genase activity between fibres were not as distinct as the differences in adenosine triphosphatase activity. The proportion of aerobic and anaerobic fibres varied between species, but was not influenced by body size. Phosphorylase activity tended to show an opposite trend to that of adenosine triphosphatase. The horse had nearly 100% of its fibres high in phosphorylase activity, but in the shrew diaphragm no fibres with phosphorylase activity were seen.

Fast-contracting muscle fibres were also high in phosphorylase activity, showing that faster-contracting fibres used mainly an anaerobic metabolism. In smaller animals the proportion of fibres with high adenosine triphosphatase activity exceeded that of fibres with high phosphorylase activity, so aerobic metabolism may be used by fast-contracting fibres.

Some Properties of a Protease from *Bacillus polymyxa*

By P. J. GRIFFIN and W. M. FOGARTY. (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

Preliminary investigations dealing with the elaboration of proteolytic and starch-degrading enzyme systems by *Bacillus polymyxa* N.C.I.B. 8158 and the influence of an acid-catalysed extract of peat on these processes have been described (Griffin & Fogarty, 1971a). The protease alone was secreted in high yield when arabinose was used as carbon source. The effects of different factors influencing the proteolytic and milk-clotting activity of the enzyme have been recorded (Griffin & Fogarty, 1971b).

The protein in the cell-free supernatant from a peat extract-arabinose medium was fractionated by precipitation with ammonium sulphate, dialysed and concentrated with acetone. Further purification was effected by gel filtration on Sephadex G-100. An overall 70-fold increase in specific activity was thus achieved.

Dialysis of the protease against 20 mM-phosphate buffer, pH 7.0, resulted in irreversible loss in activity. Addition of 1 mM-calcium chloride to the phosphate buffer reversed this effect. Dialysis against 20 mM-tris-HCl buffer, pH 7.0, resulted in only a slight loss in activity. The ionic composition of the buffer used had a significant effect on enzyme activity, e.g. Britton and Robinson's buffer, phosphate buffer maleate buffer, and citrate-phosphate buffer gave activities of 83, 85, 67.7 and 53% respectively, of that observed with tris-HCl buffer, pH 7.0.

The enzyme showed highest activity at pH 7.0 for casein and hide powder, 83% and 82% of the respective substrates being rendered soluble in 5% (w/v) trichloroacetic acid in 2 h. The protease caused considerable breakdown of a variety of proteins.

There was complete inhibition of activity by 1 mM-EDTA and by 1 mM-*o*-phenanthroline, 66% inhibition by 2 mM- α '-bipyridyl, 7% inhibition by 5 mM-sodium oxalate and 34% inhibition by 5 mM-sodium citrate. The effects of a variety of other inhibitors on activity of the enzyme were also examined.

Dialysis of the enzyme preparation against 1 mM-EDTA, pH 7.0, for 3 h and then against 20 mM-tris-HCl buffer, pH 7.0, for 12 h, followed by addition of Mn^{2+} (final concentration 1 mM), gave an increase of 56% in activity over the original non-dialysed material. Dialysis against 1 mM-EDTA for 12 h resulted in no activity on the addition of Mn^{2+} .

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Extracellular Enzymes of a *Bacillus* sp. Associated with Increased Permeability in Sitka Spruce (*Picea sitchensis*)

By O. P. WARD and W. M. FOGARTY. (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

Destruction of tori and bordered pit membranes in the sapwood of Sitka spruce (*Picea sitchensis*) during water storage has been shown to result in increase in permeability of the material to treatment with preservative (Dunleavy & Fogarty, 1971). Evidence indicates that destruction of the pit membranes may be caused by the action of bacterial extracellular enzymes. The importance of pectinolytic activity in the degradation of the pits has been suggested (Ward & Fogarty, 1971). Certain enzymes, e.g. cellulases, are known to have highly detrimental effects on the structural properties of wood. We now report the results of a study of the nutritional factors influencing the elaboration of extracellular enzymes by an organism associated with permeability increase in water-stored spruce.

The bacterium, a *Bacillus* sp., isolated from water-stored Sitka spruce (W. M. Fogarty, unpublished work), was grown at 27°C in liquid media

in an orbital shaker-incubator. To establish the effect of carbon substrate on enzyme elaboration, various carbohydrates were incorporated in (1) a basal salts medium and (2) a salts-yeast extract medium. Glucose, sodium polypectate and pectin, in that order, induced highest polygalacturonate lyase activity in the basal salts medium. Xylanase induction was greatest with xylan or cellobiose. Some amylase and protease activity was induced by a range of carbohydrates. Carboxymethyl-cellulase activity was markedly low in all media examined.

Because of the association of pectate degradation with increase in permeability, sodium polypectate was used as carbon source in investigations of the effect of nitrogen source on enzyme production. Complex nitrogen sources were found to induce largest amounts of polygalacturonate lyase, amylase, protease and xylanase activities. Enzyme production in media containing individual amino acids was extremely low. However, with casamino acids (casein hydrolysate) simultaneous production of the four enzymes occurred.

The very low cellulolytic activity is noteworthy. The demonstration that the four extracellular enzymes listed above are secreted simultaneously, under conditions in which sodium polypectate is the sole carbon source, may therefore have significance in the overall process of permeability increase in water-stored Sitka spruce.

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Properties and Kinetics of Biliverdin Reductase

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Biliverdin reductase is a mammalian enzyme catalysing the reduction of biliverdin to bilirubin by transfer of electrons from both NADPH and NADH (Colleran & Ó Carra, 1970).

Purification of biliverdin reductase was severely hampered by the fact that ion-exchange and adsorption chromatographic techniques changed the properties of the enzyme, causing a loss of stability and alteration of the kinetic properties. Only non-adsorptive methods of purification were found useful. The enzyme was partly purified from guinea-

pig liver, which was homogenized in 3 vol of 0.1 M-phosphate buffer, pH 7.4, and centrifuged at 30 000g for 60 min. The resulting supernatant was subjected to preparative ultracentrifugation at 104 000g for 90 min followed by ammonium sulphate fractionation [40–60% (w/v) fraction] and gel filtration on Sephadex G-200.

The elution profile of the activity from a calibrated Sephadex G-200 column indicated an apparent molecular weight of about 70 000 for the reductase. The usual activation and inhibition studies provided no evidence in favour of a flavin, haem or metal prosthetic group, nor for the involvement of an active-site thiol group.

Double-reciprocal plots and replots of the data obtained by varying the concentration of each substrate [NAD(P)H and biliverdin IX α] at various fixed concentrations of the other, and kinetic analysis of the product inhibitions by NAD⁺ and by bilirubin, indicated an ordered sequential Bi Bi mechanism (cf. Cleland, 1963a,b,c). The reduced nicotinamide nucleotide is the obligatory first substrate in the formation of the ternary complex, its binding causing the induction or formation of a high-affinity binding site for the biliverdin.

As previously reported (Colleran & Ó Carra, 1970), NADPH (K_m 2–5 μ M) is a far more effective substrate than is NADH (K_m 200–500 μ M). NADPH also seems to promote tighter binding of biliverdin, since the K_m for biliverdin is 1–2 μ M with NADH as co-substrate but is below 0.2 μ M with NADPH as co-substrate. It is unusual for the second substrate adding to the enzyme to have a lower K_m than the obligatory first substrate. Biliverdin reductase displays a high, but not absolute, specificity for the 'natural' IX α isomeric form of biliverdin (Colleran & Ó Carra, 1970). The main factor determining this specificity is the location of the propionate side chains. These straddle the electron-accepting central methine bridge in biliverdin IX α , but not in the IX β , IX γ or IX δ isomers. Only one of the two propionate side chains is essential for effective binding, since esterification of one of them decreases substrate effectiveness only slightly, whereas esterification of both abolishes substrate effectiveness entirely.

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