *R. communis* agglutinin-

Fig. 10. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the affinity-purified glycoproteins of MOLT-4B cells labelled with periodate NaB³H₄
Experimental details are described in the text. (a) The specifically eluted fraction from a *R. communis* agglutinin-Sepharose 4B column. The abbreviations are as for Fig. 4.

Sephacose column were applied in turn to a wheat-germ agglutinin-Sepharose column and the fractions specifically eluted from the column were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, no significant radioactive peaks, except the one near the tracking dye, were observed. These observations indicate that the major glycoprotein adsorbed on wheat germ agglutinin-Sepharose 6MB could also be adsorbed on both *R. communis* agglutinin- and concanavalin A-Sepharose 4B columns.

Discussion

In a previous paper (Saito *et al.*, 1977) we reported the isolation of two types of glycopeptides from the surface of lectin-stimulated human peripheral-blood lymphocytes. One of these glycopeptides contains sugar chains of the type found in serum glycoproteins and the other contains sugar chains like those of mucins. This indicates that sialoglycoproteins such as glycophorins of human erythrocyte membranes may exist also in the membranes of human lymphocytes.

In this work, we isolated a sialoglycoprotein from human lymphoblastoid cells of a MOLT-4B cell line that had originated from T-lymphocytes. This glycoprotein, having an approx. mol.wt. of 95 000, is strongly periodate/Schiff-positive and has the same electrophoretic mobility as that of the major glycoprotein of the isolated MOLT-4B plasma-membrane fraction. The localization of the isolated glycoprotein of an approx. mol.wt. 95 000 on the cell surface was also suggested by surface-labelling by means of periodate/ NaB^3H_4 , which is known as a method for specific ^3H -labelling of cell-surface sialic acids (Gahmberg & Andersson, 1977), and lactoperoxidase-catalysed iodination. Possible contamination of the glycoprotein with a cytoplasmic glycoprotein with the same molecular weight cannot completely be excluded. However, the fact that the isolated glycoprotein is homogeneous in terms of the *N*-terminal amino acid (aspartic acid or asparagine) strongly suggests that the glycoprotein is a single entity.

Recently, Andersson *et al.* (1977) analysed glycoprotein of MOLT-4 cells and observed a major glycoprotein of approx. mol.wt. 130 000. The discrepancy in the molecular weights of our result and theirs might be due partly to the fact that they analysed the glycoproteins in neuraminidase-treated cells and partly to errors inherent in the estimation of molecular weights of glycoproteins by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Carbohydrate analysis of the glycoprotein indicated that it contains both *O*-glycosidically linked and *N*-glycosidically linked sugar chains. However, the total carbohydrate content is considerably smaller

than that of the glycophorins of human erythrocyte membranes, and relative proportions of the *N*-acetylglucosamine and *N*-acetylgalactosamine contents indicate that *O*-glycosidically linked sugar chains are not as abundant as in glycophorins, and several *N*-glycosidically linked sugar chains can be expected in this glycoprotein. As for the structure of the *O*-glycosidically linked sugar chains, we isolated two kinds of reduced oligosaccharides by alkaline borohydride treatment. One of these oligosaccharides has the same carbohydrate composition as that of the trisaccharide released by alkaline borohydride treatment of the trypsin fragment of human erythrocytes (Thomas & Winzler, 1969), but the other has one additional fucose residue. Fucose-containing *O*-glycosidically linked sugar chains have not yet been found in sialoglycoproteins of human erythrocyte membranes.

As expected from the fact that two types of sugar chains exist in this glycoprotein, it binds to *R. communis* agglutinin and concanavalin A as well as to wheat-germ agglutinin, and appears to have multiple receptor activity to various lectins, as do glycophorins of human erythrocyte membranes (Fukuda & Osawa, 1973). It does not necessarily mean, however, that this glycoprotein is a major binding site for all of these lectins. In fact, lactoperoxidase-catalysed iodination of MOLT-4B cells showed that the proteins that were strongly positive for lactoperoxidase-catalysed iodination, but only weakly positive for periodate/Schiff staining, were also binding sites for wheat-germ agglutinin, as shown in Fig. 7.

Our preliminary studies have indicated that a similar sialoglycoprotein with mol.wt. approx. 95 000 is present in the membranes of normal T-lymphocytes (M. Saito & T. Osawa, unpublished work). Andersson *et al.* (1977) have also demonstrated, by the galactose oxidase/ NaB^3H_4 method, a major glycoprotein of mol.wt. approx. 120 000 on the surface of human T-lymphocytes after neuraminidase treatment of the cells.

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