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THE MAJOR SIALOGLYCOPROTEIN OF HUMAN T-LYMPHOCYTES*

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ABSTRACT

Human, peripheral-blood T-lymphocytes and human, T-lymphoblastoid cells of a MOLT 4B cell-line were surface-labeled by lactoperoxidase-catalyzed iodination, periodate and sodium borotritide, and galactose oxidase and sodium borotritide, and analyzed by dodecyl sodium sulfate–polyacrylamide gel-electrophoresis. Both types of cells were found to show a major, cell-surface sialoglycoprotein with an apparent mol. wt. of 95 000. After neuraminidase treatment, this glycoprotein showed a higher mol. wt. of 120 000. The major sialoglycoprotein of both types of cells bound to wheat-germ agglutinin and concanavalin A and, after neuraminidase treatment, to *Arachis hypogaea* agglutinin. The glycopeptides obtained from these glycoproteins by Pronase digestion gave similar elution-profiles on Sephadex G-50 gel filtration. These results suggest that the major sialoglycoprotein of normal T cells and that of MOLT 4B cells are very similar, if not identical.

INTRODUCTION

In previous work[1], we isolated a sialoglycoprotein from human T-lymphoblastoid cells of a MOLT 4B cell-line. This glycoprotein with a mol. wt. of ~95 000 was found to be located at the cell surface, and to contain two types of sugar chain, one having an *O*-glycosyl linkage and the other an *N*-glycosyl linkage. In the present paper, we report that a major sialoglycoprotein with a mol. wt. of ~95 000 is present also at the surface of human, normal, peripheral-blood T-lymphocytes, and that this glycoprotein is similar in many respects to the one found at the cell surface of the MOLT 4B cell-line cells.

EXPERIMENTAL

Human lymphoid cell-lines. — The human lymphoblastoid cells of a MOLT 4B cell-line, which had been established and classified as T-lymphocytes by Minowada

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et al.[2], were kindly donated by Dr. Y. Hinuma, Kumamoto University Medical School, Kumamoto, Japan. The cells were grown in RPMI-1640 medium (Gibco Diagnostics, Gibco Invener Div., Chagrin Park, OH 44022) supplemented with 10% (v/v) heat-inactivated (56°, 30 min) fetal-calf serum (Gibco) containing Kanamycin- (60 μg/mL; Meji Seika Co., Tokyo, Japan).

Human, peripheral-blood T-lymphocytes. – Human, peripheral-blood lymphocytes were purified from fresh venous blood by Ficoll–Urografin density gradient centrifugation as previously described[3]. T-Lymphocytes were enriched by passage through a column of Leukopac nylon wool according to the method of Greaves and Brown[4]. About 95% of the nylon wool-nonadherent cells were found to form rosettes with sheep red-blood-cells by use of the method of Galili and Schlesinger[5], and were considered to be T-lymphocytes.

Labeling of cells. — Lactoperoxidase-catalyzed iodination of the cell surface was performed by the method of Hubbard and Cohn[6]. Sialic acid was labeled at the cell surface by the method of Gahmberg and Andersson[7]. Cell-surface labeling by the D-galactose oxidase method was done according to the method of Andersson *et al.*[8].

Lectin-affinity columns and proteins binding to lectin-affinity columns. — Wheat-germ agglutinin–Sepharose 6MB was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Concanavalin A–Sepharose 4B and *Arachis hypogaea* (peanut) agglutinin–Sepharose 4B were prepared by the method of Matsumoto and Osawa[9]. Concanavalin A was purified from jack-bean meal (Sigma Chemical Co., St. Louis, MO 63178) by the method of Agrawal and Goldstein[10]. *Arachis hypogaea* agglutinin was purchased from E. Y. Laboratories (San Mateo, CA 94401).

Labeled T cells or MOLT 4B cells were suspended in 10mM Tris · HCl buffer, pH 7.5, containing 0.2% Triton X-100 and 2mM phenylmethanesulfonyl fluoride for 2 h at 4°. After brief homogenization with a glass Potter–Elvehjem homogenizer, the homogenate was centrifuged at 12 000*g* for 30 min, and the supernatant solution was applied to the lectin-affinity columns. The absorbed glycoproteins were eluted with 0.2M 2-acetamido-2-deoxy-D-glucose for wheat-germ agglutinin–Sepharose 6MB, 0.2M methyl α-D-mannopyranoside for concanavalin A–Sepharose 4B, and 0.2M lactose for *Arachis hypogaea* agglutinin–Sepharose 4B, and dialyzed against distilled water and freeze-dried.

Dodecyl sodium sulfate–polyacrylamide gel electrophoresis. — Labeled cells or materials eluted specifically from the lectin-affinity columns were solubilized in 0.06M Tris · HCl buffer, pH 6.8, containing 2% dodecyl sodium sulfate, 5% 2-mercaptoethanol, 10% glycerol, and 0.00015% Bromophenol Blue for 5 min at 100°. A discontinuous dodecyl sodium sulfate–polyacrylamide gel electrophoresis-system was used as described by Laemmli[11]. The polyacrylamide gel concentration was 7.5%. The gels were sliced into 1-mm slices with a gel slicer, and the radioactivity of each slice was counted directly in an Alloka well-type autogamma counter (^{125}I), or in a Packard Tricarb liquid-scintillation counter (^{3}H), after incubation of the gel slices in 0.5 mL of NCS solubilizer (Amersham Corp., Arlington Heights, IL 60005)

for 2 h at 65°. To determine the mol. wt. of the components, the following proteins were used as standards (mol. wt. shown in parentheses): human γ-globulin (160 000), bovine serum albumin (68 000), ovalbumin (45 000), and α-chymotrypsinogen A (25 000). These standard proteins were purchased from Schwarz/Mann Div., Orangeburg, NY 10962). In order to elute proteins from the gel, the slices were incubated in 0.05M Tris · HCl buffer, pH 7.5, containing 0.1% dodecyl sodium sulfate and 2mM phenylmethanesulfonyl fluoride overnight at 37°. The eluted materials were dialyzed against distilled water and freeze-dried.

Pronase digestion and gel filtration. — Glycoproteins eluted from gel slices were solubilized in 0.05M Tris · HCl buffer (0.5 mL, pH 8.0) containing 0.01M calcium chloride and Pronase (2 mg, Kaken Kagaku Co., Tokyo, Japan), and incubated for 24 h at 37° under a toluene layer. After an addition of Pronase (2 mg), the reaction was continued for an additional 48 h. The reaction was stopped by placing the tube in boiling water for 1 min. The digests were then filtered through a column of Sephadex G-50 (1.2 × 107 cm) that had been equilibrated and eluted with 0.05M acetic acid buffered with ammonium hydroxide to pH 6.0.

Neuraminidase treatment. — Glycoproteins eluted from gel slices were treated with *Vibrio cholerae* neuraminidase (Calbiochem–Behring Corp., San Diego, CA 92112) for 2 h at 37° in Dulbecco's phosphate buffered saline.

RESULTS

Cell-surface proteins of T and MOLT 4B cells. — Patterns of dodecyl sodium sulfate–polyacrylamide gel electrophoresis of surface-labeled T and MOLT 4B cells are shown in Fig. 1. In both types of cells, the region with an apparent mol. wt. of 95 000 was strongly labeled by periodate and sodium borotritide treatment (Fig. 1b,c). With the lactoperoxidase-catalyzed iodination method, this major sialoglycoprotein was not so strongly labeled in MOLT 4B cells, but was most strongly labeled in T cells (Fig. 1a,d). Fig. 1c,f, shows the labeling patterns after the D-galactose oxidase method. With this method combined with the neuraminidase treatment, the region with an apparent mol. wt. of 120 000 was most intensely labeled in both types of cells, and the glycoproteins having an apparent mol. wt. of 95 000, which were intensely labeled by the periodate and sodium borotritide treatment, could not be detected.

Extraction of the major sialoglycoproteins from gel slices after dodecyl sodium sulfate–polyacrylamide gel electrophoresis. — The glycoproteins that bound to wheat germ agglutinin–Sepharose 6MB were analyzed by dodecyl sodium sulfate–polyacrylamide gel electrophoresis (Fig. 2). The region of the gel corresponding to the major sialoglycoprotein was extracted. The yield of the major sialoglycoprotein was about 30 μg of protein from 2 g of packed cells in both types of cells.

Decrease in mobility of the major sialoglycoprotein on dodecyl sodium sulfate–polyacrylamide gel electrophoresis after neuraminidase treatment. — ^{125}I-Labeled major sialoglycoproteins of T and MOLT 4B cells, purified as described earlier,

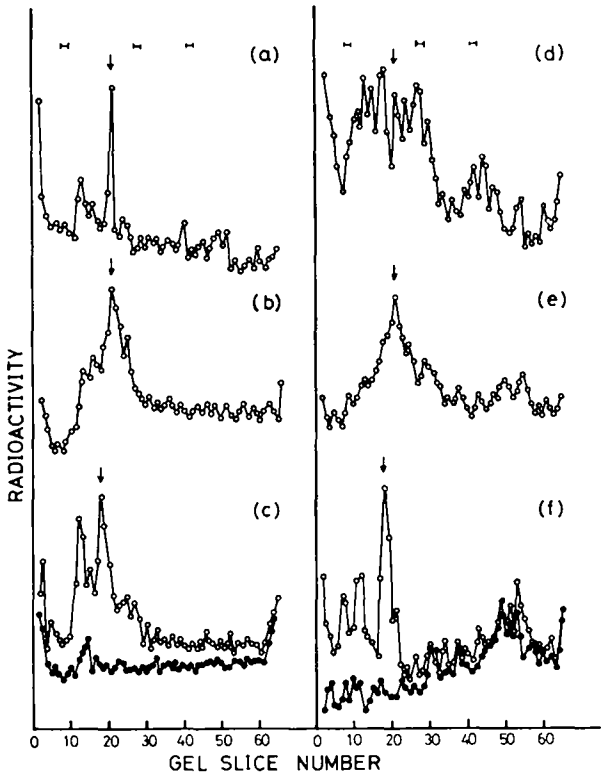

Fig. 1. Dodecyl sodium sulfate–polyacrylamide gel electrophoresis of ^{125}I- or ^{3}H-labeled, cell-surface glycoproteins of T and MOLT 4B cells: (a) T cells labeled by lactoperoxidase-catalyzed iodination; (b) T cells labeled by periodate and sodium borotritide treatment; (c) ○——○ T cells labeled by D-galactose oxidase and sodium borotritide after neuraminidase treatment, ●——● T cells labeled by D-galactose oxidase and sodium borotritide treatment; (d) MOLT 4B cells labeled by lactoperoxidase-catalyzed iodination; (e) MOLT 4B cells labeled by periodate and sodium borotritide treatment; and (f) ○——○ MOLT 4B cells labeled by D-galactose oxidase and sodium borotritide after neuraminidase treatment, ●——● MOLT 4B cells labeled by D-galactose oxidase and sodium borotritide treatment. Vertical arrows indicate the position of elution of the major sialoglycoproteins. The position of elution of standard markers (⊢⊣) are indicated in the following order (from left to right): human immunoglobulin G, bovine serum albumin, and ovalbumin.

were treated with neuraminidase and again analyzed by dodecyl sodium sulfate–polyacrylamide gel electrophoresis. Fig. 3 shows the patterns of the major sialoglycoproteins before and after neuraminidase treatment. Before neuraminidase treatment, the major sialoglycoproteins from both types of cells were seen at the position of an apparent mol. wt. of 95 000. After neuraminidase treatment, however, these glycoproteins having an apparent mol. wt. of 95 000 were found to have moved to the region of mol. wt. ~120 000.

Glycopeptide analysis of major sialoglycoproteins. — The major sialoglycoproteins labeled by periodate and sodium borotritide treatment were digested with Pronase, and the glycopeptides thus formed were analyzed by Sephadex G-50 gel-

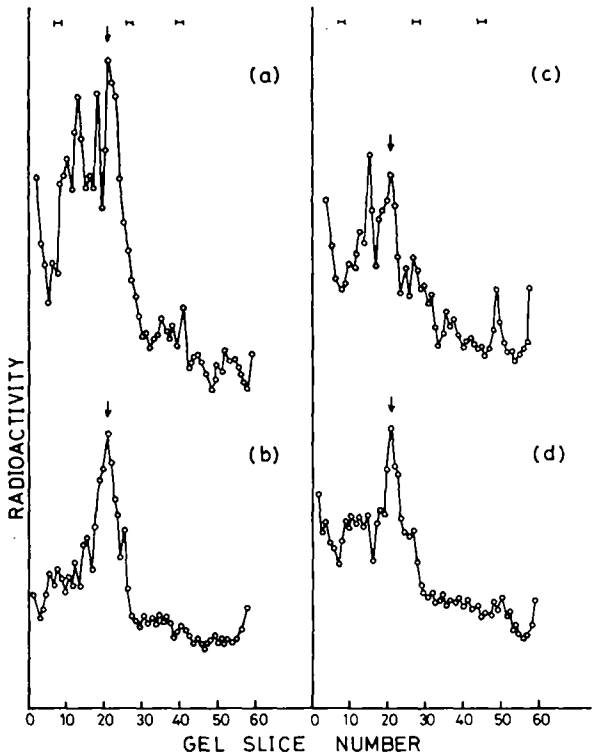

Fig. 2. Dodecyl sodium sulfate–polyacrylamide gel electrophoresis of glycoproteins eluted from wheat germ agglutinin–Sepharose 4B: (a) from T cells labeled by lactoperoxidase-catalyzed iodination; (b) from T cells labeled by periodate–sodium borotritide treatment; (c) from MOLT 4B cells labeled by lactoperoxidase-catalyzed iodination; and (d) from MOLT 4B cells labeled by periodate–sodium borotritide treatment.

filtration. As shown in Fig. 4, the glycopeptides obtained from both T and MOLT 4B cells gave similar chromatographic profiles.

Affinity chromatography on lectin–Sepharose columns. — Fig. 5 shows the results of dodecyl sodium sulfate–polyacrylamide gel electrophoresis of the materials bound to concanavalin A–Sepharose 4B and *Arachis hypogaea* agglutinin–Sepharose 4B. In the case of *Arachis hypogaea* agglutinin–Sepharose 4B, the lyzates of ^{125}I-labeled T and MOLT 4B cells were treated with neuraminidase before application to the affinity columns. The major sialoglycoproteins of T and MOLT 4B cells were found to bind to both lectin-affinity columns. *Arachis hypogaea* agglutinin specifically recognized major sialoglycoproteins treated with neuraminidase.

DISCUSSION

Previously, we isolated and partially characterized a major sialoglycoprotein of human, leukemic cell-line MOLT 4B cells[1]. In this work, we demonstrated that a

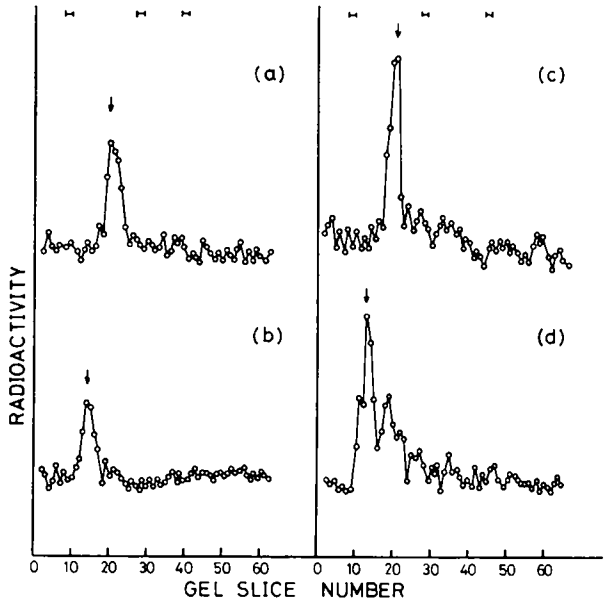

Fig. 3. Dodecyl sodium sulfate–polyacrylamide gel electrophoresis of the major sialoglycoproteins before and after neuraminidase treatment: (a) ^{125}I-Labeled major sialoglycoprotein of T cells before neuraminidase treatment; (b) ^{125}I-labeled major sialoglycoprotein of T cells after neuraminidase treatment; (c) ^{125}I-labeled major sialoglycoprotein of MOLT 4B cells before neuraminidase treatment; and (d) ^{125}I-labeled major sialoglycoprotein of MOLT 4B cells after neuraminidase treatment.

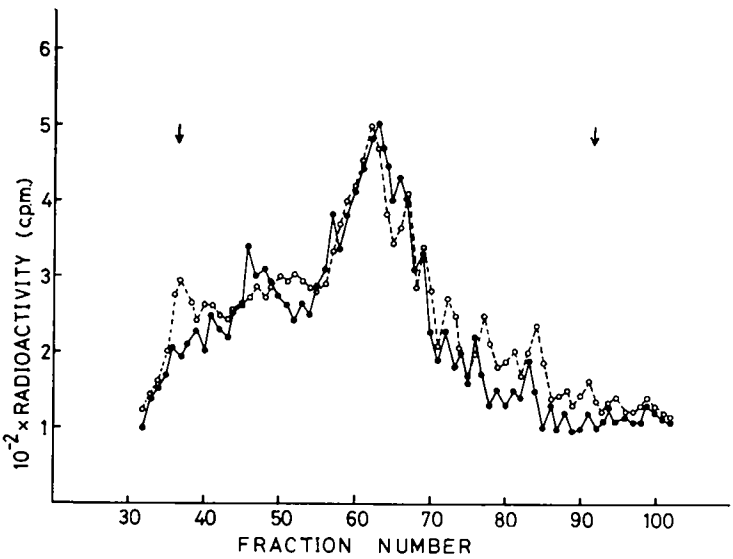

Fig. 4. Sephadex G-50 gel-filtration of Pronase-digests of major sialoglycoproteins obtained from T (●——●) and MOLT 4B (○- - -○) cells, both labeled by periodate–sodium borotritide treatment. Experimental details are described in the text. Vertical arrows indicate the position of elution of Blue Dextran (Fractions 36–37) and D-glucose (Fractions 91–92).

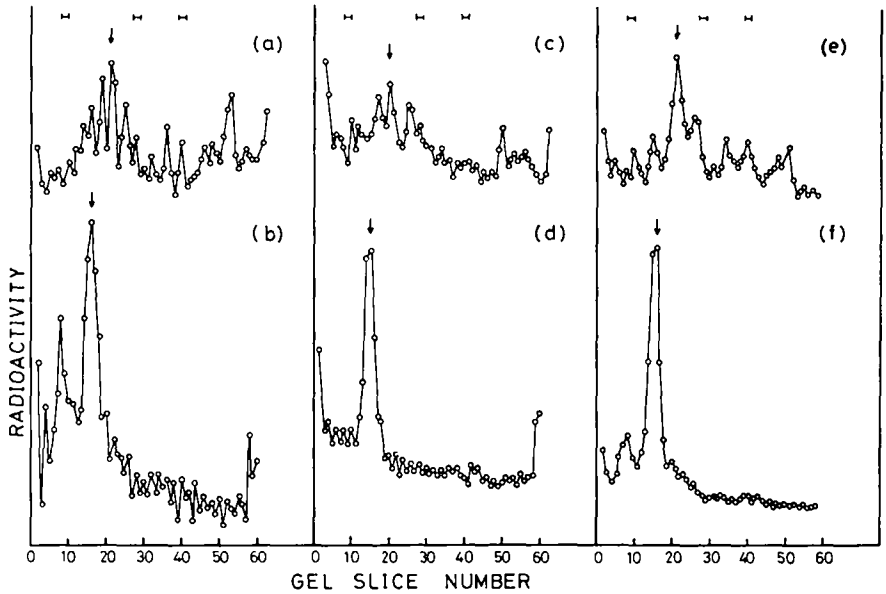

Fig. 5. Dodecyl sodium sulfate–polyacrylamide gel electrophoresis of the glycoproteins specifically eluted from concanavalin A–Sepharose 4B and *Arachis hypogaea* agglutinin–Sepharose 4B: (a) ^{125}I-Labeled T cell glycoproteins after affinity chromatography on a concanavalin A–Sepharose 4B column; (b) ^{125}I-labeled T cell glycoproteins after treatment with neuraminidase and affinity chromatography on an *Arachis hypogaea* agglutinin–Sepharose 4B column; (c) ^{125}I-labeled MOLT 4B cell glycoproteins after affinity chromatography on a concanavalin A–Sepharose 4B column; (d) ^{125}I-labeled MOLT 4B cell glycoproteins after treatment with neuraminidase and affinity chromatography on an *Arachis hypogaea* agglutinin–Sepharose 4B column; (e) periodate–sodium borotritide-labeled MOLT 4B cell glycoproteins after affinity chromatography on a concanavalin A–Sepharose 4B column; and (f) glycoproteins from neuraminidase-treated and D-galactose oxidase–sodium borotritide-labeled MOLT 4B cells after affinity chromatography on an *Arachis hypogaea* agglutinin–Sepharose 4B column. The same standard markers were used as described in the legend to Fig. 1.

cell-surface major sialoglycoprotein of similar properties was present at the surface of normal human T-lymphocytes. Both glycoproteins are similar in apparent mol. wt. (before and after neuraminidase treatment), affinity to lectins, and size distribution of the glycopeptides obtained from the Pronase-digests of these glycoproteins. It is, however, possible that differences of several carbohydrate and amino acid residues exist.

Andersson *et al.*[12] have demonstrated, by the D-galactose oxidase and sodium borotritide method, the presence of a major glycoprotein of mol. wt. ~120 000 at the surface of human T-lymphocytes and of a major glycoprotein of mol. wt. ~130 000 at the surface of leukemic, T cell-line cells. The major sialoglycoproteins from T and MOLT 4B cells, however, exhibited the same degree of decrease in electrophoretic mobility after neuraminidase treatment. Such a decrease after desialosylation has been observed with cell-surface sialoglycoproteins of mouse[7], and human lymphocytes[13,14] and HeLa cells[15]. From these results, we suggest that the major sialoglycoprotein of mol. wt. ~95 000 obtained in the previous work[1] is the same glycoprotein

as that of mol. wt. ~120 000 reported by Andersson *et al.*[12]. Furthermore, our studies indicate that the same glycoprotein may exist also at the cell surface of normal T cells. This suggestion was supported by the observation that the glycopeptides obtained from the Pronase-digest of major sialoglycoproteins of either T or MOLT 4B cells showed similar size-distributions on Sephadex G-50 gel-filtration. It remains to be established whether the glycoprotein of mol. wt. ~95 000 is one of the glycoproteins that are specific for human T cells.

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