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, 1963*a,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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