The Effect of Thiol Compounds on Rat Liver Sulphotransferases

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There is conflicting evidence about the role of thiol groups in mammalian sulphotransferases. GSH and cysteine have been found necessary for the maximum activity of phenol sulphotransferases from rabbit and rat livers respectively (Gregory & Lipmann, 1957; Subba Rao, Seshadri Sastry & Ganguly, 1963). Conversely, GSH, 2-mercaptoethanol and dithiothreitol have been shown to inhibit the sulphation of *p*-nitrophenol but to enhance the sulphation of *L*-tyrosyl derivatives by rat liver enzymes (Mattock & Jones, 1970). Dehydroepiandrosterone sulphotransferase from human adrenal glands is either activated or inhibited by cysteine according to the concentration of substrate employed for its assay (Adams & Edwards, 1968). The present communication offers a possible common explanation for these observations.

Enzymes (stage 3; Mattock & Jones, 1970) were chromatographed on DEAE-cellulose with a linear 0–0.4M-NaCl concentration gradient in 0.01M-tris-HCl buffer, pH 7.5, containing 0.2mM-EDTA and 0.01M-2-mercaptoethanol. Two peaks (A and B) of *L*-tyrosine methyl ester sulphotransferase activity were obtained. There was no evidence of inter-conversion of enzymes A and B, and both possessed phenol sulphotransferase activity whereas enzyme A also possessed dehydroepiandrosterone sulphotransferase activity.

With enzyme B at pH 7.5 the K_m values for *L*-tyrosine methyl ester and *p*-nitrophenol were 2mM and 2 μ M respectively. After 2 weeks at 0°C the preparation was inactive towards *L*-tyrosine methyl ester, whereas the V_{max} for the sulphation of *p*-nitrophenol was increased fourfold and the K_m was 0.34mM. All of the original kinetic properties of enzyme B were restored when this preparation was fully reduced by 0.01M-dithiothreitol. Shorter periods of storage merely decreased the activity towards *L*-tyrosine methyl ester but complicated the kinetics of the sulphation of *p*-nitrophenol. The sulphotransferase activity of freshly prepared rat liver supernatant towards *L*-tyrosine methyl ester or *p*-nitrophenol was unaffected by dithiothreitol. The collective results confirm that *L*-tyrosine methyl ester and *p*-nitrophenol sulphotransferases can exist in a reduced and an oxidized form. In particular, the kinetic parameters for the sulphation of *p*-nitrophenol will depend on the state of oxidation of the enzyme, and the effect of thiols on the activity of the enzyme will therefore

vary with the concentration of substrate employed for its assay.

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Lysosomal Arylsulphatases A and B of Rat Liver

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Arylsulphatases A and B are lysosomal enzymes that appear to be found in most mammalian tissues. Both enzymes show high activity towards potassium 2-hydroxy-5-nitrophenyl sulphate, although certain differences are to be found in their kinetic responses towards this unnatural substrate (see, e.g., Dodgson, Spencer & Thomas, 1955; Baum, Dodgson & Spencer, 1958). With the human enzymes these differences are sufficiently great to enable the independent assay of the enzymes (Baum, Dodgson, Spencer, 1959), and application of this assay method to human patients subsequently led to the recognition that arylsulphatase A was absent from the urine and tissues of patients with metachromatic leucodystrophy (see, e.g., Austin *et al.* 1964). Unfortunately, for a variety of reasons (M. Worwood, unpublished work), this assay method cannot be applied to rat liver.

Partial separation of rat liver arylsulphatases A and B can be obtained by ion-exchange chromatography on CM-cellulose or DEAE-cellulose, or by gel filtration on Sephadex G-200, and a relative measure of the amounts of arylsulphatases A and B may be obtained by a method involving fractionation on DEAE-cellulose, followed by assay of fractions under different kinetic circumstances (M. Worwood, unpublished work). This method has been applied to the study of the enzymes in liver cells.

The method of Howard, Christensen, Gibbs & Pesch (1967) was used to obtain preparations of parenchymal cells of high homogeneity (approx. 95%) and viability (approx. 90%). The remaining liver cells were isolated by centrifugal techniques, and attempts were made to separate them into