ON THE STRUCTURE OF THE CARBOHYDRATE CHAINS OF BLOOD GROUP SUBSTANCES

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A scheme for the general composition of the carbohydrate chains in blood group substances is suggested. The principal peculiarities of this scheme are the high degree of carbohydrate chain branching and the presence in the region close to the peptide backbone of a fragment built up of only N-acetylhexosamines.

The biological specificity of blood group substances (BGS) is known to be determined by the structure of their carbohydrate chains [1] which are attached to the central peptide backbone [2]. The nature of the terminal nonreducing monosaccharides and the structure of the neighbouring regions has been established by means of immunochemical methods as well as by partial degradation of BGS [1]. No data have been available up to now concerning the general structure of carbohydrate chains in BGS. A structural scheme for the nonreducing terminal parts of the carbohydrate chains and the part close to the peptide backbone has been recently put forward by Lloyd, Kabat and Licerio [3].

On the basis of our present studies of BGS (A + H) (isolated from hog stomach lining according to a previously described procedure [4]) together with data on the composition of oligosaccharides isolated earlier [1,3,5,6], we suggest a general scheme for the structure of BGS carbohydrate chains.

The main approaches to the elucidation of the structure of the carbohydrate chains were as follows: stepwise Smith degradation, methylation studies, a combined chemical and enzymatic degradation and a study of alkaline degradation products. The latter consisted in the determination of the formation rate of degradation products of N-acetylhexosamine units, 3-acetamido-5-(1,2-dihydroxyethyl)-furan (chromogen), and galactose units, saccharinic acids and 2-substituted hex-2-enose.

During the stepwise Smith degradation of BGS the first and second periodate oxidations proceeded to completion within 5 hours, the amount of oxidant consumed being 2.5 and 1.3 moles per 1000 g of BGS. The lowmolecular weight products contained only traces of galactose and N-acetylhexosamine derivatives, thus demonstrating that the oxidation proceeded only from the nonreducing termini of chains. Two successive Smith degradations result in destruction of ca. 70% of the original galactose content and ca. 50% of that of N-acetylhexosamine [7].

The total number of carbohydrate chains in BGS should be approximately 60 as deduced from [8-10], and taking into account the chain branching into 2 determinants [3]. Consequently the high degree of galactose and hexosamines oxidation can only be explained on the basis of a high degree of branching of the carbohydrate chains.

The data on methylation of BGS are also in accord with the complexity and high degree of branching of its carbohydrate chains. Several O-methyl-derivatives of galactose (2,3,4,6--tetra-, 3,4,6-tri-, 2,4,6-tri-, 2,3,6-tri-, 2,3,4-tri-, 4,6-di-, 2,6-di-, 3,6-di-O-Me-Gal), hexosamines (3,4,6-tri-, 3,6-di-, 3-, 6- O-Me-GN, 3,4,6-tri-O-Me-GalN) and fucose (2,3,4-tri-O-Me-Fuc) were identified among the products of hydrolysis of

the methylated BGS thus revealing a large variety of intermonomeric linkages [11,12]. According to these data, fucose, and in part, galactose, 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose occupy terminal positions, while galactose and 2-acetamido-2-deoxy-D-glucose are also located in branch-points. The presence of galactose with substituents at C-4 and C-3 or C-2 follows also from the identification of threitol among the lowmolecular weight products of the second Smith degradation of BGS [7].

Treatment of BGS with 0.05 M Na_2CO_3 at 70° for 4 hrs causes rapid destruction of N-acetylhexosamines which give rise to chromogen production. Galactose degradation products appear and accumulate very slowly. Such a treatment is accompanied with destruction of ca. 30% of the total content of N-acetylhexosamine and of ca. 4% of that of galactose [13]. Hence galactose seems to be practically absent from the carbohydrate chain region that is subjected to degradation under conditions used, and consequently does not appear to enter the carbohydrate-peptide linkage. These data also give an unambigous proof that the monosaccharide attached to serine and/or threonine residues is N-acetylhexosamine. It should be pointed out as well that the reducing end of the carbohydrate chain consists of several (no less than 3-4) N-acetylhexosamine residues linked with 1→3 bonds, this being the backbone of this chain region. Characterization of chromogens formed upon alkaline degradation of BGS proved to be of value in further elucidation of carbohydrate chain structure. Two types of chromogens, viz. substituted and nonsubstituted, were formed, these corresponding to the presence of 1→3 linked N-acetylhexosamines with and without substituents at C-6 [13].

Alkaline degradation of BGS after periodate oxidation and borohydride reduction results in formation of decreased amounts of nonsubstituted chromogen /14/. This fact can be explained as being due to periodate oxidation of the terminal nonreducing N-acetylhexosamine residues in the side chain that is linked with the chain fragment formed of 1 ⟶ 3 linked N-acetylhexosamines and which is subject to degradation upon treatment with alkali.

Examination of the kinetics of substituted and unsubstituted chromogen formation (fig. I) showed that the curve of accumulation of the former had a lag-phase /14/. It follows, unequivocally, that N-acetylhexosamine at the carbohydrate-protein linkage bear no branching.

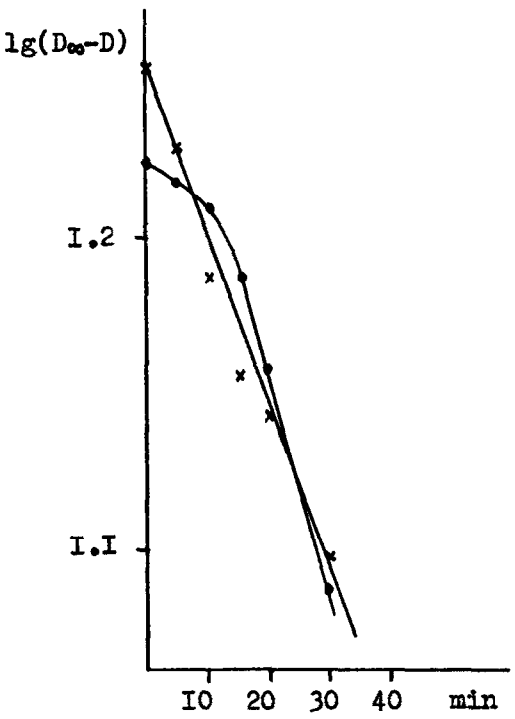
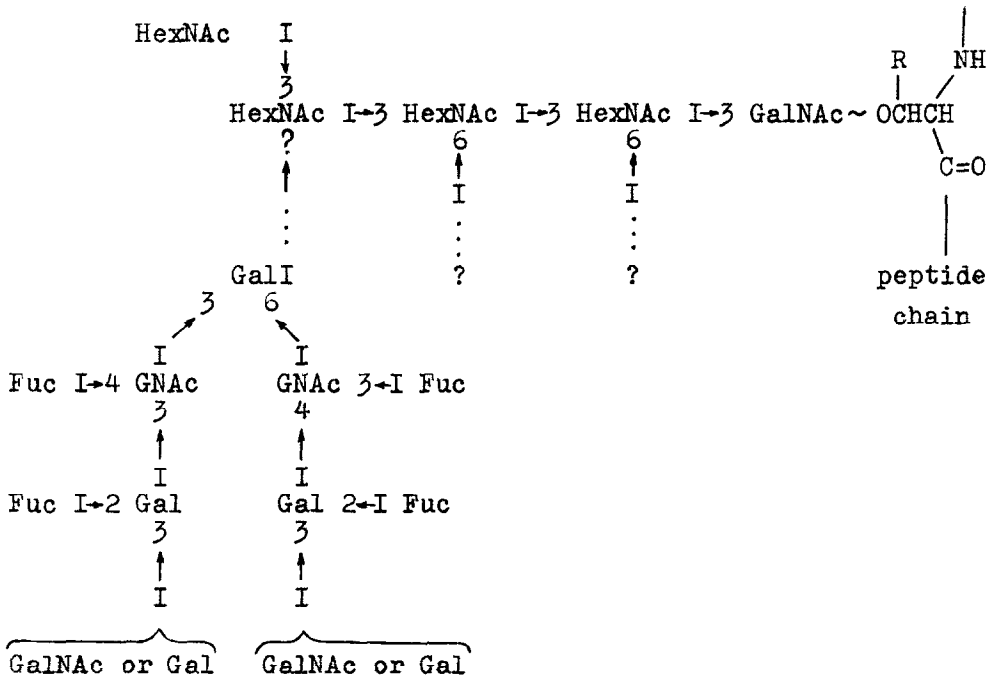


Fig. 1. The kinetics of the substituted and unsubstituted chromogen formation from BGS treated with 0.05 M aqueous Na_2CO_3 at 70°.
⟶×⟵×⟶×⟵ - unsubstituted chromogen
⟶•⟵•⟶•⟵ - substituted chromogen.

A combination of degradation methods (Smith degradation followed by enzymatic hydrolysis by a Clostridium perfringens glycosidase preparation [15]) resulted in isolation of a high-molecular weight fragment consisting mainly of amino acids (60%) and hexosamines (34%), the 2-acetamido-2-deoxy-D-galactose to 2-acetamido-2-deoxy-D-glucose ratio being equal to 7.5 : 1 [16]. Alkaline degradation and periodate oxidation of this fragment revealed that in most of O-glycosidic carbohydrate-protein linkage a single monosaccharide, viz. 2-acetamido-2-deoxy-D-galactose, was present.



The scheme for the carbohydrate chain structure of BGS presented here may, of course, be completed, altered or refined in the light of newer experimental data, particularly on the details of attachment of the part responsible for the biological activity to the fragment built up of N-acetylhexosamines.

We should like to emphasize two principal peculiarities of this scheme, viz. the high degree of carbohydrate chain branching and the presence in the region close to the peptide backbone of the fragment built up of only N-acetylhexosamines.

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