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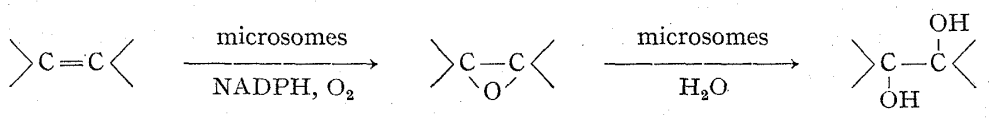
Solubilization of Epoxide Hydrolase from Liver Microsomes

The hydrolysis of a variety of 1,2-epoxides to *trans*-glycols by liver microsomes has recently been reported,[1,2] using olefins which give such epoxides as are fairly stable in water, to be essential step in the metabolism of olefins to gylcols.[3] Thus, the oxygen-requiring conversion of olefins into glycols by liver microsomes can be depicted as a two-step enzymatic process, *i.e.* epoxidation of their ethylene linkage by a mixed function oxygenase in the presence of NADPH and molecular oxygen, followed by the hydrolysis of the resulting epoxides to the corresponding *trans*-glycols by a hydrolase. Similar evidence has also been obtained in the metabolism of naphthalene by rat liver microsomes, although it is rather indirect, for naphthalene oxide formed during the reaction is so reactive as to be, in part, hydrolyzed nonenzymatically.[4] Furthermore, it was found that the same process involves not only the oxidation of foreign olefins, but also conversion of free oleic acid into *threo*-9,10-dihydroxy stearic acid.[5] The microsomal epoxide-hydrolyzing enzyme was tentatively named epoxide hydrolase by Watabe and Maynert.[1] Focusing attention on the latter half of this enzymatic process, solubilization of the hydrolase associated with the particulate fraction is a matter of primary importance as a first step in the clarification of the nature of the enzyme. In the present paper it is shown that epoxide hydrolase can be obtained in soluble and partially purified form. Moreover, evidence is presented that the enzyme solubilized could require sulfhydryl group of its own protein but no dialyzable cofactor for the hydrolysis reaction.



For the solubilization of epoxide hydrolase, 33 g of twice washed microsomes, obtained from rabbit liver, was homogenized for 10 min with 200 ml of 0.1 M phosphate buffer, pH 7.4, containing 6.4 mmoles of sodium deoxycholate. After stirring on a magnetic stirrer for 30 min, the homogenate was treated with 100 ml of aqueous solution of 6.4 mmoles of calcium chloride for removal of deoxycholate, and then centrifuged at 200000 × *g* for 2 hr. To the transparent soluble supernatant fraction containing 52% of the total enzyme activity of the microsomes, solid ammonium sulfate was added to 30% saturation, and the protein precipitated was collected by centrifugation under the same conditions. For the delipidation, this precipitate was dissolved in 150 ml of 0.1 M phosphate buffer, pH 7.4, and acetone was added to the final concentration of 85%. Protein precipitated was collected by centrifugation, washed successively with 200 ml each of acetone and peroxide-free ether by homogenation and subsequent centrifugation at each time, and dried *in vacuo* over anhydrous calcium chloride. The acetone powder was then extracted by homogenation with 150 ml of 0.1 M phosphate buffer, pH 7.4, and insoluble protein was removed by centrifugation. To the clear extract separated, ammonium sulfate was added to 10% saturation, and the supernatant fraction obtained by

1) T. Watabe and E.W. Maynert, *Federation Proc.*, **27**, 302 (1968).
2) K.C. Leibman and E. Ortiz, *Federation Proc.*, **27**, 302 (1968); G.T. Brooks, S.E. Lewis and A. Harrison, *Nature*, **220**, 1034 (1968).
3) T. Watabe and E.W. Maynert, *Pharmacologist*, **10**, 203 (1968).
4) D.M. Jerina, J.W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *J. Am. Chem. Soc.*, **90**, 6525 (1968).
5) T. Watabe, S. Imazumi and Y. Ueno, to be published.

Table I. Fractionation Procedure and Specific Activities of Epoxide Hydrolase

	Fraction	Total protein[a] (g)	Specific activity[b]	Purification	Total activity
I	microsomes	4.05	0.573	1.00	2320
II	deoxycholate supernate (200,000×*g* for 2 hr)	1.79	0.678	1.18	1210
III	deoxycholate supernate, 0—30% $(NH_4)_2SO_4$ precipitate	0.67	1.280	2.23	860
IV	extract of acetone powder of fraction III, 0—10% $(NH_4)_2SO_4$ supernate	0.059	2.160	3.77	127

a) equivalent to 100 g of liver or 33 g of microsomes Protein was determined by the standard method.[8]
b) Measured in μmoles of the epoxide hydrolyzed/mg protein/30 min.

centrifugation was dialyzed against distilled water for 24 hr. All the above procedures were carried out at 0—3°. At this stage the recovery of protein was 5.5% and the specific activity was 3.7 times higher than that of the microsomal fraction. The typical results are summerized in Table I.

For the assay of epoxide hydrolase activity, 30 μmoles of 1,2-epoxy-*n*-octane[1] dissolved in 0.2 ml of ethanol was added to a mixture of the enzyme preparation and 0.1 M phosphate buffer, pH 7.4, to make a final volume of 5 ml. Incubation was carried out at 37° for 30 min. During this time the hydrolysis reaction proceeds in a zero order kinetics. The reaction was terminated by the addition of 0.5 ml of 5N NaOH, and the product, 1,2-*n*-octanediol,[1] was extracted with ether, following saturation of the mixture with sodium chloride, and analyzed by gas chromatography.[6] The glycol was eluted at 7.5 min from a 20% Apiezon L-Gas-Chrom Q column[7] at the column temperature of 150° and the flow rate of 45 ml/min of nitrogen as the carrier gas. Under these conditions no detectable peak other than those of the substrate (at 2.8 min) and the glycol formed appeared.

The hydrolase is stable when it is stored as solution at 0° or frozen rapidly at −20° and especially as the dry acetone powder,[9] although it loses activity on repeated freezing and thawing. The activities of the soluble enzyme preparations are unaffected by dialysis. EDTA, 1,2-iminocyclohexane, and monoiodoacetamide failed to inhibit enzyme activity at the concentration of 10^{-3}M. Mercuric chloride (10^{-3}M) strongly inhibited the enzyme, but the activity was completely restored by the coexistence of glutathione (10^{-3}M). However, glutathione alone did not show any effect. These facts could imply that no dialyzable cofactor is required for the hydrolysis reaction and be strongly suggestive that sulfhydryl group of the enzyme is essential for the activity. Further studies on purification of this enzyme are now in progress.

Biochemical Laboratory
Division of Organic Chemistry,
Tokyo College of Pharmacy
Ueno-sakuragi, Taito-ku, Tokyo, 110, Japan

Tadashi Watabe
Shyukuko Kanehira

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6) Blank experiments which were carried out using mixtures consisting of the heat-inactivated enzyme preparations and the epoxide did not show the formation of any detectable amount of the glycol.
7) Apiezon L grease: Shell Oil Co., England; Gas-Chrom Q: Applied Science Co., U.S.A.
8) H.O. Lowry, N.J. Rosenbrough, A.L. Faar and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
9) All of the soluble enzyme preparations have no activity to convert 1-octane to the glycol in the presence of NADPH and O_2.