A LARGE SCALE PURIFICATION OF CRYSTALLINE PIG LIVER CARBOXYLESTERASE

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In order to carry out an extensive investigation of the mechanism of action of the carboxylesterases from animal livers*, we required a procedure suitable for the large scale preparation of highly purified enzyme. A crystalline "microsomal liver esterase" has been isolated from pig liver by Krisch (1963), whose starting material was the isolated microsomal fraction. The reported yield was low (14 mg.) and the method does not allow of easy scaling up because of the prohibitive times of ultracentrifugation involved. Moreover, Bernhammer and Krisch (1965) have expressed doubt as to whether this enzyme is the commonly identified pig liver carboxylesterase (E.C. 3.1.1.1).

For this reason we investigated the use of acetone powder as the starting material. Acetone powders have been used previously for liver esterase preparations (Connors et al., 1950; Burch, 1954; Adler and Kistiakowsky, 1961; Kibardin, 1962). However, none of

* For previous work on these enzymes, see (a) PIG: A.J. Adler and G.B. Kistiakowsky (1961); S.A. Kibardin (1962); K. Krisch, (1963). (b) HORSE: J.C. Boursnell and E.C. Webb (1949); W.M. Connors et al. (1950); J. Burch (1954). (c) OX: E.C. Webb (1948); B.T. White (1956); R.J.A. Kirkland (1963). (d) CHICKEN: G.I. Drummond and J.R. Stern (1961).

these purifications has led to a crystalline or homogeneous enzyme. A conspicuous contaminant of partially purified liver esterase preparations is a red pigment which has an absorption maximum at 410 mμ. Chloroform has been used to remove haemoglobin from acetone powders in the preparation of leucine aminopeptidase (Hill et al., 1958).

We now wish to report a simple procedure starting from a $CHCl_3$/acetone powder which produces 250 mg. of crystalline pig liver carboxylesterase in a single batch. Each kilogram of pig liver mince is homogenzied with 4 l. of chloroform (0°), filtered, and the resulting paste is then homogenized with 8-10 l. acetone (-10°) in an explosion proof blendor. The acetone powder is dried under vacuum and final traces of acetone are removed with conc. H_2SO_4. The thoroughly dried powder is stable for six months at 4°.

The purification procedure is summarized in TABLE I.

The enzyme from the final stage can be readily crystallized in the following way at pH 5.5 or 7. Solid ammonium sulfate is added until the first turbidity appears. This is removed with the minimum amount of buffer and the solution is slowly evaporated in a refrigerator at 4°. The enzyme crystallizes as large thin plates which are extremely fragile, and which fragment while under observation (see Figure 1). The crystals appear to be similar to, but better defined than, the crystals of the "microsomal liver esterase" of Krisch (1963).

The data given in TABLE I do not represent the best results obtained to date. A_{280}/A_{410} ratios of 360 have been obtained and the highest specific activity is 530. Further, other work has shown that CM sephadex chromatography frequently lowers the A_{280}/A_{260} ratio.

TABLE I

PURIFICATION OF PIG LIVER CARBOXYLESTERASE[a]

Step	A_{280}/A_{260}	A_{280}/A_{410}	Specific[g] Activity	% Yield
Powder[b] Extract	0.83	9	8	100
45-70% A.S. cut[c]	1.42	20	30	88
CM Cellulose[d]	1.66	23	230	50
Bio-Gel P-150[e]	1.70	75	330	45
CM Sephadex C-50[f]	1.63	170	500	20

[a] at 4°; [b] 10 g. powder per 100 ml. 0.1 M pH 3.9 citrate buffer; pH rises to 4.5 during extraction and the supernatant is then adjusted to pH 8 with 2 M NaOH; [c] dialyzed against 0.05 M acetate buffer, pH 5.65; [d] CM cellulose, type 20, Brown Paper Company; column dimensions, 5.5 x 90 cm.; the enzyme is retarded but is eluted with the starting buffer, 0.05 M acetate, pH 5.65; [e] after concentration with A.S.; column dimensions 5 x 90 cm.; elution buffer, 0.01 M phosphate, pH 7; [f] column dimensions, 2.4 x 80 cm.; starting buffer 0.1 M acetate, pH 5; eluted with a linear salt gradient provided by starting buffer 0.25 M with respect to ammonium sulfate; [g] activity is expressed as μmoles of 1.25×10^{-3} M ethyl butyrate hydrolyzed per minute at pH 7.5 and 38°; specific activity is defined as activity/A_{280}.

The enzyme is homogeneous on starch gel electrophoresis at pH 8.6 and 5.5 and on rechromatography on Sephadex G-200. A detailed ultracentrifugal analysis carried out by Dr J.R. Dunstone shows the enzyme to be at least 90% homogeneous with a molecular weight of 163,000 (measured partial specific volume, 0.733). This result may be compared with 174,000 (partial specific volume assumed, 0.75) for the "microsomal liver esterase" (Boguth et al., 1965). However, calculation of our data using the partial specific volume assumed by

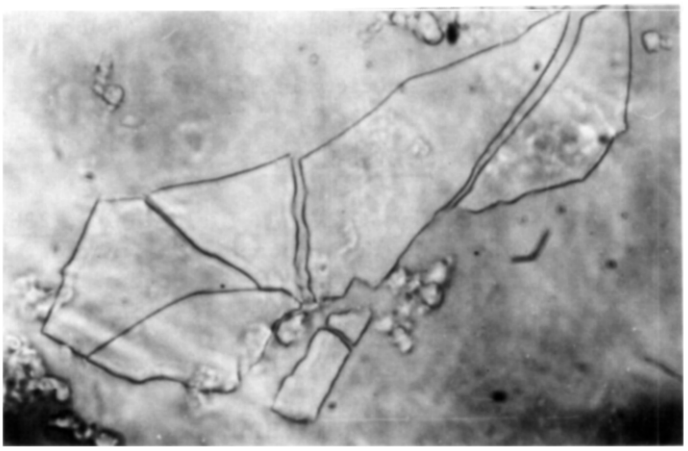

Fig. 1. Crystalline Pig Liver Carboxylesterase (x 500)

Krisch, gives a molecular weight of 172,000. It seems probable, therefore, that the "microsomal liver esterase" is indeed pig liver carboxylesterase (E.C. 3.1.1.1), and that the $CHCl_3$/acetone powder procedure introduces no artifacts.

Similar, although not identical, procedures have also led to homogeneous preparations of the carboxylesterases from horse and ox livers. These preparations have enabled us to investigate the chemistry of the enzymes and in the following communication we report on the determination of the normality of a pig liver carboxylesterase solution.

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