Biochemical Properties of the High-Molecular-Weight Glycopeptides Released from the Cell Surface of Human Teratocarcinoma Cells

Hisako Muramatsu, Takashi Muramatsu, and Philip Avner

Department of Biochemistry, Kagoshima University School of Medicine, Usukicho 1208-1, Kagoshima, Japan [H. M., T. M.] and Laboratoire Immunologie et Virologie des Tumeurs, Groupe INSERM U152, ERA CNRS 781 Hôpital Cochin, 27 rue du Faubourg Saint-Jacques 75674 Paris Cedex 14, France [P. A.]

ABSTRACT

The present study deals with the biochemical properties of high-molecular-weight glycopeptides isolated from the surface of human teratocarcinoma cells. This cell surface material released by mild trypsin digestion from galactose-labeled human teratocarcinoma cells, Tera I and PA1, was digested extensively with pronase. Most of the resulting glycopeptides were large and were excluded from a Sephadex G-50 column. The properties of these large cell surface glycopeptides isolated from Tera I cells have been examined in detail. It is clear from these experiments that they are neither acidic mucopolysaccharides nor mucin-type glycopeptides with short oligosaccharide chains. Although the glycopeptides are hardly hydrolyzed by β-galactosidase even after prior digestion with neuraminidase, around 30% of the glycopeptides are depolymerized by treatment with endo-β-galactosidase from Escherichia freundii. The large cell surface glycopeptides from Tera I cells therefore appear to be very similar to the large glycopeptides seen on mouse embryonal carcinoma cells, which have core structures composed of galactose and N-acetylglucosamine. Like the mouse cell glycopeptides, a fraction of the large glycopeptides from these human cells bind to agarose-conjugated fucose-binding proteins and peanut agglutinin.

INTRODUCTION

Four lines of human teratocarcinoma cells have been shown to release high-molecular-weight fucosyl glycopeptides after extensive pronase digestion (10, 14). Such large fucosyl glycopeptides eluting in the excluded volume region on Sephadex G-50 column chromatography have not been found to be present, at least in large amounts, in other cancer cells or in a variety of normal cells (10, 13). The large glycopeptides from human teratocarcinoma thus appear to be of possible use for the diagnosis of human teratocarcinomas. However, the large glycopeptides from human teratocarcinoma cells were studied mainly by gel filtration profiles. Therefore, the biochemical nature of the unusual glycopeptides remained unresolved.

Mouse EC cells also strongly express such large fucosyl carbohydrates (13). In case of the murine system, the biochemical properties of these large carbohydrates have been partially resolved (11, 12). The glycopeptides have repeated and branched arrangements of galactose and N-acetylgalcosamine in the core structure and fucose and other sugars in the nonreducing terminal positions (11).

Here we describe the biochemical properties of the large glycopeptide found on human cells. Instead of using fucose-labeled large glycopeptides prepared from whole cells, we have chosen to analyze galactose-labeled large glycopeptides prepared from cell surface materials released by mild trypsin treatment. This protocol was adopted, as we wished to analyze exclusively material located on the cell surface. Galactose-labeled material was used since the large glycopeptides are labeled more efficiently by it. The isolated large glycopeptides were analyzed by several methods. Of these, most efficient was digestion with endo-β-galactosidase from Escherichia freundii (5). Susceptibility to this enzyme was the key information indicating that the large glycopeptides from human teratocarcinoma cells were structurally similar to those from murine EC2 cells. In addition, we have shown that the large glycopeptides from human cells carry binding sites for some specific lectins.

MATERIALS AND METHODS

Preparation of Galactose-labeled Cell Surface Glycopeptides. The human testicular teratocarcinoma cell line Tera I was kindly provided by Dr. J. Fogh of the Sloan-Kettering Institute for Cancer Research. PA1, an ovarian teratocarcinoma cell line, was obtained from Dr. J. Zeuthen. The cells were cultured as described previously (10). To freshly replicated cultures, [1-3H]-galactose (12.4 mCi; Radiochemical Centre) was added to a final concentration of 10 μCi/ml. After 3 to 4 days, the labeled cells were trypsin treated for 20 min at 37° using a 1:4 Dulbecco’s phosphate-buffered saline dilution of trypsin:EDTA (Grand Island Biological Co.). Cell death was controlled by the trypan blue exclusion test and was less than 5%. The supernatant obtained was concentrated and recentrifuged to get rid of any remaining cell membrane fragments prior to pronase digestion (10). The cell surface material thus obtained was digested extensively with 40 mg of pronase (E grade; Kaken Chemical Co.) in 4 ml of 0.05 M Tris-HCl buffer, pH 8.0, at 37° for 24 hr. Forty mg of fresh pronase in 2 ml H2O were added 24 and 48 hr later, and the digestion was terminated after 72 hr. Digested materials were heated at 100° for 10 min and centrifuged, and the supernatant was lyophilized. This was then applied to a column (2.0 x 70 cm) of Sephadex G-50 (fine) which had been equilibrated with 0.05 M ammonium acetate buffer, pH 6.0. Four-mi fractions were collected after elution with the same buffer. The glycopeptides eluted in Fractions 23 to 28 were collected as high-molecular-weight glycopeptides and lyophilized (Chart 1).

Biochemical Analysis. Digestion with endo-β-galactosidase from E. freundii was performed using the enzyme prepared by Seikagaku Kogyo Co., Ltd., as described previously (11), except that 10 μM galactono-1→5-lactone was added to the reaction mixture in order to inhibit the trace amounts of β-galactosidase present in the enzyme preparation. Digestion with β-galactosidase and β-N-acetylgalcosaminidase was performed as described before (11).

PNA:agarose and FBP:agarose were purchased from E. Y. Labora-
RESULTS AND DISCUSSION

Using mild trypsin digestion, cell surface material was released from 2 human teratocarcinoma cell lines, Tera I and PA1, which had been labeled with \(^{3}H\)galactose. The cell surface material was digested extensively with pronase, and the resulting galactosyl glycopeptides were analyzed by Sephadex G-50 column chromatography. In both cases, most of the cell surface glycopeptides were eluted in the excluded volume (Chart 1). The elution profiles were very similar to those of galactose-labeled membrane glycopeptides isolated from mouse EC cells (13).

The large cell surface glycopeptides eluted in the excluded volume from Tera I cells were pooled and analyzed further. The glycopeptides could not be extracted at all with chloroform: methanol (2:1) and thus were not glycolipids of the usual sugar length. The large glycopeptides were scarcely depolymerized by treatment with 0.2 N NaOH in the presence of 0.4 M NaBH\(_4\) at 37\(^\circ\) for 48 hr (Chart 2). Under these conditions, protein-carbohydrate linkages of mucin-type glycopeptides isolated by affinity chromatography on Dolichos lectin:agarose from murine small intestine are cleaved completely, yielding oligosaccharides of low molecular weight.\(^3\) The former experiment, therefore, excludes the possibility that the carbohydrate portion of the glycoproteins is mainly mucin type with short oligosaccharide chains. On DEAE-Sephadex A-25 column chromatography, the large glycopeptides behave mostly as neutral or weakly acidic glycopeptides (Table 1). Thus, it appears that the majority of the large glycopeptides are not glycosaminoglycans, as otherwise they should behave as strongly acidic material and should not be eluted from the column by buffers containing less than 0.1 M NaCl.

Digestion with \(\beta\)-galactosidase released only 5.2% of the total galactose label. Even after pretreatment with neuraminidase, only 9.1% of the galactose label was released. The galactosyl residues are not therefore those usually found in complex-type glycopeptides. The galactosyl residues in such glycopeptides are usually located in the penultimate position and are covered solely by sialic acid.

Key information on the structure of the galactose-labeled large glycopeptides of human teratocarcinoma cells was obtained by digestion with endo-\(\beta\)-galactosidase from E. freundii (5). By the enzyme treatment, about 30% of the large glycopeptides were depolymerized, yielding oligosaccharides having...
Large Glycopeptides from Human Teratocarcinomas

A significant fraction (38.5%) of the large glycopeptides was bound to Con A:Sepharose and could be eluted from it using 0.1 M methyl α-mannoside. It contrasts with the results obtained using material from the F9 cell line of mouse EC cells; only 5% of the large glycopeptides described herein may assist in the classification of human teratocarcinoma cells. The large glycopeptides from human EC cells may assist in the classification of human teratocarcinoma cells.

From results newly described in this paper, namely endo-β-galactosidase susceptibility, resistance to treatment with β-galactosidase in the presence of neuraminidase, resistance to mild alkaline treatment, and behavior upon DEAE-Sephadex column chromatography, we concluded that the large glycopeptides from human teratocarcinoma cells Tera I are similar to the large glycopeptides of murine EC cells. The latter glycopeptides are known to have core structure composed of galactose and N-acetylglucosamine. The structure of the mouse teratocarcinoma cells is of significant interest for diagnostic, since germinal cancers have diverse properties and the introduction of new biochemical markers such as the large glycopeptides described herein may assist in the classification of these cancers.

Isolation of the large glycopeptides from human teratocarcinoma cells is also of interest from the embryological point of view. Our results suggest strongly that the large glycopeptides

---

4 H. Muramatsu, T. Muramatsu, and G. Gachelin, unpublished data.

H. Muramatsu et al.

we have characterized will be found on early human embryos just as they have been found on mouse embryos.

At least part of the large carbohydrates of teratocarcinoma cells have core structures susceptible to endo-β-galactosidase attack. Carbohydrates susceptible to endo-β-galactosidase attack include several surface antigens such as I, i, and some ABH antigens (4, 5). Thus, we might expect that the large carbohydrate chains described here contain some of the antigenic markers of early embryonic cells. The F9 antigenic sites are most probably carbohydrate in nature (3, 6), and the F9 (IgM) antigen is apparently associated with these large carbohydrates in the mouse (6). In this respect, it is interesting to note that these large carbohydrates are expressed on human teratocarcinoma cell lines such as PA1 which lack F9 antigens (1) as well as on cell lines such as Tera I which have apparently greater homology to mouse EC cells and express F9 antigens (1). The absence of F9 antigens is not therefore accompanied by an obligatory absence of the large carbohydrates. This suggests that there may be a series of large carbohydrate chains on early embryonic cells, some of them carrying the F9 antigenic marker and others carrying not the F9 antigen but other antigenic markers.

ACKNOWLEDGMENTS

We thank Kumiko Sato for her expert secretarial assistance.

REFERENCES


Biochemical Properties of the High-Molecular-Weight Glycopeptides Released from the Cell Surface of Human Teratocarcinoma Cells

Hisako Muramatsu, Takashi Muramatsu and Philip Avner


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/5/1749

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.