

used for density-gradient fractionation. The 18.7S mucoprotein, which accounts for most of the viscosity of the water-soluble mucus (Snary *et al.*, 1970), had an intrinsic viscosity of  $320 \text{ ml} \cdot \text{g}^{-1}$  in 0.2M-KCl buffer, pH 5.5, compared with  $160 \text{ ml} \cdot \text{g}^{-1}$  for the same mucoprotein isolated by CsCl-density-gradient centrifugation. Further, the considerable shear-dependence of the viscosity of the 18.7S mucoprotein was completely eliminated after CsCl-density-gradient centrifugation. Since the molecular weights of the 18.7S and 33S mucoproteins were shown to be the same and the ratio of the areas of the two sedimentation peaks for the mucoproteins in the water-soluble mucus before and after CsCl centrifugation was constant, the changes in  $s_{25,w}^0$  values and in viscosity imply changes in shape of the mucoproteins. The value for  $K_s/[\eta]$  (Creeth & Knight, 1965) was 0.8 for the 18.7S and 33S mucoproteins and their respective frictional ratios,  $f/f_0$ , were 10.4 and 1.9. This decrease in  $f/f_0$ , together with the constancy of  $K_s/[\eta]$ , indicates a decrease in the expansion and hence the hydration of the mucoprotein, giving a relatively compact structure similar in shape to that of the highly hydrated shear-dependent 18.7S mucoprotein present in the water-soluble mucus. It is noteworthy that 0.2M-CsCl produces an even higher degree of contraction of the mucoprotein than that induced by 1.5M-KCl (Snary *et al.*, 1971). These results show that the use of CsCl in density-gradient centrifugation of mucoproteins can cause large and irreversible changes in their tertiary structure. In the case of gastric mucoproteins their native tertiary structure is necessary for their function *in vivo* (Snary *et al.*, 1972).

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found in the cell envelopes of most other bacteria. Cell envelopes prepared from this organism contain approx. 10% of their dry weight as hexose and 3–4% as hexosamine. A glycolipid has been isolated from this organism (Kates *et al.*, 1967), and the results now presented indicate that glycoproteins are also present.

When cell envelopes were treated with aq. 50% (w/v) phenol the carbohydrate-containing material was extracted into the aqueous phase, from which it could be precipitated by the addition of ethanol. The precipitate was washed with ethanol and then treated with nucleases before being exhaustively dialysed and freeze-dried. The yield was approx. 70mg/g of cells.

This fraction was resolved by electrophoresis in 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulphate into three bands that could be stained by both periodate-Schiff reagent and Coomassie Brilliant Blue. The positions of the bands indicate molecular weights of 41000, 56000 and 79000, on the basis of pure protein standards. The mobilities of the periodate-Schiff-positive materials in this system is at least partly due to covalently attached proteins, since treatment of the preparation with Pronase lowers the electrophoretic mobilities of these components. These results support the suggestion that at least a proportion of the periodate-Schiff-positive material is glycoprotein.

The carbohydrate moieties present in the cell envelopes were isolated after exhaustive Pronase treatment. Mannose was the only hexose present in appreciable quantities. Molecular weights of these carbohydrate moieties were determined by gel filtration on a 2:1 mixed bed of Sephadex G-100 and G-200. About 85% of the hexose content was eluted in a broad peak at a position corresponding to an average molecular weight of 15000. The remainder of the hexose appeared to be present in compounds of molecular weights approx. 30000 and 60000.

When concanavalin A was added to washed-cell suspensions of *H. halobium*, agglutination was observed but there was no loss of u.v.-absorbing material from the cells in excess of that lost by control cells. This suggests that the glycoproteins are probably surface components with the carbohydrate moiety accessible to the phytagglutinin.

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### Glycoproteins in the Cell Envelope of *Halobacterium halobium*

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*Halobacterium halobium* is a rod-shaped Gram-negative bacterium that grows optimally at above 4M-NaCl and lacks the peptidoglycan component