reactions is in large part due to a competition with activating K^+. When Tris is used as the buffer in studies of these enzymes a K_a for K^+ will be obtained which can vary simply as a result of varying buffer concentration. Thus in the pyruvate kinase system, a K_a for K^+ of 0.011 M has been reported[4] using a Tris buffer concentration of 0.04 M. As can be seen from Fig. 3, a K_a for K^+ both higher and lower than 0.011 M has been obtained simply by varying the Tris concentration.

In order to assess the possible physiological significance of enzyme activation by univalent cations it is essential to obtain accurate data for saturation characteristics of these enzymes with K^+. It is clear that in obtaining such data for pyruvate kinase, acetaldehyde dehydrogenase and perhaps other K^+-activated enzymes, the buffer system used for assays must be accurately defined, and actively considered in the interpretation of results.

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Department of Botany, Oregon State University, Corvallis, Oreg. (U.S.A.)

GRAHAM F. BETTS*
HAROLD J. EVANS

1 H. J. EVANS AND G. J. SORGER, *Ann. Rev. Plant Physiol.*, 17 (1966) 47.
2 M. COHN AND J. MONOD, *Biochim. Biophys. Acta*, 7 (1951) 153.
3 E. R. STADTMAN, *J. Biol. Chem.*, 196 (1952) 527.
4 J. F. KACHMAR AND P. D. BOYER, *J. Biol. Chem.*, 200 (1953) 669.
5 S. BLACK, *Arch. Biochem. Biophys.*, 34 (1951) 86.
6 G. J. SORGER AND H. J. EVANS, *Biochim. Biophys. Acta*, 118 (1966) 1.
7 R. H. WILSON, H. J. EVANS AND R. R. BECKER, *J. Biol. Chem.*, 242 (1967) 3825.
8 G. MILLER AND H. J. EVANS, *Plant Physiol.*, 32 (1957) 346.
9 T. BÜCHER AND G. PFLEIDERER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, New York, 1955, p. 435.

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* Present address: School of Biological Sciences, University of Sussex, Brighton, England.
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Rat liver glycine acyltransferase: Partial purification and some properties

Glycine acyltransferase (acyl-CoA:glycine *N*-acyltransferase, EC 2.3.1.13) catalyzes the third and final step in one of the detoxication pathways for benzoic acid and similar substances[1]. Its specificity is indicated by its systematic name, and it is found in the mitochondrial fraction of liver and kidney[2]. In the course of studying the activity of the enzyme in developing rat liver[3], partial purification and characterization were performed.

Adult rats of the Sprague–Dawley strain were killed by decapitation and exsanguination. The livers were homogenized in 9 vol. of cold 0.25 M sucrose, and the mitochondria were isolated by differential centrifugation, washed 4 times and made

into acetone powders. The latter were kept in a dessicator at 2–4° until extracted with 0.2 M potassium phosphate (pH 7.5). The material precipitating between 35 and 50% saturation of ammonium sulfate was dissolved in 0.01 M phosphate buffer (pH 7.0), dialyzed and fractionated by CM-cellulose chromatography.

Enzyme activity was assayed by the method of SCHACHTER AND TAGGART[1] measuring the decrease in $A_{280\ nm}$ as the thioester bond of benzoyl-SCoA was cleaved. Protein was determined by the biuret reaction. Specific activity was expressed as $\Delta A_{280\ nm}$ per min per mg protein.

A significant degree of purification was achieved (Table I). This was primarily the result of the CM-cellulose chromatography, but when preceding steps were omitted,

TABLE I

PURIFICATION OF RAT LIVER MITOCHONDRIAL GLYCINE ACYLTRANSFERASE

The incubation medium was at 20° and contained 0.1 M Tris (pH 7), 0.1 M glycine and 7.5 μM benzoyl-CoA. The absorbance at 280 nm was measured 15 and 45 sec after the addition of enzyme. Control flasks without glycine were run to determine the presence of benzoyl-CoA deacylase; none was found after ammonium sulfate fractionation, and the data on the crude extract have been corrected for the small amount of activity present.

Fraction	*Total activity ($\Delta A_{280\ nm}$ per min)*	*Total protein (mg)*	*Specific activity ($\Delta A_{280\ nm}$ per min per mg)*	*Yield (%)*
Acetone powder extract	1850	1281	1.4	100
Ammonium sulfate	3800	751	5.1	205
Dialysis	7430	376	19.8	402
CM-cellulose column	4710	4.3	1095.3	254

the same degree of separation could not be consistently obtained. Obviously, much of the purification had already been obtained by starting out with acetone powder of mitochondria, the exclusive site of the enzyme.

The increase in total activity as the purification proceeded is of interest. This would suggest that inhibiting factors must have been present in the starting material. On this basis one might have expected a still higher yield from the last step, but the technique of chromatography itself often results in reduced activity of unstable enzymes such as this one. It is of interest that ammonium sulfate fractionation of beef liver mitochondria did not result in an increase in total activity[1].

While the separation appeared to be fairly clean (Fig. 1), the slight amount of activity present in Tubes 14 to 22 as well as the shoulder at Tube 30 were noted in all runs and indicated the likelyhood of molecular heterogeneity. This conclusion was strengthened by the elution pattern when DEAE-cellulose was employed (Fig. 2); approximately equal amounts of activity were eluted before and sometime after the addition of the NaCl gradient to the system.

The purified enzyme showed a rather sharp pH optimum at 8.7 with 50% reduction in activity at 8.1 and 9.2. This is in contrast to the beef liver enzyme which demonstrated a plateau from 7.3 to 9.7 (ref. 1).

While maximal activity was obtained with any glycine concentration between

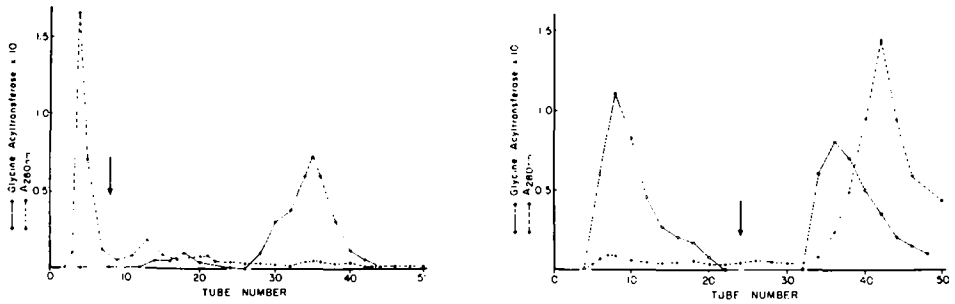

Fig. 1. CM-cellulose chromatography of glycine acyltransferase activity. A 12 mm × 160 mm column was loaded with 56 mg of protein in 5 mM sodium phosphate (pH 7.0) and initially eluted with the same buffer. The arrow indicates where a convex NaCl gradient (0 to 0.3 M in the same buffer) was started. The fractions were 4.5 ml.

Fig. 2. DEAE-cellulose chromatography of glycine acyltransferase activity. A 12 mm × 280 mm column was loaded with 146 mg of protein in 10 mM glycine (pH 9.0) and initially eluted with the same buffer. The arrow indicates where a linear NaCl gradient (0 to 0.5 M in the same buffer) was initiated. The fractions were 4 ml.

0.17 and 0.36 M (the highest used), apparent substrate inhibition was noted with respect to the benzoyl-CoA. Activity reached a plateau at a concentration of 60 μM and remained at that level until 200 μM. Above this there was a gradual decrease until at 300 μM the activity was 40% of the maximum.

Both products were shown to inhibit the reaction. Addition of either was associated with a progressive inhibition of enzyme activity to 56% at 0.5 mM CoA and to 73% at 1.3 mM hippurate. These values approximate those reported for beef liver enzyme by SCHACHTER AND TAGGART[4].

A number of substances could be added to the incubation medium without significantly altering the enzyme activity (all mM): KF, 10; $HgCl_2$, 1; *p*-chloromercuribenzoate, 0.3; α-iodoacetamide, 2; $MgSO_4$, 2; $CaCl_2$, 2; KCN, 100; 2,4-dinitrophenol, 5. This is in agreement with the findings when beef liver enzyme was studied previously[1].

The preparation exhibited appreciable temperature instability. At 38° essentially all of the activity was lost in 30 min. At room temperature half of the activity was lost in 24 h.

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Department of Pediatrics,
Yale University School of Medicine,
New Haven, Conn. 06510 (U.S.A.)

IRA K. BRANDT*
PEGGY SIMMONS
TAMAR GUTFINGER

1 D. SCHACHTER AND J. V. TAGGART, *J. Biol. Chem.*, 208 (1954) 263.
2 R. K. KIELLEY AND W. C. SCHNEIDER, *J. Biol. Chem.*, 185 (1950) 869.
3 I. K. BRANDT, *Biochem. Pharmacol.*, 15 (1966) 994.
4 D. SCHACHTER AND J. V. TAGGART, *J. Biol. Chem.*, 211 (1954) 271.

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* New address: Children's Hospital, San Francisco, Calif. 94119, U.S.A.