have been made after some vibrational relaxation of the observed species has occurred.

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[1] Basco, N., and Norrish, R. G. W., *Nature*, **189**, 455 (1961); *Proc. Roy. Soc.*, A, **260**, 293 (1961); *Canad. J. Chem.*, **38**, 1769 (1960).
[2] Smith, F. T., *J. Chem. Phys.*, **31**, 1352 (1959).
[3] Jones, W. M., and Davidson, N., *J. Amer. Chem. Soc.* (in the press).
[4] Cross, P. C., and Van Vleck, J. H., *J. Chem. Phys.*, **1**, 350 (1933); Eq. 6.
[5] Glasstone, S., Laidler, K. J., and Eyring, H., *The Theory of Rate Processes*, 101 (McGraw-Hill Book Co., Inc., New York, 1941).
[6] Polanyi, J. C., *J. Chem. Phys.*, **31**, 1338 (1959).

BIOCHEMISTRY

Structure of the Carbohydrate Moiety of Orosomucoid

OROSOMUCOID isolated from serum has been the subject of many investigations in recent years[1–4]. Popenoe[5] isolated an α_1-glycoprotein from the urine of patients with the nephrotic syndrome and showed it to be immunochemically identical to serum orosomucoid. More recently the glycoprotein has been isolated from the same source by means of cellulose ion-exchangers[6]. This glycoprotein was shown also to be identical to serum orosomucoid by immunoelectrophoresis and gel-diffusion techniques, and was found to be homogeneous by physical and chemical techniques[7].

The present investigations were concerned with the chemical structure of the carbohydrate portion of the latter glycoprotein by means of periodate oxidation.

Oxidation of the urinary orosomucoid in 0·04 *M* sodium metaperiodate (15 molar excess) at ambient temperature is complete after 10 days. Eighteen moles of formaldehyde and 36 moles of formic acid are liberated rapidly. During further slow oxidation an additional 54 moles of formic acid are liberated, but no additional formaldehyde. The final consumption of periodate is 190 moles per mole of orosomucoid (mol. wt. 46,500). The ratio formaldehyde/formic acid/periodate consumed after oxidation for 15 hr. is 2 : 4 : 9. Titratable non-dialysable acid increased from 18 equiv./mole of orosomucoid to 28 equiv./mole during the oxidation. The material contains no *O*-acetyl groups as determined by the method of Lipmann and Tuttle[8].

The urinary orosomucoid sample contained 18 *N*-acetyl neuraminic acid (NANA) residues per mole. When it was treated with 36 moles of sodium metaperiodate, 18 moles of formaldehyde and 18 moles of formic acid were liberated after 10 hr.

The carbohydrate residues remaining and fragments formed during the oxidation were investigated chromatographically after oxidation for 1 hr. and after total oxidation. The oxidized samples were reduced with sodium borohydride, hydrolysed (3 *N* H_2SO_4 for 4 hr.) and the amino-acids were removed by use of 'Deacidite *FF*' (carbonate).

Fucose and NANA were destroyed during the first hour of oxidation. After total oxidation appreciable amounts of galactose and mannose remained unoxidized, these substances being present in nearly equal quantities before and after oxidation, while the *N*-acetyl glucosamine residues appeared largely resistant.

A spot migrating between galactose and mannose (butanol/ethanol/water, 5 : 1 : 4) was present in the hydrolysate of oxidized orosomucoid, and was shown to be glucose by oxidation to gluconic acid with the specific enzyme glucose oxidase. The absence of glucose in the hydrolysate of native urinary orosomucoid is consistent with the fact that it has not been reported by previous workers. One explanation for its release after oxidation is that its neighbouring unit(s) is oxidized, causing a weakening of an acid-resistant linkage, but the possibility of it arising by epimerization from mannose has not been excluded.

The fragments of the oxidized sugars contain, *inter alia*, appreciable quantities of glycerol and glycolaldehyde. The glycerol concentration appears to diminish during the slow stage of oxidation, indicating that oxidation of the activated carbon-5 hydrogen follows a cleavage between carbons-3 and -4, giving then additional formic acid with resultant loss of glycerol. Ionophoresis in molybdate buffer[9] showed the presence of a trace of threitol but no erythritol.

The amino-acid composition of the protein moiety remains qualitatively unchanged during the oxidation as shown by hydrolysis (5 *N* HCl for 17 hr.) and two-dimensional chromatography.

From these observations the following conclusions are indicated. *N*-acetyl glucosamine must be linked at position 3 or 4 or branched. The production of large amounts of glycerol and formic acid favours a 1–6 linkage for some of the galactose and/or mannose. The absence of erythritol proves that no mannose is linked at positions 1 and 4 alone, while the presence of a trace of threitol shows that only a small amount of galactose is linked at 1 and 4. Some of the mannose as well as galactose must be substituted at 1 and 3 or branched to account for that which resists oxidation.

In a recent paper, Eylar and Jeanloz[10] proposed the following structure for an octasaccharide obtained by mild hydrolysis of serum orosomucoid:

β-D-Gal *p* 1–4 D-GN *Ac p* 1–4 (or 2 ?) Man *p* 1–4 D-GN *Ac p*
1–2 (or 3 ?) Man *p* 1–4 D-GN *Ac*
4
|
β-D-Gal *p* 1–4 D-GN *Ac p* 1

(GN *Ac* is *N*-acetyl glucosamine)

The above findings are in accord with this structure first from the point of view of resistance of hexosamine units, and secondly, that only part of the mannose is susceptible to periodate oxidation.

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[1] Popenoe, E. A., *Biochim. Biophys. Acta*, **32**, 584 (1959).
[2] Weimer, H. E., Mehl, J. W., and Winzler, R. J., *J. Biol. Chem.*, **185**, 561 (1950).
[3] Popenoe, E. A., and Drew, R. M., *J. Biol. Chem.*, **228**, 673 (1957).
[4] Schmid, K., Bencze, W. L., Nussbaumer, T., and Wehrmuller, J. O., *J. Biol. Chem.*, **234**, 529 (1959).
[5] Popenoe, E. A., *J. Biol. Chem.*, **217**, 61 (1955).
[6] Hardwicke, J., and de Vaux St. Cyr, Ch., *Clin. Chim. Acta*, **6**, 503 (1961).
[7] Whitehead, P. H. (unpublished results).
[8] Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **159**, 21 (1945).
[9] Bourne, E. J., Hutson, D. H., and Weigel, H., *J. Chem. Soc.*, 35 (1961).
[10] Eylar, E. H., and Jeanloz, R. W., *J. Biol. Chem.*, **237**, 622 (1962).