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THE OCCURRENCE OF MOUSE-TYPE OLIGOSACCHARIDES IN MOUSE-HUMAN CHIMERIC IMMUNOGLOBULIN G

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The asparagine-linked sugar chains of mouse-human chimeric IgG which is composed of the variable regions derived from mouse and the constant regions derived from human and produced in mouse cells were quantitatively released as tritium-labelled oligosaccharides by hydrazinolysis followed by N-acetylation and NaB^3H_4-reduction. Paper electrophoresis in combination with sialidase digestion indicated that more than 70% of the oligosaccharides were neutral and the rest was sialylated oligosaccharides. Their structures were determined by sequential exoglycosidase digestions. The mouse-human chimeric IgG was shown to have same sugar chains as those of mouse IgG.

Carbohydrate moiety of antibodies (IgG) is necessary for effector functions of the antibodies such as complement binding, induction of antibody-dependent cellular cytotoxicity (ADCC), binding to macrophage Fc receptors, and rapid elimination of antigen-antibody complexes from the circulation but not for antigen binding and protein A binding (1). Recently, several groups have reported the production of very interesting chimeric antibodies which may have the therapeutic application against cancer (2-4). The chimeric antibody (IgG) is constructed with mouse-derived variable regions and human-derived constant regions. The oligosaccharides of the chimeric IgG are conserved in the constant regions derived from human. To investigate which type of oligosaccharides occur in the mouse-human chimeric IgG produced in mouse cells is important for understanding not only the effector function of the chimeric antibodies but also the mechanisms of glycosylation of proteins. We report here the oligosaccharide structures identified by enzymic micro-sequencing on the mouse-human chimeric IgG produced in mouse cells.

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MATERIALS AND METHODS

Materials---Chimeric antibody (IgG) was produced in mouse myeloma cells (SP2/0) as described previously (5). The chimeric IgG is a glycoprotein composed of the variable region derived from mouse Ig and the constant region derived from human IgG_1 (κ). The chimeric IgG was purified from the ascites by Protein A-Sepharose CL-4B column chromatography. The IgG preparation thus obtained reacted with anti-human IgG goat serum but not with anti-mouse IgG goat serum on Ouchterlony's double immunodiffusion assay. Finally, 910 μg of the chimeric IgG was obtained from 1.4 ml of the ascites.

NaB^3H_4 (320 mCi/mmol) was from New England Nuclear (Boston, MA). A lectin (RCA120) affinity HPLC column was from Honen Corporation (Tokyo). Concanavalin A Sepharose was from Pharmacia. Radioactive oligosaccharides used in this study were obtained as described previously (6,7). Sialidase purified from Arthrobacter ureafaciens was from Nakarai Tesque Inc. (Kyoto). Diplococcal β-galactosidase which cleaves the Galβ1→4GlcNAc linkage but not the Galβ1→3 GlcNAc and Galβ1→6GlcNAc linkages (8), and diplococcal β-N-acetylhexosaminidase were from Seikagaku Kogyo Co. (Tokyo). α-Mannosidase, β-galactosidase, and β-N-acetylhexosaminidase were purified from jack bean meal according to the methods of Li and Li (9). Snail β-mannosidase purified from Achatina fulica was from Seikagaku Kogyo Co. (Tokyo). α-L-Fucosidase from bovine epididymis was from Sigma Chemical Co. Digestions of the radioactive oligosaccharides ($1\text{-}6\times10^4$ cpm) with glycosidases were performed as reported previously (6).

Liberation of the asparagine-linked sugar chains of the chimeric IgG---The purified chimeric IgG (0.9 mg) was subjected to hydrazinolysis for 9 h followed by N-acetylation (10,11). The liberated oligosaccharides were reduced with NaB^3H_4 (0.5 mCi) in 160 μl of 0.05 N NaOH at 30 °C for 4 h to obtain tritium-labelled oligosaccharides. Then, 1mg of $NaBH_4$ in 100 μl of 0.05 N NaOH was added and the reaction was continued for an additional 2 h to complete the reduction. The radioactive oligosaccharide fraction (about 2×10^5 cpm) was obtained by paper chromatography as described previously (7).

Analytical methods---Paper chromatography, high voltage paper electrophoresis, Bio-Gel P-4 (extra fine) column chromatography, a lectin (RCA120) affinity HPLC, and other experimental procedures used in this study have been previously described (6,7,12).

RESULTS AND DISCUSSION

Separation of oligosaccharides liberated from the chimeric IgG by paper electrophoresis---When a mixture of the radioactive oligosaccharides from the chimeric IgG was subjected to paper electrophoresis at pH 5.4, one major neutral (N) and two minor acidic (A1 and A2) radioactive fractions were obtained (data not shown) as in the case of human and mouse IgGs (6,7). A molar ratio of N, A1, and A2 was calculated to be 72 : 20 : 8 from their radioactivities incorporated. The migration positions of A1 and A2 were the same as those of mono- and disialylated oligosaccharides, respectively, from both mouse and human IgGs. Upon incubation with sialidase, the acidic fractions were completely converted into neutral components. These results indicated that the acidic oligosaccharides A1 and A2 were monosialylated and disialylated derivatives, respectively.

Bio-Gel P-4 column chromatography of neutral oligosaccharide mixture from the chimeric IgG and its sequential exoglycosidase digests---The neutral

oligosaccharide mixture obtained by sialidase treatment of the radioactive oligosaccharide fraction from the chimeric IgG was subjected to sequential exoglycosidase digestions. The reaction mixture at each step was analyzed on a Bio-Gel P-4 column to determine the anomeric configuration and sequence of each monosaccharide in the oligosaccharides.

The radioactive neutral oligosaccharide mixture contained three major components eluted on the Bio-Gel P-4 column between about 12 and 15 glucose units (Fig. 1a). Upon incubation with jack bean β-galactosidase, the complicated pattern in Fig. 1a was converted to a simple pattern as shown in Fig. 1b. The major peak in Fig. 1b eluted at the same position as authentic GlcNAcβ1→2 Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$[2]. The shift of the elution positions corresponded to the release of 0, 1, and 2 galactose residues, respectively. Digestion of the radioactive products in Fig. 1b with jack bean β-N-acetylhexosaminidase released two N-acetylglucosamine residues and gave rise to a major and a minor peaks at a molar ratio of about 9 : 1 and eluting at about 8 and 7 glucose units, at the same positions as authentic Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ and Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$, respectively (Fig. 1c). The radioactive products in Fig. 1c were identified as trimannosyl core structures Manα1→(Manα1→)Manβ1→GlcNAcβ1→(± Fucα1→)GlcNAc$_{OT}$ by further sequential digestions with α-mannosidase, β-mannosidase, β-N-acetylhexosaminidase, and α-L-fucosidase (data not shown). The results indicated that the major and the minor peaks in Fig. 1c were derived from ±Galβ1→GlcNAcβ1→Manα1→(±Galβ1→GlcNAcβ1→Manα1→)Manβ1→GlcNAcβ1→(Fucα1→)GlcNAc$_{OT}$ and ±Galβ1→GlcNAcβ1→Manα1→(±Galβ1→GlcNAcβ1→Manα1→)Manβ1→GlcNAcβ1→GlcNAc$_{OT}$, respectively.

In separate experiments, the radioactive neutral oligosaccharides mixture gave the same products as those in Fig. 1c upon incubation with a mixture of diplococcal β-galactosidase which hydrolyses Galβ1→4GlcNAc linkage but not Galβ1→3GlcNAc or Galβ1→6GlcNAc linkage (8) and diplococcal β-N-acetylhexosaminidase which cleaves GlcNAcβ1→2Man linkage but not GlcNAcβ1→4Man and GlcNAcβ1→6Man linkage (13). It was also found (data not shown) that the radioactive oligosaccharide mixture bound to a concanavalin-A Sepharose column which requires, for binding, the presence of at least two α-mannosyl residues non-substituted at the C-3, C-4, and C-6 positions and the absence of bisecting N-acetylglucosamine residue in the biantennary complex type oligosaccharides (14-16).

From these results, it was concluded that the desialylated oligosaccharides of the chimeric IgG have biantennary complex type structures with two

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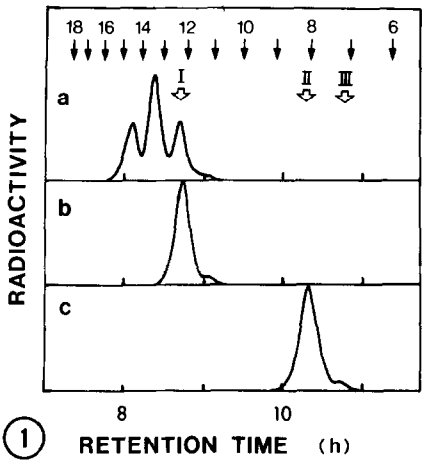
[2] Subscript OT is used to indicate NaB^3H_4-reduced oligosaccharides. All sugars mentioned in this paper are of the D-configuration except for fucose which has L-configuration.



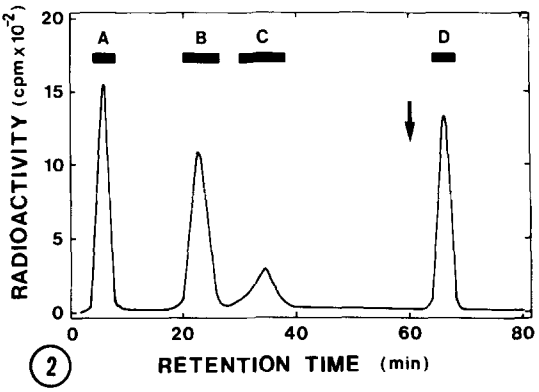


Fig. 1. Bio-Gel P-4 column chromatography of exoglycosidase digestion products. (a) The radioactive oligosaccharide mixture from the chimeric IgG after sialidase digestion; (b) the radioactive fraction in (a) after incubation with jack bean β-galactosidase; (c) the radioactive fraction in (b) after jack bean β-N-acetylhexosaminidase treatment. The radioactive oligosaccharides were subjected to Bio-Gel P-4 (extra fine) column chromatography. The black arrows indicate the elution positions of glucose oligomers (numbers indicate the glucose units) added as internal standards. The white arrows indicate the elution positions of authentic oligosaccharides: I, GlcNAcβ1→2Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$; II, Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$; III, Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$.

Fig. 2. Lectin (RCA120) affinity HPLC of the desialylated oligosaccharide mixture from the chimeric IgG. The radioactive oligosaccharide mixture obtained by sialidase treatment of the radioactive oligosaccharides liberated from the chimeric IgG by hydrazinolysis were subjected to lectin (RCA120) affinity HPLC. The arrow indicates the start of elution with lactose-containing buffer.

outer chain moieties of ±Galβ1→4GlcNAcβ1→2Man and without bisecting N-acetylglucosamine residue which is detected in human IgG oligosaccharides.

Structures of oligosaccharides of the chimeric IgG---In order to determine the ratio of the galactosylation in the oligosaccharides, the desialylated oligosaccharide mixture was subjected to a lectin (RCA120) affinity HPLC (Fig. 2). It was separated into four oligosaccharides A, B, C, and D at a molar ratio of 31 : 31 : 14 : 24. From the knowledge of the behaviour of biantennary complex type oligosaccharides without bisecting N-acetylglucosamine residue on this column (7), it was suggested that the four oligosaccharides A, B, C, and D were GlcNAcβ1→2Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)GlcNAc$_{OT}$, Galβ1→4GlcNAcβ1→2Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)GlcNAc$_{OT}$, GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)GlcNAc$_{OT}$, and Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)GlcNAc$_{OT}$, respectively. The proposed structures of desialylated oligosaccharides of the mouse-human chimeric IgG are shown in Fig. 3 together with those of human and mouse IgGs (6,7).

Fig. 3. Proposed structures of desialylated oligosaccharides of the mouse-human chimeric IgG (A-D) and oligosaccharide structures of human and mouse IgGs (6,7). The fucosylated oligosaccharides account for 90% of the total chimeric IgG oligosaccharides.

Comparison of oligosaccharides of the mouse-human chimeric IgG with mouse and human IgGs---The results obtained in this study indicated that the oligosaccharides of the mouse-human chimeric IgG produced in mouse cells and composed of the variable region from mouse and the constant region from human are identical to those of mouse IgG (Fig. 3). The ratio of each oligosaccharide is similar to that of mouse IgG previously reported (7). The oligosaccharides with bisecting N-acetylglucosamine residue which are identified in human IgG (6) but not in mouse IgG does not occur in the chimeric IgG. The present study suggests that glycosylation of proteins is regulated by the cells in which the glycoproteins are produced. It is also known that the different oligosaccharide structures occur among glycoproteins produced in the same cell (10,17-19). Further studies seem to be necessary for understanding the mechanisms of glycosylation of proteins.

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