X-100 (1·0%, w/v), approx. 50% of the sulphamidase originally applied to the column was recovered early in the elution. An overall purification of the sulphamidase of approx. 60-fold was achieved, although the preparation was still contaminated with *O*-sulphatase activity.

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The Linkage of Corneal Keratosulphate to Protein

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After exhaustive digestion with proteolytic enzymes, corneal keratosulphate contains aspartic acid as the predominant remaining amino acid. In view of the known alkali-stability of the linkage to protein, this finding led to the suggestion that the polysaccharide chains are linked *N*-glycosidically through the amide group of asparagine (Seno, Meyer, Anderson & Hoffman, 1965). Our aim has been to isolate the linkage fragment and determine its structure.

Corneal keratosulphate was hydrolysed in 1M-HCl at 100° for 15min. After removal of the acid, the hydrolysate was adjusted to pH 2 and passed over Dowex 50 (X2). The material eluted by 0·25M-HCl was subjected to high-voltage electrophoresis at pH 6·5 and 1·9, permitting the isolation of a compound with the same mobility and ninhydrin-staining properties as 2-acetamido-1-(L-β-aspartamido)-1,2-dideoxy-β-D-glucose. The isolated and authentic compounds migrated identically on t.l.c. in two solvent systems and electrophoresis in borate buffer, pH 10. Incubation with glycopeptide amidohydrolase (Makino, Kojima & Yamashina, 1966), which specifically cleaves 2-acetamido-1-(L-β-aspartamido) - 1,2 - dideoxy - β - D - glucose, yielded *N*-acetylglucosamine and aspartic acid in the molar ratio 1·0:1·0. Further, the 1-dimethylaminonaphthalene-5-sulphonyl derivatives of the isolated and authentic compounds were electrophoretically indistinguishable.

The yield of isolated linkage fragment was approx. 10% of keratosulphate-bound aspartic acid. Such a yield is consistent with the view that all aspartic acid residues are similarly linked to polysaccharide chains.

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Observations on the Enzymic Sulphurylation of Tyrosyl Derivatives

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An enzyme catalysing the sulphurylation of derivatives of L-tyrosine has been partially purified from rat liver and is believed to be separate from the enzyme responsible for the sulphurylation of simple phenols (Dodgson, Basford, Jones & Mattock, 1967). Kinetic experiments have now been carried out on this enzyme with adenosine 3′-phosphate 5′-sulphatophosphate and various derivatives of L-tyrosine as sulphuryl donor and acceptors respectively.

At pH 7·5 the K_m values for L-tyrosine methyl ester and adenosine 3′-phosphate 5′-sulphatophosphate were 0·3mM and 0·007mM respectively. The two values of K_m were independent of one another, and analysis of the experimental results (Dalziel, 1957) shows that the data are consistent with a rapid-equilibrium random Bi Bi reaction. A similar mechanism has been postulated for phenol sulphotransferase from guinea-pig liver (Banerjee & Roy, 1968).

Variation with pH in the K_m for L-tyrosine methyl ester and tyramine, with a fixed concentration of adenosine 3′-phosphate 5′-sulphatophosphate, shows that the enzyme is specific for an unprotonated α-amino group on the acceptor molecule. The ionization of this group is also apparent in the enzyme–substrate complexes and affects both K_m and $V_{max.}$. *N*-Acetyl-L-tyrosine ethyl ester is not a good substrate for this enzyme, and the low rate of its sulphurylation is independent of pH in the range 7·0–8·2. Hence the enzyme appears to be specific for compounds having a free and unprotonated amino group. This suggestion had already been made on the basis of some preliminary investigations by Segal & Mologne (1959). Variations in K_m for adenosine 3′-phosphate 5′-sulphatophosphate with pH at a fixed concentration

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