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.

P.M. and D.B. are grateful to the Science Research Council for research studentships. This work was supported by U.S. Public Health Service Research Grant no. AM-01982 from the Arthritis and Metabolic Diseases Division.

- Banerjee, R. K. & Roy, A. B. (1968). *Biochim. biophys. Acta*, **151**, 573.
 Dalziel, K. (1957). *Acta chem. scand.* **11**, 1706.
 Dodgson, K. S., Basford, J. M., Jones, J. G. & Mattock, P. (1967). *Indian J. Biochem.* **4** (Suppl.), 7.
 Segal, H. L. & Mologne, L. A. (1959). *J. biol. Chem.* **234**, 909.

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.

M.J.M. thanks the Department of Education, Dublin, for a Research Maintenance Grant.

- Coval, M. L. & Taurog, A. (1967). *J. biol. Chem.* **242**, 5510.
 Craigie, J. S. & Gruenig, D. E. (1967). *Science*, **157**, 1058.
 Evans, J. J. (1968). *Plant Physiol.* **43**, 1037.
 Kaplan, N. O., Colowick, S. P. & Neufeld, E. F. (1953). *J. biol. Chem.* **205**, 1.
 Morris, D. R. & Hager, L. P. (1966). *J. biol. Chem.* **241**, 1763.
 Ó hEocha, C. (1961). *Colloq. int. Cent. nat. Rech. sci. Paris*, no. 103, p. 121.
 Shannon, L. M., Kay, E. & Lew, J. Y. (1966). *J. biol. Chem.* **241**, 2166.

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-

131 Val-Thr-Asn-Pro-Glu-Arg-Phe-Glu-Pro-Glu-
 141 Leu-Asn-Glu-Val-Asn-Pro-Gly-Glu-Thr-Gln-
 151 Asp-Thr-Ser-Thr-Ala-Arg-Ala-Leu-Val-Thr-
 161 Ser-Leu-Arg-Ala-Phe-Ala-Leu-Glu-Asp-Lys-
 171 Leu-Pro-Ser-Glu-Lys-Arg-Glu-Leu-Leu-Ile-
 181 Asp-Trp-Met-Lys-Arg-Asn-Thr-Thr-Gly-Asp-
 191 Ala-Leu-Ile-Arg-Ala-Gly-Val-Pro-Asp-Gly-
 201 Trp-Glu-Val-Ala-Asp-Lys-Thr-Gly-Ala-Ala-
 211 ----Ser-Tyr-Gly-Thr-Arg-Asn-Asp-Ile-Ala-
 221 Ile-Ile-Trp-Pro-----Pro-Lys-Gly-Asp-Pro-
 231 Val-Val-Leu-Ala-Val-Leu-Ser-Ser-Arg-Asp-
 241 Lys-Lys-Asp-Ala-Lys-Tyr-Asp-Asp-Lys-Leu-
 251 Ile-Ala-Glu-Ala-Thr-Lys-Val-Val-Met-Lys-
 261 Ala-Leu-Asn-Met-Asn-Gly-Lys.

Direct evidence linking residues 54-55, 220-221 and 233-234 is lacking, but the homology makes alternative structures very unlikely. If deletions at 211 and 225 are allowed, 110 residues (43%) are identical in matching positions.

Tetranitromethane is a reagent specific for tyrosine (Sokolovsky, Riordan & Vallee, 1966). After 1 hr. at pH 8.5 with 1 mole of tetranitromethane/mole penicillinase, activity fell 40% and nitrotyrosine was found only at residue 77.

I am grateful to Professor M. R. Pollock and Dr R. P. Ambler for their interest and encouragement, and to the Medical Research Council for a Studentship.

Ambler, R. P. & Meadway, R. J. (1969). *Nature, Lond.*, **222**, 24.

Sokolovsky, M., Riordan, J. F. & Vallee, B. L. (1966). *Biochemistry*, **5**, 3582.

Breakdown of Pyridine Haemichrome by Liver Extracts

By EMER COLLERAN and P. Ó CARRA. (*Department of Biochemistry, University College, Galway, Irish Republic*)

Pyridine haemichrome is converted by liver extracts into a green product that is readily converted into biliverdin. Nakajima, Takemura, Nakajima & Yamaoka (1963) and Nakajima & Gray (1967) report that this reaction is enzymic, whereas Murphy, Ó hEocha & Ó Carra (1967) and Levin (1967) consider that it is mostly, if not entirely, non-enzymic and possibly similar to the coupled oxidation of pyridine haemichrome with ascorbate.

Re-examining the general extraction and assay procedures of the above authors, we find that the pyridine haemichrome-cleaving activity of dilute liver extracts is labile to heating at 100° for 15 min., giving a semblance of enzymic activity that may partly account for the claims of Nakajima *et al.* (1963). However, the activity survives shorter periods of heating, and dilute solutions of ascorbate

(e.g. 0.03%) display an almost identical lability. Both systems display greater heat-stability at higher concentrations and an identical non-linearity in the relationship between concentration and pyridine haemichrome-cleaving activity. Careful analysis of the complex results gave no indication of an enzymic component in the pyridine haemichrome-cleaving activity of extracts from ox, guinea-pig or rat liver or from guinea-pig or rat kidney.

Whereas coupled oxidation of pyridine haemichrome with ascorbate yields an almost random mixture of the four biliverdin isomers (Rüdiger, 1968), Nakajima & Gray (1967) report that liver extracts specifically cleave pyridine haemichrome to the IX α -isomer of biliverdin. This is also the biliverdin isomer specifically produced *in vivo*. However, using the chromatographic technique described in the accompanying communication (Ó Carra & Colleran, 1969), we can find no difference between the isomeric composition of the products formed with liver extracts or with ascorbate. Both produce mixtures of all four isomers. This result is supported by the results obtained by W. Rüdiger (personal communication) by using a dichromate micro-degradation technique (Rüdiger, 1968).

It seems unlikely therefore that pyridine haemichrome breakdown is a valid model for haem breakdown *in vivo*.

E.C. thanks the Department of Education, Dublin, for a Research Maintenance Grant.

Levin, E. Y. (1967). *Biochim. biophys. Acta*, **136**, 155.

Murphy, R. F., Ó hEocha, C. & Ó Carra, P. (1967). *Biochem. J.* **104**, 6c.

Nakajima, O. & Gray, C. H. (1967). *Biochem. J.* **104**, 20.

Nakajima, H., Takemura, T., Nakajima, O. & Yamaoka, K. (1963). *J. biol. Chem.* **238**, 3784.

Ó Carra, P. & Colleran, E. (1969). *Biochem. J.* **115**, 13 P.

Rüdiger, W. (1968). In *Porphyrins and Related Compounds*, p. 121. Ed. by Goodwin, T. W. London and New York: Academic Press.

Coupled Oxidation of Myoglobin with Ascorbate as a Model of Haem Breakdown *in vivo*

By P. Ó CARRA and EMER COLLERAN. (*Department of Biochemistry, University College, Galway, Irish Republic*)

When haemoproteins are incubated with ascorbate at physiological pH and temperature the haem groups undergo coupled oxidation and are released as biliverdin on acidification (cf. Kench & Gardikas, 1952). We have reinvestigated these systems, since the much-studied breakdown of pyridine haemichrome now appears to be an inadequate model for the mechanism of haem breakdown *in vivo* (see