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Abstract

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STRUCTURE OF BLOOD GROUP SUBSTANCES

ALKALINE HYDROLYSIS OF BLOOD GROUP SUBSTANCE A + H

The study of mixed biopolymers, in particular glycopeptides of such complex structure as blood group substances (BGS), requires above all the development of general concepts of their structure. For this purpose one usually resorts to various methods of destruction of the biopolymer with analysis of the fragments obtained. However, the technique used for this—the qualitative assessment, by paper chromatography, of the mixture of fragments obtained upon destruction of the polymer—as a rule gives very limited information about the overall structure of the polymer. In this connection we have developed a new approach: the mixture of degradation products from a small amount of substance is separated by gel filtration, and the resulting fractions are analyzed for the content of the various components of the mixture. Analytical curves constructed on this basis make it possible to compare the monomeric composition of the fractions and the molecular weight of the fragments obtained, which provides very valuable information about the structure of the polymer and subsequently permits the selection of the most suitable conditions for carrying out fragmentation of the biopolymer on a preparative scale, in order to study the structure of the resulting fragments.

In the present work this method has been used to study the alkaline hydrolysis of BGS obtained from pig stomachs.

Experimental Part

BGS (A + H)* from the mucous membranes of pooled pig stomachs was obtained by Kabat' s method ([1]) with subsequent fractionation from phenol ([2]), and was characterized by gel filtration and ultracentrifugation ([3]).

Gel filtration was carried out on Sephadex G-25 in 0.1 N CH_3COOH, and on G-100 and 7% agar gel (60 mesh) ([4])—in 0.01 N acetate buffer (pH 5.0) containing 0.1 N NaCl.

In the fractions the following were determined: the sum of galactose and fucose by the anthrone method, fucose by Dische' s method, hexosamines by the Elson-

Morgan method after hydrolysis with 2 N HCl for 2 h at 100° ([1]). The free amino group was determined by reaction with trinitrobenzenesulfonic acid (TNBS) ([5]).

Destruction of BGS with 0.1–0.15 N NaOH in the presence of 1% $NaBH_4$ ([6]). 200 mg of BGS are dissolved in 4 ml of 0.1 N NaOH, 40 mg of $NaBH_4$ are added, and the mixture is kept at temperatures of 17, 20, or 25° for 6–15 days; it is acidified with 1 N CH_3COOH, 0.5-ml portions are taken, and subjected to gel filtration.

The amino-acid content was determined on an automatic analyzer.

Destruction of BGS with 0.05 M Na_2CO_3 solution. 50 mg of BGS are dissolved in 5 ml of 0.05 M Na_2CO_3 solution (pH 10.8), heated for 4–10 min in a boiling water bath, cooled, neutralized with 1 N CH_3COOH, 1.5-ml portions are taken, and subjected to gel filtration.

* As is known, BGS A obtained by this method also possesses a certain H activity, which cannot affect the results in addressing the question of the general architecture and the principal types of bonds in BGS.

Determination of the total amino-acid content after hydrolysis of the fractions with 5.7 N HCl, 100°, 20 h. To separate them from hexosamines, the hydrolysate is evaporated in vacuo to dryness and passed through a Dowex 50X8 column (Na form) (1.2 × 15 cm) in 0.35 M citrate buffer, pH 5.28; the first 20 ml are collected in a volumetric flask, made up to 25 ml, and amino acids are determined in an aliquot by TNBS, with valine as the standard.

Results and discussion

Although the study of BGS was begun comparatively long ago, data on the general architecture of the biopolymer and on the principal types of bonds are still lacking. One of the main chemical features of BGS is their high lability to the action of alkalis (7–9). It should be noted that even in distilled water at 20° BGS gradually undergoes destruction, whereas at pH 3 no appreciable destruction is observed (3).

Alkaline cleavage of BGS in the presence of $NaBH_4$ was studied by Kabat (6, 9). Along with other fragments, dulcitol and 2-acetamido-2-deoxydulcitol, formed upon reduction of the corresponding monosaccharides, were isolated from the dialysate. Since rupture of ordinary glycosidic bonds of polysaccharide chains under these conditions is practically excluded, we assumed that the formation of hexitols is associated with rupture of O-glycosidic bonds with hydroxyamino acids, which are present in BGS in large amounts. The lability of such bonds toward alkalis is well known (see, e.g., (10)) and was verified by us on specially synthesized models of O-glycosides of serine (11).

Fig. 1. Gel filtration of BGS after treatment with 0.1 N NaOH + 1% $NaBH_4$ (6 days, 17°): *a* —on Sephadex G-25 (2 × 117 cm); *b* —on Sephadex G-100 (1.8 × 66 cm). 1 —optical density in the reaction with anthrone in 65% H_2SO_4; 2 —

Figure 1

Figure 1: Figure 1

the same in the Dische reaction; 3 –the same in the Elson–Morgan reaction; 4 –the same in the reaction with Ehrlich' s reagent.

To test this hypothesis we studied in detail the alkaline destruction of BGS using gel filtration.

When BGS is cleaved with 0.1 N NaOH in the presence of 1% $NaBH_4$ at 17° for 6 days (Fig. 1), two fractions are formed that differ greatly in molecular weight: a high-molecular-weight fragment (~50% of the weight of the original polymer) and an oligomeric fraction. The bulk of the fragments of the oligomeric fraction have a rather similar molecular weight (Fig. 1a); monosaccharides and lower oligosaccharides are practically not formed. The main part of the high-molecular-weight fraction, during gel filtration on Sephadex G-100, comes out with the front (Fig. 1b) and gives a peak sharply separated from the low-molecular-weight fraction.

Determination of the ratio of the contents of the different components in the two fractions obtained from Sephadex G-100 (Fig. 1b) showed that this ratio is, for fucose, 1 : 1; for neutral sugars, 1 : 1; for hexosamines, 0.78 : 1; and for NH_2 groups, 0.45 : 1. The oligomeric fraction contains practically no amino acids. The absence of amino acids in the cleaved-off portion

polymer indicates the stability of peptide bonds under the conditions of alkaline cleavage. The similarity of the carbohydrate composition of the fractions obtained is also noteworthy.

Increasing the NaOH concentration to 0.15 N and the temperature to 20°, and also carrying out alkaline cleavage in 0.1 N NaOH at 25° for 15 days (Fig. 2), although it leads to further destruction of BGS, does not change the general pattern of cleavage (cf. Figs. 1a and 2). Kabat and co-workers indicate ([9]) that the addition of $NaBH_4$ during alkaline cleavage of BGS made it possible, by preventing further destruction of the cleaved oligomers, to isolate fragments containing up to 10 monosaccharide residues. The parallel treatment of BGS with 0.1 N NaOH without addition of $NaBH_4$, carried out by us, showed (cf. Figs. 1a and 3) that the main portion of the oligosaccharides formed is not destroyed further and has molecular weights close to the products of reductive cleavage. This indicates the stability of the oligomeric fraction under the conditions of polymer cleavage.

Fig. 2
Fig. 2. Gel filtration of BGS after treatment with 0.1 N NaOH + 1% $NaBH_4$ (15 days, 25°) on Sephadex G-25 (1.8 × 66 cm)

Fig. 3
Fig. 3. Gel filtration of BGS after treatment with 0.1 N NaOH (6 days, 17°)

on Sephadex G-25 (2 × 117 cm)

Fig. 4

Fig. 4. Gel filtration of BGS on Sephadex G-25 (1.8 × 66 cm) after treatment with 0.05 M Na_2CO_3: *a* –100°, 3 min; *b* –100°, 10 min

Proceeding from the assumption that the polymer contains alkali-labile O-glycosidic bonds with oxyamino acids, we analyzed the amino acids in the polymer before and after alkaline cleavage. Under the action of alkali, the O-glycosides of serine and threonine are cleaved by the β-elimination mechanism with formation, respectively, of α-aminoacrylic and α-aminocrotonic acids, which are reduced by $NaBH_4$ to alanine and α-aminobutyric acid ([12]). Comparison of the amino-acid composition of the original BGS and the products of its cleavage by 0.1 N NaOH in the presence of 1% $NaBH_4$

shows (Table 1) a considerable decrease in the content of serine and threonine and, simultaneously, an increase in the content of alanine.

The results obtained by us, in combination with Kabat' s data (9) on the isolation of hexitols from the cleavage products of BGS under analogous conditions, clearly indicate the presence in BGS of O-glycosidic bonds with serine and threonine as one of the types of linkage between the carbohydrate and peptide parts of the polymer.

Table 1

Change in the content of hydroxyamino acids* during destruction of BGS

	Amino acid content in µmoles in the original BGS	Amino acid content in µmoles after destruction	Amino acid content in µmoles difference in µmoles
Aspartic acid	0.241	0.245**	–
Threonine	1.988	1.567	−0.421
Serine	0.999	0.609	−0.390
Proline	1.218	1.199	–
Glycine	0.316	0.314	–
Alanine	0.563	0.833	+0.270
Glutamic acid	0.352–	0.346	–

* The analysis was performed by Ts. A. Egorov, to whom the authors express their appreciation.

** The absence of changes in the content of dicarboxylic amino acids does not indicate the absence of ester bonds in the polymer, since special experiments have shown that, under the described conditions, cleavage of the ester bond is not accompanied by reduction.

The considerable content of hydroxyamino acids in glycopeptides of this class and, consequently, the multiplicity of such bonds make understandable the high lability of BGS in an alkaline medium.

It was of interest to compare the cleavage of BGS in alkali according to Kabat with the pattern of cleavage of BGS in 0.05 N Na_2CO_3 at 100° for 3–4 min (Fig. 4, *a*), described earlier by Morgan (7). Comparison of Figs. 4*a* and 1*a* shows that the pattern of cleavage of BGS by soda very much resembles the results obtained at a definite stage of reductive alkaline cleavage.

The high-molecular-weight fragment, in molecular weight, considerably exceeds that obtained earlier (7), since it is not retained by Sephadex G-100 and, as gel filtration on agar gel showed, is not completely homogeneous.

Some increase in the time of treatment of BGS with soda solution does not lead to a noticeable increase in the amount of low-molecular-weight products (Fig. 4, *b*) and, accordingly, to cleavage of the polymer as deep as in the case of alkaline-reductive cleavage, which indirectly indicates the existence of different types of alkali-labile bonds.

The absence, among the products of alkaline cleavage of BGS, of a broad spectrum of fragments differing in molecular weight testifies to the existence in BGS of a molecular skeleton bearing cleavable fragments that are close to one another in size and composition.

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Note: Figure translations are in progress. See original paper for figures.