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

homogeneous cell-type fractions by isopycnic centrifuging on both sucrose and ficoll gradients. Although fractions were obtained that were considerably enriched in endothelial lining cells or Kupffer cells, homogeneous preparations suitable for precise analysis have not yet been obtained. However, analysis of these fractions indicated that the relative proportion of arylsulphatases A and B in endothelial lining and Kupffer cells was markedly different from that observed for parenchymal cells. Thus, on the basis of the arbitrary method used to express enzyme activity, the ratios of arylsulphatase A to arylsulphatase B in whole liver and in the various fractions were as follows: whole liver, 1:13; parenchymal cells, 1:13; fraction enriched in Kupffer cells, 1:30; fraction enriched in endothelial lining cells, 1:45.

Mehl & Jatzkewitz (1968) have suggested that arylsulphatase A is a component of a more complex cerebroside sulphatase. On the other hand, the natural substrate for arylsulphatase B is quite obscure. A knowledge of the relative distribution and proportions of the two enzymes in mammalian tissues may provide a clue as to the role of the B enzyme and hence to the discovery of its natural substrate.

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Sulphation of Simple Aliphatic Alcohols and Bile Alcohols by the Toad *Xenopus laevis*

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The synthesis of activated forms of sulphate, adenosine 3'-phosphate 5'-sulphatophosphate and adenosine 5'-sulphatophosphate, has been shown to occur in certain micro-organisms and the tissues of plants and animals. In bacteria and other micro-organisms these compounds are principally used for the production of reduced forms of sulphur, whereas

in the higher animals they are used mainly in the synthesis of various types of sulphuric acid ester. The transition in the principal metabolic role of 'active sulphate' between these two extremes is of considerable interest from an evolutionary point of view and, as part of a comparative investigation of sulphate activation and utilization, these processes are currently being studied in the toad *Xenopus laevis*.

Sulphate activation occurs readily in toad liver, although the optimum conditions for the process (pH 8.0, 30mM-ATP and 12mM-Mg²⁺) differ quite markedly from those found for the rat liver system (pH 7.5, 45mM-ATP and 12mM-Mg²⁺). As yet it has not been possible to detect utilization of adenosine 5'-sulphatophosphate and adenosine 3'-phosphate 5'-sulphatophosphate for the production of reduced sulphur compounds in the cytosol of male and female toad livers. However, such preparations readily transferred sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to a wide variety of acceptors, including phenols, primary and secondary aliphatic alcohols, steroids and aromatic amines. Generally speaking this situation is similar to that noted for the rat by Spencer (1960), and suggests the presence of a number of sulphotransferase enzymes of different specificity. However, one striking difference between the two species was apparent in that toad liver preparations were able to sulphate the bile alcohols, β -cyprinol and scymnol, as well as simple aliphatic alcohols such as propan-1-ol and butan-1-ol, whereas rat liver preparations could sulphate only the latter acceptors. A similar lack of ability to sulphate these bile alcohols was also observed in chick liver.

Sulphotransferase activity of toad liver towards simple aliphatic alcohols could be separated from that towards bile alcohols by chromatography on DEAE-cellulose. The activity of the simple alcohol sulphotransferase fraction towards a series of simple aliphatic alcohols was optimum with pentan-1-ol, although, for routine assay purposes, butan-1-ol was a more convenient acceptor (50mM, pH 8.0, in the presence of 75 μ M-adenosine 3'-phosphate 5'-sulphatophosphate). The enzyme showed no requirement for Mg²⁺ but was activated up to 30% by mercaptoethanol and dithiothreitol.

The fraction active towards scymnol and β -cyprinol yielded the C-26/27 sulphates already recognized as being present in certain invertebrate biles (Haslewood, 1967), and was assayed as a routine with 100 μ M- β -cyprinol at pH 8.0 in the presence of 75 μ M-adenosine 3'-phosphate 5'-sulphatophosphate. This enzyme showed a requirement for Mg²⁺ (5mM) but was unaffected by thiols.

Sulphate activation in the toad, as in higher animals, therefore appears to be mainly directed towards sulphate ester biosynthesis, although there