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Note

Evidence for the degradation of sugars during the resin hydrolysis of glycoproteins

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Quantitative gas—liquid chromatography (GLC) of alditol acetates has been used frequently to determine the carbohydrate composition of glycoproteins [1—7]. Of the methods available for hydrolysis that using dilute acid in the presence of a cation-exchange resin [1, 2, 4, 6, 7] offers the advantages of mild conditions, minimization of losses due to the interaction of amino acids and carbohydrates and no destruction of the liberated sugars. In connection with a study of the carbohydrate composition of α_2-macroglobulin (α_2-M) isolated from the plasma of patients with cystic fibrosis and normal controls [8] we have had occasion to reinvestigate the resin hydrolysis procedure. The results of this study indicate that, in contrast to the investigations of Lehnhardt and Winzler [2] and Porter [6], there was significant degradation of sugars during hydrolysis.

MATERIALS AND METHODS

Materials

L-Fucose, D-mannosamine hydrochloride and bovine serum albumin were

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obtained from Sigma (St. Louis, MO, U.S.A.) and lactose from Fisher Scientific (Vancouver, Canada). All other carbohydrates were Calbiochem A grade (Calbiochem, La Jolla, CA, U.S.A.). Methanol (A.C.S. Spectranalyzed), pyridine and acetic anhydride (A.C.S.) from Fisher Scientific were used without further purification. Anion-exchange resin AG 1-X8, 200—400 mesh (Cl^-) and cation-exchange resin AG 50W-X8, 200—400 mesh (H^+) were obtained from Bio-Rad Labs. Canada (Mississauga, Canada).

α_2-Macroglobulin was isolated from normal plasma according to the method of Bridges et al. [9] and the Tris buffer removed by exchange dialysis against sodium bicarbonate pH 8.3. A gift of pure, lyophilized α_1-acid glycoprotein from Drs. G. Strecker and B. Fournet (Laboratoire de Chimie Biologique, Université de Sciences et Techniques de Lille, Lille, France) is gratefully acknowledged.

Analysis of sugar mixtures and glycoproteins

Analyses were performed on hydrolysates prepared essentially as described by Lehnhardt and Winzler [2] using arabinose [3] and mannosamine [5] as the internal standards for the neutral sugars and hexosamines, respectively. Elution of the hexosamines from the cation-exchange resin was achieved using 0.3 *M* hydrochloric acid. The molar response factor for each monosaccharide was determined relative to the appropriate internal standard by the above procedure with omission of the hydrolysis step [2].

Gas—liquid chromatography

Alditol acetates were separated on a 183 cm × 2 mm I.D. siliconized glass column containing GP 3% SP-2340 on 100—120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.) using a Hewlett-Packard 7610A chromatograph fitted with a flame-ionization detector and on-column injection; nitrogen was used as the carrier gas. All separations were carried out isothermally; neutral sugars and hexosamines were analyzed at 200°C and 240°C, respectively. Peak areas were recorded with a 3370B electronic integrator.

RESULTS

Fig. 1A and B demonstrate that, as shown previously [2, 6], sugars were released from α_1-acid glycoprotein and α_2-M at different rates. However, in contrast to these reports, loss of the released sugars was observed with extended hydrolysis times. This was apparently not due to interaction of the free sugars with amino acids as studies on the hydrolysis of lactose demonstrated that the release and subsequent loss of galactose were not affected by the presence of bovine serum albumin. Presumably, the observed loss of the released sugars was a consequence of acid degradation since it was also observed when mixtures of free sugars were hydrolysed (Fig. 1C).

Analysis of the data obtained from the hydrolysis of the free sugars (Fig. 1C) indicated that there were significant differences in the rates of degradation of the various sugars. The data shown were reanalyzed by expressing them as the percentage of the maximal value and regression analyses were performed. Comparison of the slopes of the regression lines [10] indicated

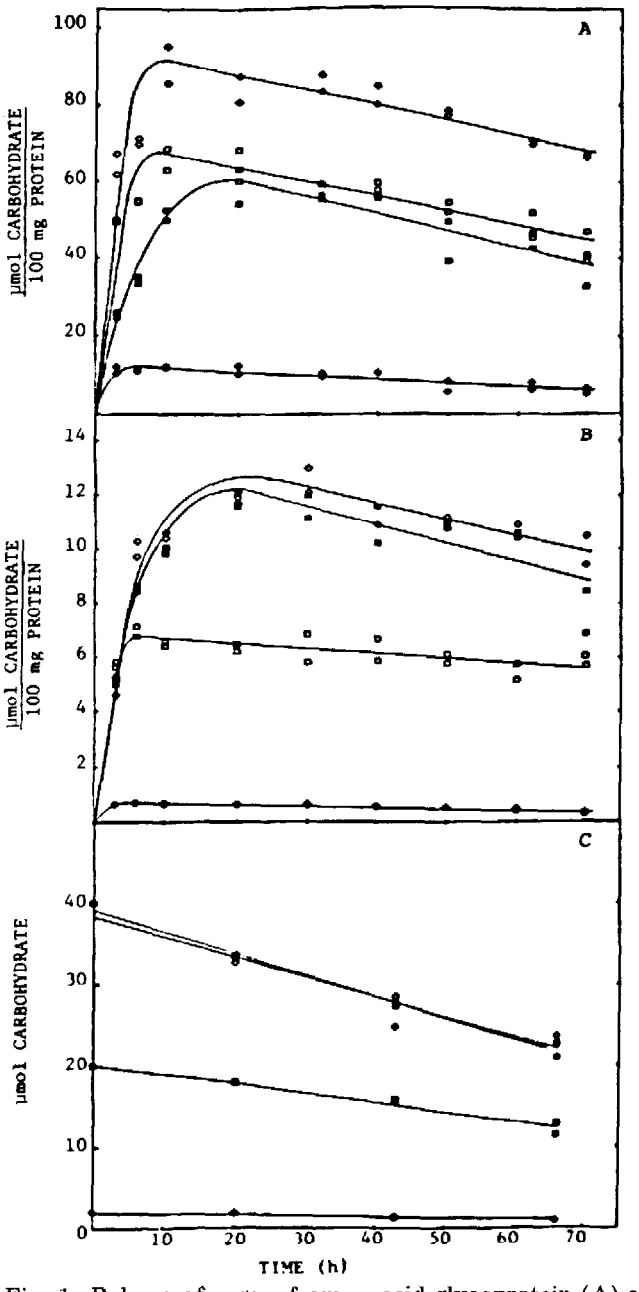

Fig. 1. Release of sugars from α_1-acid glycoprotein (A) and α_2-M (B) by acid hydrolysis in the presence of AG 50W resin. Duplicate aliquots of α_1-acid glycoprotein (180 μg) and α_2-M (360 μg) were hydrolyzed in 20% (w/v) AG 50W-X8, 200—400 mesh (H^+) in 0.01 *M* hydrochloric acid for the time periods indicated. Following hydrolysis and cooling, the internal standards were added and the neutral and amino sugars were separated and estimated by GLC of the derived alditol acetates [2, 3, 5]. The straight line portions of the graphs were obtained by regression analysis; correlation coefficients were greater than 0.87. (C) Degradation of free sugars during resin hydrolysis. Duplicate aliquots of a mixture of glucosamine, mannose, galactose and fucose in amounts similar to those found in α_2-M were hydrolyzed as described above and regression analysis was performed. The zero-time values were obtained by analyzing aliquots of the mixture which were mixed with the resin but not subjected to acid hydrolysis. Correlation coefficients were greater than 0.97. (◇) Glucosamine; (■) mannose; (□) galactose; (◆) fucose.

that fucose was degraded at a significantly different rate to mannose ($p < 0.001$), galactose ($p < 0.001$) and glucosamine ($p < 0.001$). In addition, mannose was degraded at a different rate to galactose ($p < 0.01$).

DISCUSSION

Acid-catalyzed degradation of sugars is well documented [2, 4, 11]. Previous investigations of resin hydrolysis methods indicated, however, that there was no degradation of the sugars released from glycoproteins with extended hydrolysis time [2, 6]; therefore the hydrolysis times selected were those at which maximal release of all sugars was obtained. The results presented in this study indicate, however, that degradation of sugars is a continuous process and, furthermore, that the rates of degradation of the released sugars may be different. As a consequence, the maximal values obtained for the individual components must be less than the actual values and analyses performed at a single hydrolysis time will result in incorrect molar ratios of the components. Each glycoprotein will therefore require careful investigation of appropriate hydrolysis times to take into account differences in the rate of release and degradation of each sugar. The contributions will probably be different for each glycoprotein.

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