Carbohydrate Research, 235 (1992) C9–C12
Elsevier Science Publishers B.V., Amsterdam

Preliminary communication

A study of possible sulfate loss during the chemical release of sulfated oligosaccharides from glycoproteins

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(Received January 31st, 1992; accepted June 10th, 1992)

Sulfated oligosaccharides are present in certain mucus glycoproteins[1] and other glycoproteins such as lutropin, follitropin, and thyrotropin[2]. There is evidence that the sulfate ester groups are important serologically[3,4], that they are responsible for the inhibition of bacterial growth in the alimentary tract[5], and that they differ in diseased tissue compared with normal tissue[4]. Although sulfated polysaccharides such as heparin and some sulfated glycoproteins have been thoroughly studied, some investigations of the oligosaccharide moieties of mucus glycoproteins have ignored the sulfate groups[6].

In a study of the sulfated oligosaccharides of human colonic mucus glycoproteins, it was necessary to establish whether or not any sulfate loss occurred under the conditions used to release the oligosaccharides. The step most likely to lead to sulfate loss is the alkali-promoted β-elimination, which is used to release the serine-bound and threonine-bound oligosaccharides from glycopolypeptide. The latter are generated from the high molecular weight glycoproteins under mild conditions using proteolytic enzymes. Typical conditions for the β-elimination involve 0.05 M sodium hydroxide and M sodium borohydride at 45° for 15 h, the borohydride being included to reduce the released oligosaccharides and avoid alkali-induced degradations[7].

Sulfate loss from saccharide sulfates can occur under alkaline conditions as a result of anhydride formation[8]. For the monosaccharides that are common constituents of glycoproteins, there are two main possibilities: (*a*) 3,6-anhydride formation from hexopyranose 6-sulfates and (*b*) oxirane formation from sulfates of secondary alcohols with a neighbouring trans-related free hydroxyl group.

Model experiments were designed to determine the extent of sulfate loss. First,

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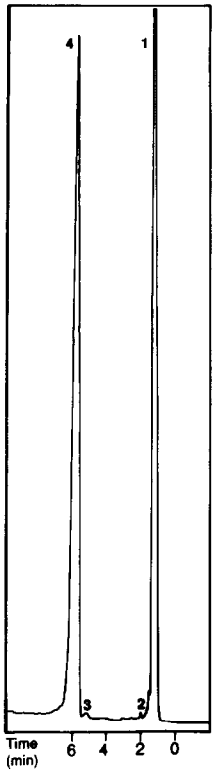


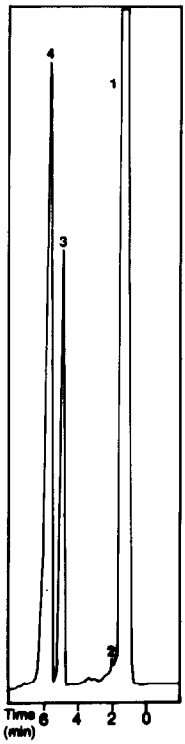
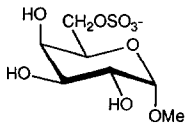


Fig. 1. (left). LC analysis of the products resulting from exposure of methyl α-D-galactopyranoside 6-sulphate to 0.05 M NaOH at 45° for 30 h. Column, 25×0.5 cm ODS/Spherisorb (5 micron); eluant, water; detector, refractive index. Peak 1 = starting material and inorganic salt; peak 2 = methyl α-D-galactopyranoside; peak 3 = methyl 3,6-anhydro-α-D-galactopyranoside; peak 4 = 1-propanol (internal standard).

Fig. 2. (right). As for Fig. 1, for more vigorous conditions (1 M NaOH at 100° for 5 h).

3,6-anhydride formation was monitored by exposing methyl α-D-galactopyranoside 6-sulfate (**1**), synthesised from methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside by sulfation followed by debenzylation, to 0.05 M sodium hydroxide at 45° for the longer reaction time of 30 h described by Carter et al.[9]. Aliquots of the solution were neutralised with hydrochloric acid and analysed by liquid chromatography, using a reverse phase column, water as eluent, and detection by refractive index. To quantify the results, the experiment was repeated using 1-propanol as internal standard. Only 2% of methyl 3,6-anhydro-α-D-galactopyranoside (**3**) was formed, with a trace of methyl α-D-galactopyranoside (**2**), which was not quantified (Fig. 1).



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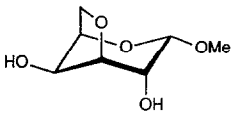

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More vigorous conditions have been used to release asparagine-bound oligosaccharides from glycoproteins[10], and these conditions were mimicked in a parallel experiment using methyl α-D-galactopyranoside 6-sulfate in M sodium hydroxide at 100° for 5 h. Under these conditions, 86% anhydride formation was observed (Fig. 2).

Borohydride was excluded from these model experiments in order to simplify the analysis. Borate is a by-product of the reduction, and borate complex formation at positions 3 and 4 of methyl α-D-galactopyranoside 6-sulfate is possible in a reductive cleavage. However, complex formation is reversible, whereas 3,6-anhydride formation is irreversible, and thus the presence of borate would not be expected to influence anhydride formation.

In a model experiment to test for loss of sulfate via oxirane formation, dextran sulfate (average MW 5000, Sigma), purified by chromatography on BioGel-P4, was used as a model compound. ^{13}C NMR measurements showed that this material had sulfate groups at positions 2 (C-1 at δ 96.9, C-2 at 79.8) and 3 (C-3 at 82.3) in separate glucose residues. Exposure to the same conditions (0.05 M NaOH, 45°, 30 h) followed by the normal workup (removal of Na^+ by cation-exchange resin)[9] resulted in a loss of approximately 15% of the sulfate (assayed according to Silvestri et al.[11]). Amendment of the workup, so that cation-exchange resin was added only to neutralise the solution, reduced the loss of sulfate (which fell from 7.6 to 7.4% SO_4). Any sulfate released was separated from dextran sulfate by gel-permeation chromatography on BioGel-P4. A separate experiment demonstrated that this small loss resulted during the workup and not during the alkaline digestion.

Since the rate of oxirane formation is likely to depend on the position of the sulfate group and the configuration of the sulfated monosaccharide unit, a control experiment has also been carried out using mucus glycoprotein metabolically labelled with sodium [^{35}S]sulfate and D-[6-^{3}H]glucosamine. Human rectal mucosa biopsies were cultured with radiolabelled sulfate and glucosamine, and the mucus glycoproteins were isolated[12]. The mucus glycoproteins were subjected to alkaline borohydride digestion under Carlson conditions[7] and the released oligosaccharides fractionated by gel-permeation chromatography on Sepharose CL 4B and BioGel-P6. Analysis of six individual samples treated in this manner showed the presence of less than 1% of the original mucus glycoprotein bound [^{35}S]-label eluting as free sulfate.

These experiments show that the standard Carlson alkaline digestion conditions[7] for the release of *O*-linked oligosaccharides do not cause significant loss of sulfate. However, the more vigorous conditions used to release *N*-linked oligosaccharides can cause sulfate loss.

ACKNOWLEDGMENTS

The financial support of the Medical Research Council is gratefully acknowledged, and we are indebted to Mr. Gareth Llewellyn for the preparation of galactose derivatives.

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