activators and inhibitors on the rates of sulphurylation of *p*-nitrophenol, dehydroepiandrosterone and L-tyrosine methyl ester, suggested that different enzymes were involved in these reactions. This communication describes the isolation of an enzyme that catalyses the sulphurylation of phenol but not of dehydroepiandrosterone or L-tyrosine methyl ester.

Livers from male Wistar rats were homogenized in 0.15M-KCl in a Potter-Elvehjem homogenizer and centrifuged at 100000g for 60 min. The supernatant was made 10mM with respect to 2-mercaptoethanol and the protein concentration was 20mg/ml. The phenol sulphotransferase was precipitated at 45% saturation of ammonium sulphate, and the precipitated protein was dissolved in 5mM-tris-HCl buffer, pH 8, and dialysed against large volumes of the same buffer for 3 days. The enzyme was adsorbed on alumina gel C_γ and the phenol sulphotransferase was eluted from the gel with high concentrations of EDTA at pH 8. The EDTA was removed by ultrafiltration and the enzyme chromatographed on a column of DEAE-cellulose. Preparative electrophoresis on polyacrylamide gel yielded a phenol sulphotransferase with a specific activity 150-fold higher than that of the crude supernatant and the overall recovery was 20%.

This partially purified enzyme catalysed the sulphurylation of phenol, *p*-nitrophenol and *m*-aminophenol, but had no activity towards dehydroepiandrosterone, L-tyrosine methyl ester, butan-1-ol or 1-naphthylamine. The enzyme preparation displayed linear Michaelis-Menten kinetics for the phenol and nucleotide substrates. It was stabilized by EDTA and converted into a less active form by thiols.

This work was supported by U.S. Public Health Service Grant 5R05TW00314-03.

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The Activation of Liver Phenol Sulphotransferase

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Phenol sulphotransferase (adenosine 3'-phosphate 5'-sulphatophosphate-phenol sulphotransferase, EC 2.8.2.1) exists in various molecular sizes (Carroll, 1969) and the state of aggregation of this

enzyme appears to be influenced by the free thiol content of the enzyme solution. The relationship between the enzyme's size and its catalytic activity has been investigated.

The supernatant fraction was prepared by homogenizing the liver of a male Wistar rat in 0.15M-KCl and centrifuging at 100000g for 60 min. The phenol sulphotransferase of this preparation existed mainly with a particle size of 70000 as determined by gel-filtration and sedimentation-equilibrium studies, and the enzyme's activity was increased at least twofold by treatment with *o*-iodosobenzoate or with thiol-binding agents *p*-chloromercuribenzoate and mercuric chloride: maximum stimulation of enzymic activity was observed when the concentration of these reagents in the assay medium was 0.25mM. The particle size of the enzyme was not altered significantly by treatment with *o*-iodosobenzoate. *o*-Iodosobenzoate inhibited sulphurylation of dehydroepiandrosterone, 1-naphthylamine, butan-1-ol and L-tyrosine methyl ester. The phenol sulphotransferase activity of fresh liver preparations was increased by ultrafiltration, gel filtration or storage in the cold, whereas the rate of transfer to the other acceptors was unaltered or diminished by these treatments. The phenol sulphotransferase eluted from Sephadex G-25 was inhibited by either added thiol-binding agent or added thiol. The crude enzyme aggregated to a particle size of 300000 on storage in the cold, and this aggregated form was both inhibited and dissociated by added thiol.

Phenol sulphotransferase, but not the other sulphotransferase activities, was activated by heating at 37°C. This activation required the presence of metal ion, and Mg²⁺, Mn²⁺, Fe²⁺, Fe³⁺ and Ca²⁺ were all equally effective in promoting the increase in activity. The heat-activated phenol sulphotransferase was inhibited by added thiols or thiol-binding agents.

There does not appear to be a direct relationship between the size of phenol sulphotransferase and its activity, but activation of this enzyme requires the masking of protein thiol groups. These results are of interest in view of the demonstration (Wellers-Leluan & Chevan, 1964) that there is an association between the urinary excretion of ethereal sulphates and the glutathione concentrations in the tissues.

This work was supported by U.S. Public Health Service Grant 5R05TW00314-03.

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