Carbohydrate Compositions of Bovine Colostrum IgG Subclass Molecules

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SUMMARY

The carbohydrate compositions of bovine IgG subclass molecules prepared from colostrum and of their proteolytic fragments were studied by gas-liquid chromatography. Fucose, Mannose, galactose, and N-acetylglucosamine were found to be the constituent monosaccharides. The monosaccharide composition of bovine colostrum IgG2, reported for the first time in this study, was found to be almost identical with that of IgG1. The monosaccharides were found in the Fc fragments of both subclass molecules, but not in the Fab fragments.

Introduction

Virtually all immunoglobulins contain significant amounts of carbohydrate. There is, however, considerable quantitative and qualitative variation, even among molecules from the same species and of the same class[1]. The biological role of the carbohydrate in immunoglobulins is yet unknown. It is postulated that it may play a role in the passage of immunoglobulins through biological membranes, as during their secretion from the cell or their passage through the placental barrier, and also in certain other biological functions of the immunoglobulns[2].

Only limited information is now available on the characteristics of the oligosaccharides bound to bovine IgG. The monosaccharide composition in one subclass (IgG2) from colostrum has not been reported in the literature. In this study we investigated the composition of the carbohydrate in the individual bovine IgG subclasses prepared from colostrum by gas-liquid chromatography. We also investigated the distribution of the monosaccharides in the subclass molecules by analyzing the composition of the carbohydrate in their proteolytic fragments.

Materials and Methods

IgG subclasses

IgG1 and IgG2 were prepared from colostrum by ammonium sulfate fractionation, anion-exchange chromatography, and gel filtration[3].

Preparation of the proteolytic fragments of the subclasses was done by papain digestion followed by anion-exchange chromatography on DEAE-cellulose and gel filtration on Bio-Gel P-100[4].

Carbohydrate analysis

Neutral and amino hexoses were determined by gas-liquid chromatography of alditol acetates according to the methods of Niedermeier[5] and Tomana et al.[6] with slight modifications.

Alditol acetates in CH_2Cl_2 were analysed with a Shimadzu GC-5A gas chromatograph equipped with a dual-flame ionization detector. The neutral sugars were chromatographed on a glass column (0.3 x 200 cm) packed with 0.2% polyethylene glycol succinate, 0.2% polyethylene glycol adipate and 0.4% silicone XF-1150 on 100/120 mesh Gas-Chrom P (purchased from Wako Pure Chemical Indus-

tries, Ltd., Osaka). The starting temperature of 140℃ was programmed to 200℃ at the rate of 5℃/min. Arabinose was used as the internal standard. The amino sugars were chromatographed on a glass column (0.3 x 200 cm) packed with 3% poly-A 103 on 100/120 mesh Gas-Chrom Q (purchased also from Wako Pure Chemical Industries) using D-mannosamine as the internal standard. The starting temperature of 210℃ was programmed to 260℃ at the rate of 5℃/min.

Determination of protein concentration

Concentrations of bovine IgG1 and IgG2 were determined spectrophotometrically using the following extinction coefficients[7]; $E_{280}=13.5$ for IgG1 and $E_{280}=12.0$ for IgG2.

Concentrations of the proteolytic fragments were determined by Bio-Red protein assay using IgG1 as standard.

Results and Discussion

Carbohydrates are labile in acid solution at elevated temperature and the conditions for hydrolysis of the glycoprotein molecule have to be determined for the liberation of each carbohydrates. An

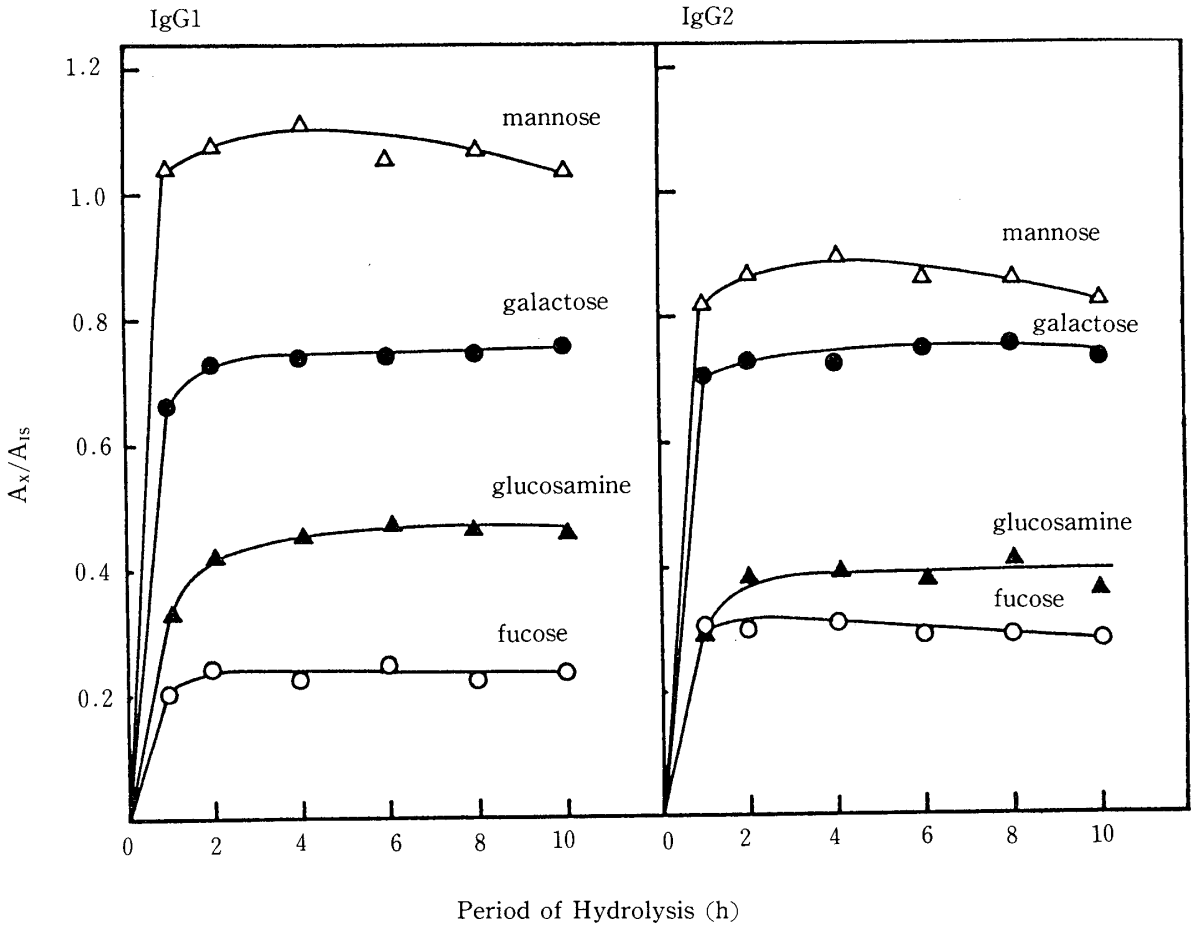

Fig. 1 Liberation of neutral and amino hexoses from bovine IgG1 and IgG2 by hydrolysis with 2 N HCl.
A_X = area of peak representing carbohydrate under analysis
A_{IS} = area of peak representing internal standard sugar

attempt was therefore made to determine the optimum conditions for hydrolysis which yield the complete cleavage of glycosidic bonds without any decomposition of the carbohydrate under consideration.

Figure 1 shows the results of the time course study of liberation of sugars from bovine IgG1 and IgG2 by hydrolysis with 2N HCl. For fucose the maximum amount of liberation was observed at 2 to 6 hr IgG1 and at 1 to 4 hr in IgG2. The amount of mannose reached maximum at 4 hr for both IgG1 and IgG2. Galactose was liberated gradually after 2 hr of hydrolysis; the maximum amount was found at 10 hr for IgG1, while some decomposition occurred at the same period for IgG2. For glucosamine the maximum was observed at 6 hr in IgG1 and 8 hr in IgG2. Taking experimental errors into consideration, it was determined that the 2 hr hydrolysate was used for the determination of fucose, the 4 hr hydrolysate for mannose, and the 8 hr hydrolysate for both galactose and glucosamine for both IgG subclasses. These conditions of hydrolysis are in good agreement with those used in the studies of human immunoglobulins[5,6].

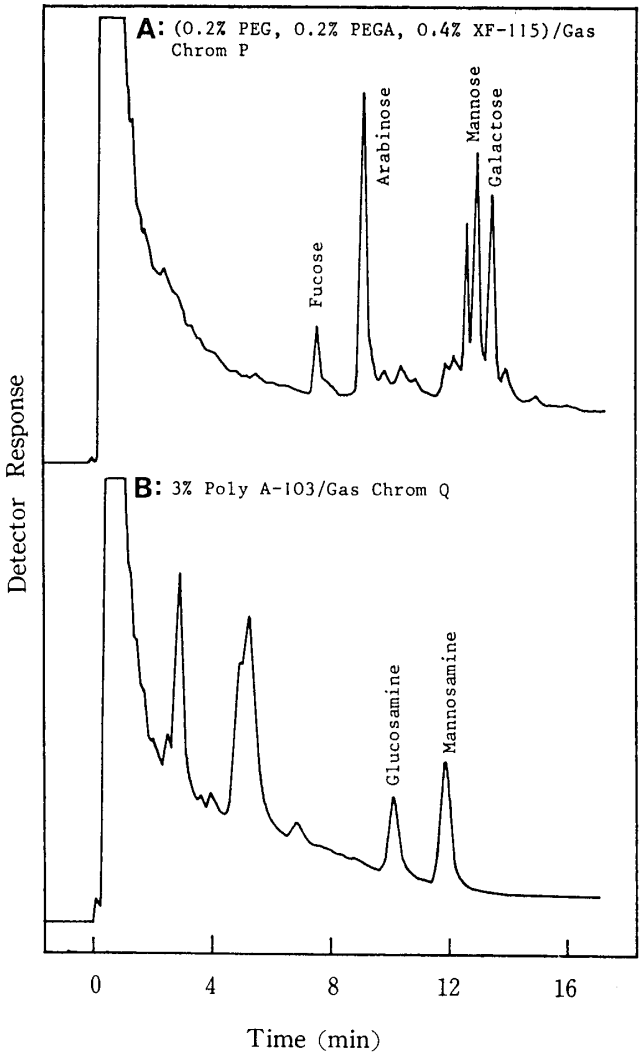

Fig. 2 Gas chromatography of alditol acetates prepared from bovine IgG1 hydrolyzate

Figure 2 exemplifies the gas chromatogram of the alditol acetates prepared from the hydrolysates of IgG1. In the subclass fucose, mannose, galactose, and glucosamine were detected as the constituent monosaccharides, supporting the data reported earlier[8,9]. The considerably large peak detected just before the peak of mannose was seen in Figure 2. What is responsible for this peak is not yet obvious, but the peak height was not proportional to the quantity of IgG1 analyzed, and no such peculiar peak was found at the identical retention time in the chromatogram of the proteoytic fragment of the subclass. Therefore, it was concluded that the peak should be neglected in the present investigation. Likewise, several small peaks were observed in the chromatogram of IgG2 between the peaks of arabinose and mannose (result not shown). They were also neglected for the same reason.

In summary the carbohydrate compositions of IgG1 and IgG2 and their proteolytic fragments are presented in Table 1. Although sialic acid has been found in bovine IgG1, we did not determine its content in this study. When compared with the sugar composition of IgG1 reported previously[8,9], the contents of both hexose and hexosamine were found to be comparable. The carbohydrate composition of another bovine sublass, IgG2, was initially investigated in this study. Although the glucosamine content of IgG2 was slightly less than in IgG1, no fundamental difference was found between the

Table 1. Carbohydrate composition of bovine colostrum IgG1 and IgG2

		Fuc	Man	Gal	GlcNAc	Total Carbohydrate
G1Fc	%	0•22	1•36	0•86	2•27	4•71
	Mols/Fc	0•6	3•4	2•1	4•6	10•7
G2Fc	%	0•29	1•31	0•79	2•30	4•69
	Mols/Fc	0•8	3•5	2•1	5•0	11•4
IgG1	%	0•14	0•76	0•43	1•25	2•58
	Mols/IgG	1•3	6•6	3•7	8•8	20•4
IgG2	%	0•19	0•80	0•55	1•00	2•54
	Mols/IgG	1•7	6•6	4•5	6•7	19•5

The molecular weight of G1Fc and G2Fc used for the calculation of molar composition was assumed to be 45,000 and 46,000, respectively. G1Fc and G2Fc are the Fc fragments prepared from IgG1 and IgG2, respectively.

carbohydrate compositions of the two subclasses. This was supported by the data of the Fc fragments which suggest that the carbohydrate composition of the bovine IgG subclasses is vertually identical. The monosaccharides were found in the Fc fragments of both subclass molecules, but not in the Fab fragments.

IgG1 from colostrum was reported to possess two glycan moieties which are linked to the peptidic chain by an N-(β-asparty1)-N-acetylglucosaminylamine bond[9]. One oligosaccharide chain contained 0 or 1 mol of fucose, 3 mols of mannose, 2 mols of galactose, 4 mols of glucosamine, and 0 or 1 mole of sialic acid. It can, therefore, be assumed theoretically that bovine colostral IgG1 contains 0 or 2 mols of fucose, 6 mols of mannose, 4 mols of galactose, and 8 mols of glucosamine. The molar composition shown in Table 1 is consistent with the theometical one. Accomding to Tai et al. [8], on the other hand, a sugar chain in which deletion occurred in one galactose residue was also present in IgG from bovine serum. The data presented in Table 1 suggest that such deletion may not occur in the oligosaccharide chains of IgG1 and IgG2 from bovine colostrum.

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ウシ初乳の IgG サブクラス分子の糖組成

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要　　約

初乳から調製したウシの IgG サブクラス分子とその消化フラグメントの糖組成をガスクロマトグラフィーで調べた。いずれのサブクラスも，フコース，マンノース，ガラクトース，N-アセチルグルコサミンを持つことが明らかにされた。本研究によって初めて明らかにされたウシ初乳の IgG2 の糖組成は，IgG1 とほとんど同一であることがわかった。いずれのサブクラスでも，糖は Fc フラグメントに検出され，Fab フラグメントには見出されなかった。

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