The linkage of neuraminic acid in orosomucoid

Although neuraminic acid in its various forms occurs in many diverse components throughout animal organisms, the nature of its linkage has been established in only a few cases. For example, GOTTSCHALK has shown that it is linked glycosidically to galactosamine residues in bovine submaxillary mucoid[1]. A trisaccharide composed of N-acetylneuraminic acid and lactose occurs in human milk, and KUHN AND BROSSMER[2] have shown that in this compound there is an α-ketoside linkage between NANA and carbon 3 of the galactose residue. The present report presents evidence that, in orosomucoid, the α_1-acid glycoprotein of human plasma, NANA is likewise linked to galactose residues.

Previous studies from this laboratory[3] have shown that 15–16 NANA residues can be cleaved from orosomucoid by an enzyme present in filtrates of *Clostridium*

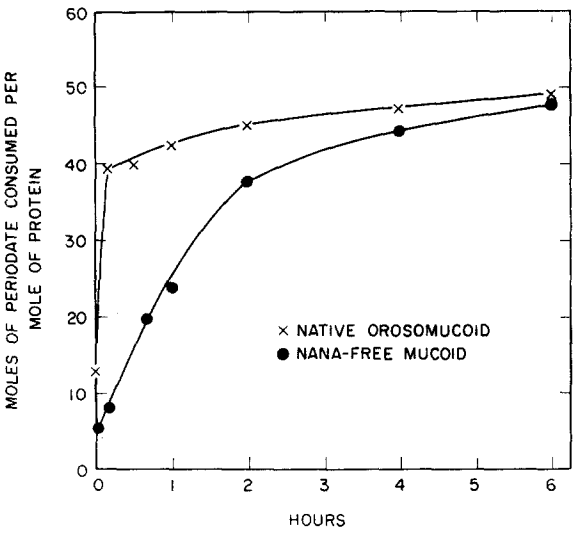

Fig. 1. Periodate consumption by orosomucoid and by NANA-free mucoid. For experimental details see the text. Excess periodate was determined by arsenite titration as described by DYER[8].

perfringens and can be removed by dialysis to leave a mucoid essentially free of NANA. It was further shown[4] that sodium periodate reacts rapidly with native orosomucoid, apparently primarily with its NANA residues. The action of periodate on the NANA-free mucoid is much slower, but the ultimate periodate consumption of the two proteins (from about 5 h onward) is the same on a molar basis (Fig. 1). Thus, in the enzymic cleavage of NANA from orosomucoid, new groupings susceptible to periodate oxidation are released. An analysis for the various carbohydrate constituents of the NANA-free mucoid before and after periodate oxidation should show which one was destroyed by oxidation and thus identify the constituent to which NANA was linked in native orosomucoid. To this end purified orosomucoid prepared from pooled normal human plasma and the NANA-free mucoid prepared as previously described[3] were analyzed before and after periodate oxidation. The oxidations were carried out in acetate buffer pH 4.8, ionic strength 0.02, at 0°. The initial periodate

Abbreviation: NANA, N-acetylneuraminic acid.

concentration was 0.006 *M* and the protein concentration was approximately 0.5 %. Glucosamine was determined according to BOAS[5] after hydrolysis of the samples in 3 *N* HCl for 18 h at the boiling point. The analysis of the periodate-oxidized NANA-free mucoid was also confirmed chromatographically according to GARDELL[6]. Galactose and mannose were determined by a combination of the two cysteine-sulfuric acid reactions designated PCyRI and SCyRI by DISCHE, SHETTLES AND OSNOS[7]. The results of these analyses are shown in Table I. It is clear that the galactose residues are susceptible to attack by periodate only after enzymic removal of NANA from the orosomucoid, hence the NANA must be linked to this glycoprotein through the galactose residues. Furthermore, since the galactose residues of native orosomucoid are immune to periodate oxidation it is probable that the linkage is through carbon number 3 of the galactose.

TABLE I

CARBOHYDRATE ANALYSES OF OROSOMUCOID AND ITS REACTION PRODUCTS

Results are expressed as moles/mole of protein. 1 mole of orosomucoid is assumed to be 45,000 g (anhydrous). Molar concentrations of the NANA-free mucoid were determined spectrophotometrically as previously described[3].

	Periodate oxidation	*Carbohydrate content*		
		Glucosamine	*Mannose*	*Galactose*
Native orosomucoid	None	28.5	18.8	18.0
	30 min	28.5	15.5	17.4
	5 h	29.9	21.1	16.2
NANA-free mucoid	None	31,0	18.4	18.9
	5 h	27.5	17.2	5.1

A discussion of the hexose analyses with DR. ZACHARIAS DISCHE was most helpful. It is hoped to publish a complete description of these experiments and of some related experiments on the state of NANA in orosomucoid in the near future.

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Medical Research Center, Brookhaven National Laboratory, Upton, N.Y. (U.S.A.) EDWIN A. POPENOE

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