The Nature of the Enzymically Catalysed Binding of Glucose to the Protein Coat of *Colpoda steinii* Cysts

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The cyst coat of the protozoan *Colpoda steinii* is a protein rich in glutamic acid (Tibbs, 1966). In results not explicable as arising from bacterial contamination or adsorption, the coat has been shown, *in vitro*, to bind [U-¹⁴C]glucose in a reaction that appears to be enzymically catalysed (Tibbs & Marshall, 1969).

After acid hydrolysis of the glucose-labelled coat, much of the radioactivity may be extracted by ether, behaves chromatographically as laevulinic acid and reacts with 2,4-dinitrophenylhydrazine. This is the behaviour to be expected from glucose itself after heating with mineral acid, and shows that most of the hexose backbone is retained intact on binding to the cyst coat. This has been confirmed by using [1-¹⁴C]-, [2-¹⁴C]-, [6-¹⁴C]- and [U-¹⁴C]-glucose. For every atom of C-2 bound one atom of C-6 is also bound. By comparison there is some loss of C-1, which may be up to 50%. In accordance with this, the [U-¹⁴C]glucose uptake compared with the [2-¹⁴C]glucose uptake showed that more than 5 carbon atoms of the hexose were bound but not the complete 6.

The loss of some C-1 suggests that the bonding is not via glycosidic or aldol links or via a Schiff-base type of compound. Confirming the latter, m-pyruvate at pH 7.4 and 45° for 11 hr. did not remove any radioactivity from coat labelled with [U-¹⁴C]-glucose. Fragmentation of the bound molecule by periodate oxidation should result in the loss of those carbon atoms that are more than one removed from any involved in binding. None of the label was removed by 0.01 M-sodium periodate (pH about 4.5) for up to 20 hr. at room temperature, suggesting that several of the sugar hydroxyl groups may be involved in the linking of the glucose to the coat protein.

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Tibbs, J. (1966). *Biochem. J.* **98**, 645.

Tibbs, J. & Marshall, B. J. (1969). *Abstr. 6th Meet. Fed. Europ. biochem. Soc., Madrid*, p. 63.

Studies on the Particle-Bound Carbohydrate Sulphoamide Sulphohydrolase and Carbohydrate Sulphate Sulphohydrolase of *Flavobacterium heparinum*

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Previous reports from these laboratories (Lloyd, Law, Fowler & Embery, 1968; Lloyd, Fowler, Embery & Law, 1968) summarized preliminary observations on the carbohydrate sulphoamide sulphohydrolase (syn. sulphamidase) and carbohydrate sulphohydrolase (syn. O-sulphatase) of *Flavobacterium heparinum* N.C.I.B. 9290. The finding (Dietrich, 1968) that both of these enzymes were sedimented when sonicated preparations of the organism were centrifuged at 6×10^6 g-min. raised the possibility that both enzyme proteins were particle-bound. This view was supported by experiments that showed that the sulphamidase and O-sulphatase were both excluded during gel filtration on columns of Sepharose 2B (mol.wt. exclusion limit 25×10^6).

The effects of various pretreatments on the sedimentation behaviour, gel-filtration properties and activities of the sulphamidase and O-sulphatase were investigated with a view to 'solubilizing' the enzymes before further purification studies. Neither was obtained in a 'soluble' active form after extended sonication, acetone-drying or treatment with 25 mM-sodium acetate solutions, pH 7.5, containing sodium cholate (0.5–1.0%, w/v), sodium dodecyl sulphate (0.1–1.0%, w/v), Nonidet P.40 (1.0%, w/v) or Tween 80 (1.0%, w/v). On the other hand, treatment of enzyme-containing pellets with Triton X-100 (1.5%, w/v) at 4° for 10 min. resulted in the 'solubilization' of both enzymes. These were retained in the supernatant fraction after centrifuging at 6×10^6 g-min., were retarded on columns of Sepharose 2B and 4B (mol.wt. exclusion limit 3×10^6), but were still excluded from Sephadex G-200 even in Triton X-100 (1.0%, w/v) solution.

Preparations of the enzymes in Triton X-100 (1.0%, w/v) solution liberated inorganic SO_4^{2-} ions from 2-deoxy-2-[³⁵S]-sulphoamino-D-glucose 6-O-sulphate (O-sulphatase) and inorganic $^{35}\text{SO}_4^{2-}$ ions from 2-deoxy-2-[³⁵S]-sulphoamino-D-glucose (sulphamidase). However, removal of the detergent from the solubilized enzyme preparation by chromatography on DEAE-Sephadex previously suspended in 10 mM-tris-HCl buffer, pH 7.5, was accompanied by the inactivation of both enzymes. When the enzymes were chromatographed on DEAE-Sephadex previously equilibrated with 10 mM-tris-HCl buffer pH 7.5, containing Triton