Communication

Developmental Change and Genetic Defect in the Carbohydrate Structure of Band 3 Glycoprotein of Human Erythrocyte Membrane*

(Received for publication, February 21, 1979, and in revised form, March 19, 1979)

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SUMMARY

The chemical structure of Band 3 glycopeptide prepared from erythrocytes of normal adult (blood group Oi), umbilical cord vessels (Oi), and an i adult variant who fails to develop I antigen (Oi), has been compared. Band 3 glycopeptide of cord erythrocytes gave, on permethylation analysis, predominantly 2,4,6-tri-O-methylgalactose and 3,6-di-O-methyl-2-N-methylacetamido-2-deoxyglucose, whereas the same glycopeptide of normal adult erythrocytes gave much higher amounts of 2,3,4,6-tetra-O-methylgalactose and 2,4-di-O-methylgalactose as compared with that of cord erythrocytes. Band 3 glycopeptide from i adult showed the same methylation pattern as cord-Band 3 glycopeptide. In accordance with these results, Band 3 glycopeptide of cord and i adult erythrocytes were hydrolyzed to mostly small oligosaccharides by endo-β-galactosidase from Escherichia freundii, whereas that of normal adult produced a number of oligosaccharides with various sizes which was caused by branched structures.

Based on these results and structures of released oligosaccharides, the major developmental change of carbohydrate structure in the erythrocyte membrane is the conversion of linear repeating Gal1→ 3Gal to a branched Gal1→ 3Gal structure. i individual may result from the lack of the branching enzyme.

Membrane changes associated with the process of ontogenesis have been clearly demonstrated through the orderly appearance or disappearance of antigen markers such as "Fₐ" (1), blood group ABH (2), Forssman (3), and II antigens (4). The antigen i is converted to I during the development of fetal to adult erythrocytes (4) although rare individuals with a genetic defect cannot develop I antigen (adult i) (5). The II antigens have been regarded as the precursors of blood group ABH antigens (6), and a linear repeating Gal1→ 3Gal structure for I (7) and a branched Gal1→ 4GlcNAcβ1→ 3Gal for I specificities (8) have been assigned recently. A similar progressive branching process in the carrier carbohydrate chains for glycolipid A and H determinants has been implicated as being associated with the developmental process (9).

Recently, Band 3, the major intrinsic membrane glycoprotein of human erythrocyte (for review, see Refs. 10 and 11), has been assigned by us as I antigen carrier (12). We have found also that endo-β-galactosidase from Escherichia freundii (13) abolished Ii antigenic activity of human erythrocytes resulting from shortening the carbohydrate chains of Band 3 as one of the most significant modifications (14, 15). These observations prompted us to compare the carbohydrate structure of Band 3 from normal adult, fetus (umbilical cord), and adult i variant erythrocytes. A remarkable difference due to the branching structure was clearly demonstrated on methylation analysis and endo-β-galactosidase digestion.

MATERIALS AND METHODS

Erythrocytes of normal adult (blood group Oi) were obtained from the courtesy of the Dr. E. Giblett, Puget Sound Central Blood Bank, Seattle, WA. Erythrocytes of umbilical cord blood vessels (blood group Oi) were obtained from Group Health Cooperative of Puget Sound Hospital, Seattle, WA, and those from a very rare adult individual who fails to express I antigen (blood group Oi) were obtained through the courtesy of Spectra Biological Co., Ltd., Oxnard, CA. Band 3 proteins were purified as described previously (16) from these sources of erythrocytes and are designated as indicated in Footnote 1. SDS-polyacrylamide gel electrophoresis was performed according to the procedure of Laemmli (17). The glycopeptide from Band 3 protein was prepared by pronase digestion as described (18). A portion of the glycopeptide was labeled by galactose oxidase/NaB[H] as described (19). With this procedure, only the nonreducing terminal Gal or GalNAc was labeled (19). Endo-β-galactosidase was purified from the culture filtrate of E. freundii and was free from proteases and other glycosidases (13). The glycopeptide was digested with endo-β-galactosidase (125 munit/ml, final concentration) in 0.2 m sodium acetate buffer, pH 5.5, at 37°C for 18 h as described (13).

Glycopeptides and oligosaccharides were permethylated by the method of Hakomori (20) and permethylated products were hydrolyzed and analyzed by the modified method (21) of Stellner et al. (22). Partially O-methylated sugars were analyzed by Finnigan 3300-6110 gas chromatograph-mass spectrometer under the described condition (21). Paper chromatography was performed in a solvent system of ethyl acetate/pyridine/water (12:5:4, v/v) and radioactivity of chromatogram was determined as described (13).

RESULTS

Isolation of Glycopeptides from Band 3—Band 3 protein purified from erythrocytes of each source showed essentially a single band with a similar mobility upon examination by SDS-polyacrylamide gel electrophoresis (data not shown). When the pronase digest of Band 3 glycoprotein labeled with galactose oxidase/NaB[H] was subjected to gel filtration, two major radioactive peaks were observed (Fig. 1). Chemical analysis, however, showed that only the first peak contained detectable amounts of carbohydrates, which is consistent with the result reported previously (23). The major peak (indicated by horizontal arrow in Fig. 1) was, therefore, used for further studies. The chemical quantity of the glycopeptide eluted at Fractions 55 to 65 was small and variable, and the glycopeptide...
as alditol acetate derivatives. As shown in Fig. 2, i-Band 3 and methylated and partially O-methylated sugars were analyzed. Thus, i-Band 3 produced mainly 3-O-substituted galactose and nonreducing terminal fucose. i-Band 3 showed distinctly different gas chromatography patterns. More than 75% of galactose present in the original glycopeptide was recovered in the di-, tri-, and tetrasaccharide fractions based on neutral sugar quantitation using the unreduced digest.

In contrast to this, I-Band 3 glycopeptide was hydrolyzed to oligosaccharides with various sizes as well as small oligosaccharides (Fig. 3D). The disaccharide, trisaccharide, and tetrasaccharide were further purified by a Bio-Gel P-2 column and identified as GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 (Fig. 4). The carbohydrate chain of i-Band 3 was found to be a mixture of siac acid-containing tetrasaccharides and higher neutral oligosaccharides.

Overall Structure of I-, Cord-, and i-Band 3 Glycopeptide—Based on the permethylation of the glycopeptide (Fig. 2) and structural characterization of oligosaccharides (Fig. 3), it can be concluded that the carbohydrate side chains of i- and cord-Band 3 are mainly made up of the straight chain structures, R → Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4, whereas that of I-Band 3 contains the branched structure, R → Galβ1 → 3GlcNAcβ1 → 3 (R → 6)Gal (Fig. 4). The carbohydrate chain of i-Band 3 was extensively digested by endo-β-galactosidase to produce mostly di- to tetrasaccharides, whereas that of I-Band 3 produced oligosaccharides of various sizes (Fig. 3) because a linear chain can be degraded easily by this enzyme but branched galactose is hardly hydrolyzed (26). The greater amount of nonreducing terminal galactose in I-Band 3 glycopeptide shown by permethylation (Fig. 2) is consistent with the fact that I-Band 3 carbohydrate chain has a branched structure.

Since a negligible amount of nonreducing terminal N-acetylglucosamine was present in the starting glycopeptide (see Table 1), and an increased amount of nonreducing terminal galactose (Peak 2). In addition, both preparations showed significant amounts of 2-O-substituted mannose and 6-O-substituted galactose and nonreducing terminal fucose. Small amounts of 2,4-di-O-substituted, 3,6-di-O-substituted, and 3,4,6-tri-O-substituted mannose were noticed.

Endo-β-galactosidase Digestion of the Glycopeptide—Galactose oxidase/NaBH₄-labeled Band 3 glycopeptide was digested by endo-β-galactosidase and subjected to gel filtration on Sephadex G-50. As shown in Fig. 3, A, R, and C, the digest from i- or cord-Band 3 showed mostly the smallest component while that from I-Band 3 showed a number of oligosaccharides with various sizes. Since the above technique can show only the oligosaccharides derived from the nonreducing termini of glycopeptides, the oligosaccharide profile of the enzyme digest was separately analyzed on Bio-Gel P-4 after being labeled at the reducing termini of the oligosaccharides by reduction with NaBH₄ followed by NaB₃H₄ (15). As shown in Fig. 3, E and F, the digest from i- or cord-Band 3 glycopeptide predominantly produced disaccharide as well as small amounts of tri- and tetrasaccharide. The oligosaccharide eluted at Fractions 28 to 36 was found to be a sialic acid-containing tetrasaccharide, which is known to behave as a high molecular weight substance under this condition (21, 25). More than 75% of galactose present in the original glycopeptide was recovered in the di-, tri-, and tetrasaccharide fractions based on neutral sugar quantitation using the unreduced digest.

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results of permethylation). Galβ1 → 4GlcNAcβ1 → 3Gal, Fuca1 → 2Galβ1 → 4GlcNAcβ1 → 3Gal, and sialic acid-containing tetrasaccharides produced by endo-β-galactosidase are derived from the nonreducing terminal portion of carbohydrate chains while the disaccharide (GlcNAcβ1 → 3Gal) must be produced from the internal part of the chain (see Fig. 4). The molar ratio of disaccharide to tri- plus tetrasaccharide produced from i-Band 3 was found to be 3:2:1:0 based on paper chromatography. This indicates that the number of the GlcNAcβ1 → 3Gal repeating units per one carbohydrate side chain may not exceed 4 to 5 (see Fig. 4), since more than 75% of this chain was degraded by endo-β-galactosidase and the side chain is essentially linear in this molecule. However, the glycopeptide isolated from i-Band 3 contains about 12 residues of Gal (Table I). Therefore, the overall structure of i-Band 3 glycopeptide is envisioned as follows. Two or three carbohydrate chains, each being a linear chain and assigned as a "side chain," are linked to a "core structure" which provides the branching point for the linear side chains, probably through mannose. In fact, di-O- or tri-O-substituted mannose was detected in methylation analysis of the whole glycopeptide (Fig. 2).
Developmental Change of Band 3 Carbohydrate Structure

This paper clearly demonstrates the developmental change and genetic defect in the carbohydrate structure of Band 3. As shown in Fig. 4, the most critical change from fetus (i) to adult (l) cells is branching at position C-6 of galactose. To our knowledge, this is the first demonstration on a clear chemical basis of the structural change of cell surface carbohydrate during development. The same change might occur in cell surface glycoconjugates of human erythrocytes in general, since oligosaccharides released from surface-labeled cells by endo-β-galactosidase gave a similar characteristic pattern according to I- or i-cells as described (15).

The structure of the Band 3 carbohydrate has a few interesting features. Particularly, it has H antigenic determinants indicating that the molecule might serve as a carrier for ABH antigens as well as li antigens. Recently, glycopeptides with the structure shown here is likely based on the established structure of RB, the same as RI or RI + (Gal/β1 + 4GlcNAc/β1+ 3). Although the residue which links to C-6 of galactose is not characterized yet, for I antigenicity (8). Two or three chains shown here are linked to the same core portion (Ri). See text also.

DISCUSSION

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Acknowledgments—We wish to thank Mr. Mark Powell for the assistance in mass spectrometric analysis and Mr. Gary Ostrander for the technical assistance.

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J. Biol. Chem. 1979, 254:3700-3703.

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