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Note

Fractionation of Dns-glycopeptides by reversed-phase high-performance liquid chromatography

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One of the most perplexing features of glycoproteins is the heterogeneity of their carbohydrate chains. In order to correlate glycoproteins with their function, the nature of their complex carbohydrate chains should be thoroughly investigated. As the 5-dimethylaminonaphthalene-1-sulfonyl (Dns) residue can interact with the silica-based chemically bonded octadecyl chain, we have used reverse-phase high-performance liquid chromatography (HPLC) to fractionate Dns-asparaginyl oligosaccharides. Owing to the fluorogenic nature of the Dns group and to the high resolution of HPLC, fractionation of Dns-asparaginyl oligosaccharides by reversed-phase HPLC is far superior to conventional methods such as gel-permeation chromatography, ion-exchange chromatography and high-voltage electrophoresis.

EXPERIMENTAL

Materials

The following compounds and materials were obtained from commercial sources: crystalline ovalbumin from Nutritional Biochemicals; Dns chloride from Pierce; GlcNAc-Asn from Cyclo Chemical Co.; neuraminidase Type V from Sigma; and acetonitrile from Mallinckrodt. Human α_1-acid glycoprotein was kindly supplied by Dr. Y.L. Hao (American National Red Cross). Ovalbumin from pooled egg white was isolated by CM-cellulose chromatography as reported previously[1]. All solvents for HPLC were filtered through a Millipore filter (pore size 0.45 μm).

Preparation of Dns-asparaginyl glycopeptides

Asparaginyl glycopeptides from ovalbumin and α_1-acid glycoprotein were prepared by repeated Pronase digestion as described by Huang *et al.*[2]. Dns derivatization of glycopeptides was carried out according to the method of Gray[3]. Desialylation of Dns-glycopeptides from α_1-acid glycoprotein was performed by neuraminidase treatment and acid hydrolysis as described by Schmid *et al.*[4].

High-performance liquid chromatography

The HPLC apparatus consisted of an Altex Model 110A solvent delivery system, an LKB Model 11300 Ultragrad solvent gradient maker, an Altex Ultrasphere

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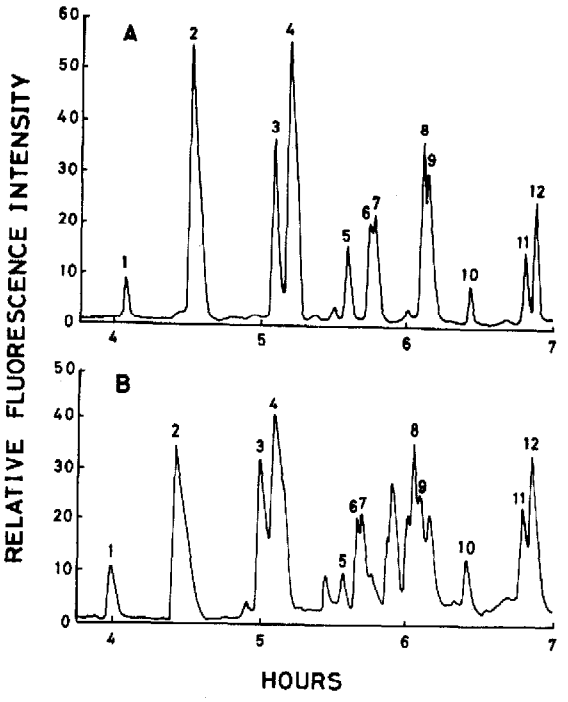

Fig. 1. Fractionation of Dns-glycopeptides derived from chicken ovalbumin by reversed-phase HPLC. Dns-glycopeptides (5 nmol) were analyzed under the conditions described in the text. (A) Dns-glycopeptides derived from purified ovalbumin by CM-cellulose chromatography; (B) Dns-glycopeptides derived from commercial crystalline ovalbumin.

ODS (5 μm) reversed-phase column (25 cm × 1 cm I.D.) protected by a Bio-Rad micro-guard column (3 cm × 4.6 mm I.D.) packed with Bio-Sil ODS (10 μm) reversed-phase hydrocarbon a Perkin-Elmer Model 7105 valve sample injector, Model 650 fluorescence monitor and Model 56 recorder. For analytical purposes, 5–15 nmol in 5 μl of Dns-glycopeptides were applied to the column. The column was eluted with a linear gradient from water to 7.5% acetonitrile containing 25 m*M* sodium borate buffer (pH 7.0) at 3.0 ml/min for 8 h. The effluent was monitored with a fluorescence monitor (excitation, 313 nm; emission, 550 nm). Owing to the lack of hydrophobicity, non-dansylated glycopeptides are not retained by the column under these conditions.

RESULTS AND DISCUSSION

Fractionation of glycopeptide mixture derived from ovalbumin

Fig. 1A shows the fractionation of Dns-glycopeptides derived from ovalbumin purified by CM-cellulose column chromatography. The glycopeptides were resolved into at least 12 peaks. In comparison, the elution profile of the Dns-glycopeptides prepared from commercial ovalbumin was much more complex and several additional peaks were detected (Fig. 1B, between peaks 5 and 9). These peaks must be derived from the contaminating glycoproteins.

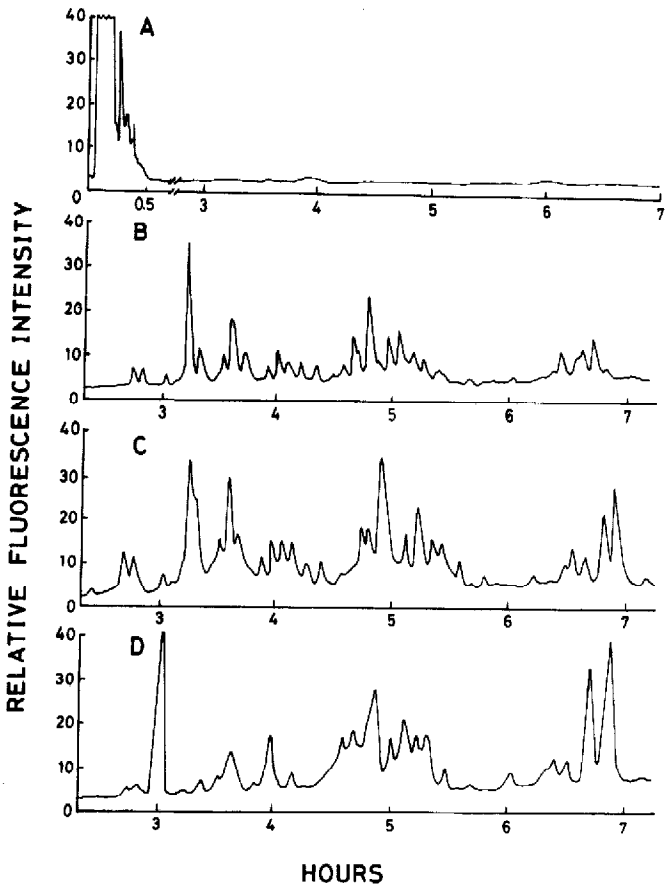

Fig. 2. Fractionation of Dns-glycopeptides derived from human α_1-acid glycoprotein by reversed-phase HPLC. Dns-glycopeptides (15 nmol) were analyzed under the same conditions in Fig. 1. (A) Untreated Dns-glycopeptides; (B) Dns-glycopeptides treated with neuraminidase; (C) Dns-glycopeptides hydrolyzed with 0.02 *N* sulfuric acid at 37°C for 24 h; (D) Dns-glycopeptides hydrolyzed with 0.1 *N* sulfuric acid at 80°C for 1 h.

Fractionation of glycopeptide mixture derived from α_1-acid glycoprotein

Owing to the presence of sialic acids, the glycopeptide mixture derived from α_1-acid glycoprotein was not retained by the octadecyl silica column (Fig. 2A). After neuraminidase treatment (Fig. 2B) or treatment with 0.02 *N* sulfuric acid at 37°C for 24 h (Fig. 2C), the asialoglycopeptides of α_1-acid glycoprotein were fractionated into at least 30 peaks by reversed-phase HPLC. When the glycopeptide mixture was heated in 0.1 *N* sulfuric acid at 80°C for 1 h, the glycopeptide profile was found to differ considerably from that obtained after neuraminidase treatment (Fig. 2D).

As shown in this study, fractionation of Dns-glycopeptides derived from ovalbumin and α_1-acid glycoprotein by reversed-phase HPLC enabled us to reveal the complexity of sugar chains in these two glycoproteins which had not been previously detected. As shown in Fig. 2, treatment of glycopeptides with 0.02 *N* sulfuric acid at 37°C for 24 h gave an elution profile similar to that prepared by neuraminidase

treatment. On the other hand, when the glycopeptides were heated in 0.1 *N* sulfuric acid at 80°C for 1 h the elution profile changed considerably.

We have also used this method to fractionate glycopeptides derived from other glycoproteins, such as ovotransferrin and human serum transferrin. As little as 30 pmol of each individual glycopeptide can be detected by this method. Owing to its high sensitivity and high resolution, this method should be useful for the fractionation of glycopeptides.

ACKNOWLEDGEMENT

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