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 15 min. 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 0.3 mM and 0.007 mM 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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of L-tyrosine methyl ester shows the presence of a group in the enzyme having a pK value of approx. 9.5. This group could conceivably be a thiol group, as the enzyme is known to be inactivated on storage and is reactivated by the addition of reduced thiols. In keeping with this hypothesis the enzyme was found to be inactivated by iodoacetamide at pH 7.5, and this inactivation is prevented by the addition of adenosine 3'-phosphate 5'-sulphato-phosphate, but not L-tyrosine methyl ester, to the alkylation mixture.

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Purification and Partial Characterization of Peroxidase from Red Algae

By M. J. MURPHY and C. Ó hEocha. (*Department of Biochemistry, University College, Galway, Irish Republic*)

Halogenated organic compounds have been reported in various red algae (see Craigie & Gruenig, 1967; Ó hEocha, 1961). Halogenation in mammals and in some fungi is catalysed by peroxidase (Coval & Taurog, 1967; Morris & Hager, 1966) and this led us to undertake a survey of this enzyme in appropriate species of the Rhodophyta.

Peroxidatic activity was determined spectrophotometrically with *o*-dianisidine as hydrogen donor (Worthington Biochemical Corp. Descriptive Manual). In a typical preparation, *Cystoclonium purpureum* was ground either in a roll mill or in a mortar and allowed to extract into 0.1 M-phosphate buffer, pH 7, for 1 hr. After centrifugation at 100 000g for 90 min. nearly all the activity was in the pellet. The particulate nature of the enzyme is unusual, as plant peroxidases are typically in the soluble fraction. The addition of polyvinylpyrrolidone during extraction did not alter this pattern. The pellet was resuspended in buffer and then centrifuged as before; this procedure was repeated. The enzyme was then solubilized by the addition to the suspended pellet of an equal volume of a 2% digitonin solution (Kaplan, Colowick & Neufeld, 1953); nearly all the activity now remained in the supernatant after centrifugation at 100 000g for 90 min. The enzyme was further purified by acetone fractionation followed by gel filtration

through a column of Sephadex G-75, with 0.02 M-glycine-NaOH buffer, pH 9.0.

The peroxidase has optimum pH between 5.2 and 5.8; maximum activity was obtained with 0.8 mM-hydrogen peroxide. The enzyme was most stable at about pH 9.0; its stability to heat was less than that of other peroxidases tested.

Gel filtration on Sephadex G-75 and G-100 suggested that the molecular weight of the algal peroxidase is approx. 40 000. The purified enzyme was resolved into five or six bands by vertical starch-gel electrophoresis in the pH range 7-9. Such heterogeneity in plant peroxidases is common (see Shannon, Kay & Lew, 1966; Evans, 1968).

The algal enzyme has unusual substrate specificities in that positive results were obtained with *o*-dianisidine, *o*-toluidine and *p*-phenylenediamine, whereas other well-known peroxidase substrates, such as benzidine and guaiacol, did not react. The possible role of peroxidases in the biosynthesis of halogenated compounds in red algae is still under investigation.

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The Amino Acid Sequence of Penicillinase from *Bacillus licheniformis*

By R. J. MEADWAY. (*Department of Molecular Biology, University of Edinburgh*)

The amino acid sequence of penicillinase from *B. licheniformis* 749/C is apparently homologous with that from *Staphylococcus aureus* (Ambler & Meadway, 1969) and is:

- 1 Lys-Thr-Glu-Met-Lys-Asp-Asp-Phe-Ala-Lys-
- 11 Leu-Glu-Glu-Gln-Phe-Asp-Ala-Lys-Leu-Gly-
- 21 Ile-Phe-Ala-Leu-Asp-Thr-Gly-Thr-Asn-Arg-
- 31 Thr-Val-Ala-Tyr-Arg-Pro-Asp-Glu-Arg-Phe-
- 41 Ala-Phe-Ala-Ser-Thr-Ile-Lys-Ala-Leu-Thr-
- 51 Val-Gly-Val-Leu-Leu-Gln-Gln-Lys-Ser-Ile-
- 61 Glu-Asp-Leu-Asn-Gln-Arg-Ile-Thr-Tyr-Thr-
- 71 Arg-Asp-Asp-Leu-Val-Asn-Tyr-Asn-Pro-Ile-
- 81 Thr-Glu-Lys-His-Val-Asp-Thr-Gly-Met-Thr-
- 91 Leu-Lys-Glu-Leu-Ala-Asp-Ala-Ser-Leu-Arg-
- 101 Tyr-Ser-Asp-Asn-Ala-Ala-Gln-Asn-Leu-Ile-
- 111 Leu-Lys-Gln-Ile-Gly-Gly-Pro-Glu-Ser-Leu-
- 121 Lys-Lys-Glu-Leu-Arg-Lys-Ile-Gly-Asp-Glu-