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Carboxylesterases (EC 3.1.1). A Large-Scale Purification of Pig Liver Carboxylesterase*

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ABSTRACT: Two procedures for the large-scale purification of pig liver carboxylesterase are described. They start from chloroform–acetone powders of minced pig liver and involve ammonium sulfate fractionation, chromatography on CM-cellulose and CM-Sephadex, and gel filtration. These procedures produce an enzyme of hitherto unobtained purity. The yield of enzyme is about 250 mg from 800 g of powder.

This is the first of a series of papers concerned with the purification, properties, and mechanism of action of carboxylesterases (carboxylic ester hydrolases, EC 3.1.1) from various animal and plant sources.

There are many reports in the literature on the preparation of mammalian liver carboxylesterases.[1] However, none of the reported procedures yields a large amount of homogeneous enzyme. Burch (1954) reported one of the more successful purifications of horse liver carboxylesterase (EC 3.1.1.1) which used an acetone-dried powder of horse liver as the source of the enzyme. Electrophoresis indicated that the protein was ~95% homogeneous. However, it was contaminated by hematin.

The pig liver enzyme has received most attention. Adler and Kistiakowsky (1962) were the first to report the preparation of a homogeneous sample of the enzyme. However, in our hands this procedure failed to yield a satisfactory preparation. Kibardin (1962a) has also reported the preparation of a "homogeneous" enzyme which he succeeded in resolving into three components (Kibardin, 1962b). His work provides the first real indication of the difficulties encountered in the purification of these enzymes.

Krisch (1963) made a significant advance on previous work. Using an isolated microsomal fraction as starting material, he succeeded in crystallizing the enzyme. However, the reported yield was low (14 mg) and the method does not permit easy scaling up because of the prohibitive times of ultracentrifugation involved.

As part of a broadly based program concerned with the mechanism of action of hydrolytic enzymes, the carboxylesterases were of immediate interest because of their reported similarities to the serine proteinases. For this reason, we required large-scale preparations of highly purified enzymes.

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[1] For previous work on these enzymes, see (a) pig: Rozengart *et al.* (1952), Adler and Kistiakowsky (1961, 1962), Kibardin (1962a,b), Krisch (1963, 1966), Boguth *et al.* (1965), Barker and Jencks (1967), and Levy and Ocken (1967); (b) horse: Boursnell and Webb (1949), Connors *et al.* (1950), Burch (1954), and Hofstee (1967); (c) ox: Webb (1948), White (1956), Kirkland (1963), and Benöhr and Krisch (1967a,b); (d) chicken: Drummond and Stern (1961); and (e) sheep: Baker and King (1935).

In this paper, we report the first large-scale purifications of pig liver carboxylesterase.

Experimental Section

Preparation of Acetone Powders. Approximately 1 kg of fresh pig liver mince was homogenized with 5 l. of redistilled acetone (−30 to −10°) in an explosion-proof Waring Blendor. The acetone was filtered through a large Büchner funnel (24 cm, Whatman No. 542 paper). The filter cake was washed with cold acetone (−30 to −10°) until the filtrate was colorless. Excess acetone was removed by compressing the cake with a rubber dam fitted over the Büchner funnel. The filter cake was then finely divided and dried *in vacuo* (10°), the residual acetone being condensed in two traps at −80°. Final traces of acetone were removed by evacuating the powder over concentrated H_2SO_4 (10°). The thoroughly dried preparation was powdered by grinding in a Waring Blendor and stored in air-tight jars at 4°.

Preparation of Chloroform–Acetone Powders. The chloroform–acetone powders were prepared in a similar manner to the acetone powders except that 1 kg of liver mince was first homogenized with 5 l. of redistilled chloroform (−30 to −10°). After the chloroform was removed by filtration, the paste was treated with acetone as described above.

Extraction of Powders. Each aliquot of powder was extracted with ten volumes of 0.1 M citrate buffer (pH 3.9) at 4°. The powder was added slowly to the buffer which was stirred continuously, while the pH was measured with a combined glass–saturated calomel electrode. As the protein was extracted the pH rose and was maintained between pH 4.3 and 4.5 by the dropwise addition of 2 N HCl. The pH became steady after about 5 min and the suspension was then stirred for a further 30 min. The suspension was centrifuged (in the 959A "45° Angle Blood Bag Head" of the International PR-2 centrifuge which holds 2400 ml) for 40 min at 3000*g* (0°). The pH of the supernatant was raised to 8.0 by the dropwise addition of 2 N NaOH. This caused the precipitation of flocculent inactive protein which could be easily removed by centrifugation, or alternately, in the ammonium sulfate fractionation.

Ammonium Sulfate Fractionation. Dixon's (1953) nomogram was used to determine the amount of ammonium sulfate to be added. The solution to be fractionated was stirred with a magnetic stirrer while solid ammonium sulfate (B. D. H. AnalaR, or Mann Research Laboratories Special Enzyme Grade) was added slowly. When all the ammonium sulfate was dissolved, the solution was allowed to stand at 4° for 30 min and then centrifuged at 3000*g* for 45 min (0°).

Chromatography. All chromatography was carried out in the cold room at 3–5°. The following media were used in the purification: DEAE-Sephadex (A-50, medium, Pharmacia, Sweden), DEAE-cellulose (type 40, Brown Paper Co., Berlin, N. H.), CM-cellulose (type 20, Brown Paper Co.), CM-Sephadex (C-50, Pharmacia), Sephadex G-100 and G-200 (Pharmacia), and Bio-Gel P150 (Bio-Rad, Calif.).

A. DEAE-SEPHADEX. DEAE-Sephadex was prepared for chromatography by washing extensively with distilled water. The suspension was stirred for a short time and allowed to settle, and the supernatant containing the fines was decanted. This was repeated up to a dozen times. With some batches of DEAE-Sephadex, the suspension at this stage was still light brown or yellow. This color could be removed by washing with 0.01 N NaOH and then with distilled water. The resin was then equilibrated with the column buffer (0.005 M Tris, 0.001 M in EDTA, pH 8.6). Equilibration was achieved by washing with the column buffer until the pH of the supernatant was constant at 8.6. The column was packed using a thick slurry of the resin. Column buffer (~2 l.) was run through the column and the pH of the eluent was measured to ensure complete equilibration. When the pH of the eluent was steady at 8.6, the enzyme solution, which had been dialyzed thoroughly against the column buffer, was added carefully to the column and was slowly washed on with the column buffer. Washing was continued until protein ceased to appear in the eluent. Gradient elution was then started. With DEAE-Sephadex an exponential gradient was used. The eluent was collected in an automatic fraction collector (L. K. B. Radirac, Sweden) and assayed for protein and enzymatic activity.

B. DEAE-CELLULOSE was prepared by washing with 0.01 N NaOH and distilled water as described for DEAE-Sephadex. Various column buffers, as described in the results section, were used. Elution was achieved with both exponential and linear gradients.

C. CM-CELLULOSE was prepared in a similar manner to DEAE-cellulose. Columns were equilibrated with 0.05 M acetate buffer at pH 5.0 and 5.5. At pH 5.5, the enzyme did not bind to the CM-cellulose and was eluted with the starting buffer. At pH 5.0, the enzyme did bind and was eluted either with a linear salt gradient or with an ascending pH gradient. The pH gradient was established by mixing 0.05 M sodium acetate and the starting buffer in a linear gradient apparatus.

D. CM-SEPHADEX was prepared by washing with dilute alkali and dilute acid as recommended by the manufacturers. It was then washed with distilled water and equilibrated with the starting buffer (0.1 M acetate, pH 5.0). Both pH and salt gradients were used to elute the enzyme from the columns.

E. GEL FILTRATION. Sephadexes G-100 and G-200 and Bio-Gel P150 were prepared by slowly adding the dry powder to an excess of distilled water stirred with a magnetic stirrer. The gels were washed by decantation several times and then allowed to swell in water overnight. They were then washed with 0.01 M phosphate buffer (pH 7) and allowed to equilibrate for at least 24 hr.

Regeneration of Media. The ion-exchange media were regenerated by emptying the columns and washing the resins thoroughly with 0.1 M trisodium phosphate (pH 12.5) to remove residual protein. This step was followed by washes with water and column buffer as described previously. The Sephadex and Bio-Gel columns were cleaned by passing an excess of phosphate buffer through them, until the eluent showed negligible absorbance at 260 and 280 mμ.

TABLE I: Purification of Pig Liver Carboxylesterase from a Chloroform–Acetone Powder. First Procedure.

Step	A_{280}/A_{260}	A_{280}/A_{410}	Sp Act.	% Yield	Total Protein[h] (g)
1 Extraction[a]	~1	~10	~8	100	~54
2 45–70% saturated ammonium sulfate cut[b]	1.4	9	26	89	14.6
3 CM-cellulose[c]	1.66	10	100 (178)[d]	58 (100)[d]	2.5 (3.0)
4 CM-Sephadex[e]	1.75	27	400	(68)	0.688
5 Sephadex G-100[f]	1.69	34	468	(66)	0.564
6 CM-Sephadex[g]	1.65	272	503	(34)	0.271

[a] 430,000 units extracted from 400 g of powder. [b] The ammonium sulfate cut was dialyzed against 0.05 M acetate buffer (pH 5.5). [c] Column dimensions 6 × 56 cm. The column buffer was 0.05 M acetate (pH 5.5). [d] 150,000 units of enzyme with specific activity of 300 (550 mg) added at this point to give a specific activity of 178 and a total activity of 400,000 units. This value (100%) was used to calculate the yields in steps 4–6. [e] Column dimensions, 4.8 × 90 cm. Enzyme concentrated with 90% saturated ammonium sulfate and dialyzed against starting buffer (0.1 M acetate, pH 5.0); enzyme eluted with a linear salt gradient of 0.5 M ammonium sulfate in 0.1 M acetate (pH 5.0). [f] Column dimensions, 5.0 × 95 cm. Enzyme concentrated with 90% saturated ammonium sulfate and dialyzed against the column buffer (0.01 M phosphate, pH 7.0). [g] Column dimensions 2.6 × 80 cm. Enzyme eluted with a pH gradient. The mixing vessel contained 0.1 M acetate (pH 5.0) (starting buffer) and the other vessel of a linear gradient system contained 0.1 M sodium acetate. Each vessel contained 1 l. of solution. [h] For the present purpose, a solution with an $A_{280} = 1$ is defined as containing 1 mg/ml of protein.

TABLE II: Purification of Pig Liver Carboxylesterase from a Chloroform–Acetone Powder. Second Procedure.[a]

Step	A_{280}/A_{260}	Sp Act.	% Yield	Total Protein[e] (g)
4 CM-Sephadex[b]	1.76	220	33	2.23
5 Bio-Gel P150[c]	1.70	367	33	1.33
6 CM-Sephadex[d]	1.70	500	14	0.436

[a] Four 400-g powders containing 2.86×10^5, 3.1×10^5, 4.7×10^5, and 4.9×10^5 units were extracted. These were purified as described in steps 1–3 of Table I resulting in a preparation with $A_{280}/A_{260} = 1.67$, a specific activity of 110, and a yield of 49% (7.6×10^5 units) (6.92 g). [b] Column dimensions, 3.5 × 100 cm. Protein eluted as described in step 4 of Table I. A column of 5-cm diameter probably would have increased the purity and yield. [c] Column dimensions 5 × 90 cm. The protein was concentrated with 90% saturated ammonium sulfate and dialyzed against the column buffer (0.01 M phosphate, pH 7.0). [d] Column size, 3.5 × 100 cm. Enzyme eluted with a linear salt gradient provided by starting buffer 0.25 M with respect to ammonium sulfate. Starting buffer, 0.1 M acetate (pH 5.0). [e] For the present purpose, a solution with an $A_{280} = 1$ is defined as containing 1 mg/ml of protein.

Starch Gel Electrophoresis. The starch gel was prepared according to the method of Smithies (1959), using hydrolyzed starch (Connaught Research Laboratories, Toronto). The starch was boiled briefly under reduced pressure, poured into Perspex trays (15 × 5 × 1.2 cm), covered with a greased Perspex sheet, and left to cool for 2 hr before use. Buffers of various concentrations were used in continuous systems.

The sample was introduced on an insert of filter paper. Electrophoresis was carried out at 4° and ~300 V using Shandon equipment. After electrophoresis, the gels were sliced in two using a taut, fine nichrome wire. The cut surfaces were stained for protein and for esterase activity, using the methods of Giri (1956, 1957) and Gomori (1952), respectively.

Polyacrylamide Gel Electrophoresis. The apparatus used is discussed in detail by Holmes (1967) and is essentially an application of the continuous system of Raymond (1964). The gel concentration was 7 or 8% and the gel was buffered at pH 8.9 with 0.03 M (total) Tris–glycine buffer. The electrophoresis was run for 3 hr at 4° (400 V, ~1.5 mA/gel). The staining techniques were the same as those described for the starch gel electrophoresis.

Enzyme Assay. Carboxylesterase activity was measured using a Radiometer TTT1c automatic titrator equipped with an SBR2c recorder. The reaction was initiated by the addition of a 100-μl aliquot of the enzyme solution to 10 ml of 0.0125 M ethyl butyrate at pH 7.5.[2] This pH was maintained by the automatic addition of 0.0100 N NaOH contained in a 0.5-ml Agla micrometer syringe buret. The reaction mixture, in a

[2] The concentration of ethyl butyrate used in the assay system was incorrectly given in a preliminary communication as 1.25×10^{-3} M (Horgan *et al.*, 1966a).

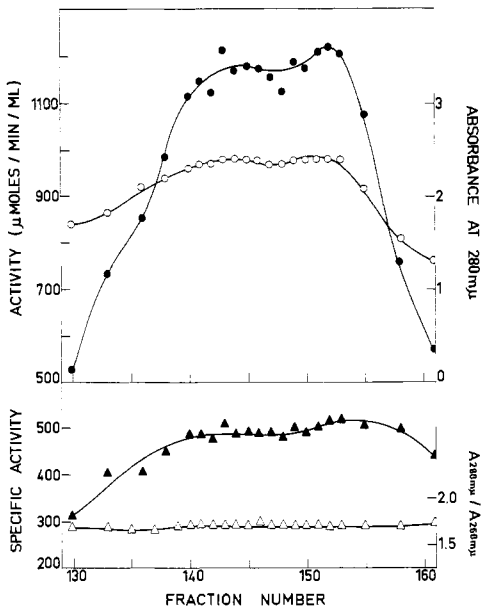

FIGURE 1: CM-Sephadex chromatogram from step 6, Table II. (○) A_{280}; (●) activity; (△) A_{280}/A_{260}; and (▲) specific activity.

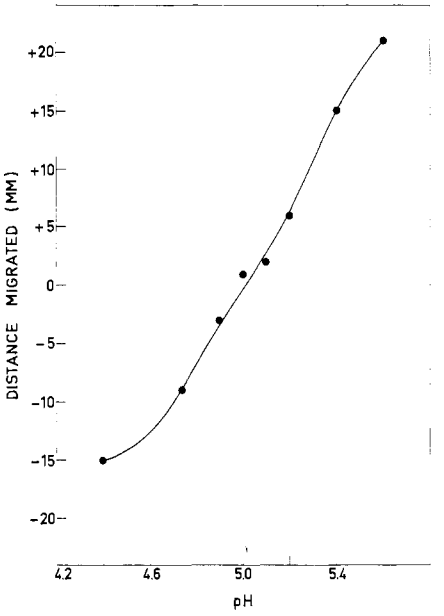

FIGURE 2: Isoelectric point determination of pig liver carboxylesterase. Gels were run in acetate buffers of constant ionic strength (μ = 0.025). Conditions: 220 V, 12–20 mA, 14 hr.

stoppered tube (2.5 × 5.7 cm), was equilibrated at 38 ± 0.1° for 15 min before the addition of enzyme. Under the conditions of the assay, no correction for spontaneous hydrolysis or for the absorption of carbon dioxide was necessary. Further, butyric acid (pK_a = 4.8) is completely ionized.

One unit of carboxylesterase activity is defined as the amount of enzyme required to catalyze the hydrolysis of 1 μmole of ethyl butyrate/min, under the assay conditions described above.

Protein Estimation. Protein concentration was estimated by measuring the absorbance at 280 mμ in a 1-cm cuvet using the Shimadzu QR-50 spectrophotometer. The purification of the enzyme was followed by measuring two ratios: (i) specific activity = activity (in units/ml)/A_{280} and (ii) A_{280}/A_{260}. The first ratio gives a measure of the amount of enzyme present relative to the total protein concentration, and the second gives a measure of the degree of fractionation of the protein (Warburg and Christian, 1941).

A red pigment was extracted from the powders and its concentration was estimated spectrophotometrically at 410 mμ.

Results

Preliminary experiments using acetone powders established that the best pH for extraction was pH 4.6. A small-scale procedure involving a heating step and DEAE-Sephadex chromatography (*cf.* Krisch, 1963) led to the first crystalline enzyme (specific activity 370). Although crystalline, the enzyme was obviously still not pure. This was shown by the low A_{280}/A_{260} ratio (1.32) and also by the large absorbance at 410 mμ (A_{280}/A_{410} = 3). In fact, the procedure actually concentrated the pigment. Krisch (1963) reported a value of ~1.58 for the A_{280}/A_{260} of his microsomal preparation, but did not give any values for absorbances at wavelengths greater than 310 mμ. Further, on a larger scale, the heating step proved to be nonreproducible. These disadvantages, coupled with the extremely slow flow rates of DEAE-Sephadex columns, led to the development of two closely related new procedures (Tables I and II).

The first procedure (Table I) yielded the first highly purified enzyme. The second procedure (Table II) is a slight modification of the first. Figure 1 shows the elution profile of the enzyme from the final CM-Sephadex step of Table II. It is evident that neither the specific activity nor the A_{280}/A_{260} ratio changes sensibly during the elution of some 320 mg of enzyme.

The enzyme from the final stages of these procedures can be readily crystallized at pH 5.5 or 7 by slow evaporation at 4° of an ammonium sulfate solution almost saturated with respect to the enzyme. The enzyme crystallizes as large thin plates which are extremely fragile and which undergo fragmentation while under observation (Horgan *et al.*, 1966a).

An isoelectric point of 5.0 was determined for the pure protein using starch gel electrophoresis (Figure 2). This value compares with the values of 5.7 and 5.0 determined by Kibardin (1962a) and Adler and Kistiakowsky (1961), respectively.

Discussion

A major problem in the purification of mammalian liver carboxylesterases is the high concentration of a red pigment in acetone powder extracts. Burch (1954)

reported the presence of hematin in her horse liver enzyme, while Krisch (1963) states that cytochrome b_5 is a contaminant in the early stages of purification of the pig liver enzyme. Further, it should be noted that the last two steps of Krisch's procedure are chromatography on DEAE-Sephadex.

The pigment extracted in the present work had an absorption maximum at 410 mμ and is likely denatured hemoglobin. Hill *et al.* (1958) reported the use of chloroform to remove hemoglobin from acetone powders of pig liver in the preparation of leucine aminopeptidase. Again, in the present work, when acetone powder extracts were shaken with chloroform, there was slow removal of the red pigment, but marked denaturation occurred when this procedure was applied at later stages of the preparation. This led us to the preparation of chloroform–acetone powders. While chloroform at 0° and acetone at −30 to −10° were initially used (Horgan *et al.*, 1966a) the procedure has been modified by routinely using chloroform and acetone initially at −30°, resulting in better powder preparations. These chloroform–acetone powders give extracts with specific activities ~50% higher than those obtained from acetone powders and there is, moreover, a three- to fourfold reduction in the concentration of the red pigment.

The data given in Tables I and II are typical results obtainable by the individual procedures. A_{280}/A_{410} ratios of 360 have been obtained, and the highest specific activity so far obtained is 530. No hard and fast rules can be set down as to which is the best procedure and which is the best sequence of the various steps. Moreover, reproducibility is made the more difficult by variations in the source of the enzyme, *i.e.*, the pig livers. It has been found that the esterase content of the livers has varied by more than a factor of 2 during the 6 years of this investigation. A large decrease (>50%) was noted at the time of a severe drought. Similar decreases were also found in the esterase content of ox and horse livers at the same time.

The first three steps of the purification (Table I) appear to be very successful and are never varied. Indeed, the key step appears to be chromatography on CM-cellulose. The specific activity of the enzyme after the CM-cellulose step is variable (specific activity ~90 to ~250). Nor is this variability logically related to the esterase content of the chloroform–acetone powders. Powders containing ~600 units of enzyme/g have yielded specific activities of 250 and 220 after CM-cellulose chromatography, while powders containing ~1100 units/g have yielded specific activities of ~100 after this step. It is possible to delete the first CM-Sephadex chromatography step of the second procedure (Table II) if the specific activity of the enzyme after CM-cellulose chromatography is greater than 200 (see Horgan *et al.*, 1966a, for an example).

After the final CM-Sephadex chromatography at pH 5.0, the pH of the solution should be raised to neutrality, since the enzyme slowly and irreversibly denatures at low pH (see below).

Adler and Kistiakowsky (1961) used DEAE-cellulose in the final stages of their purification of the enzyme.

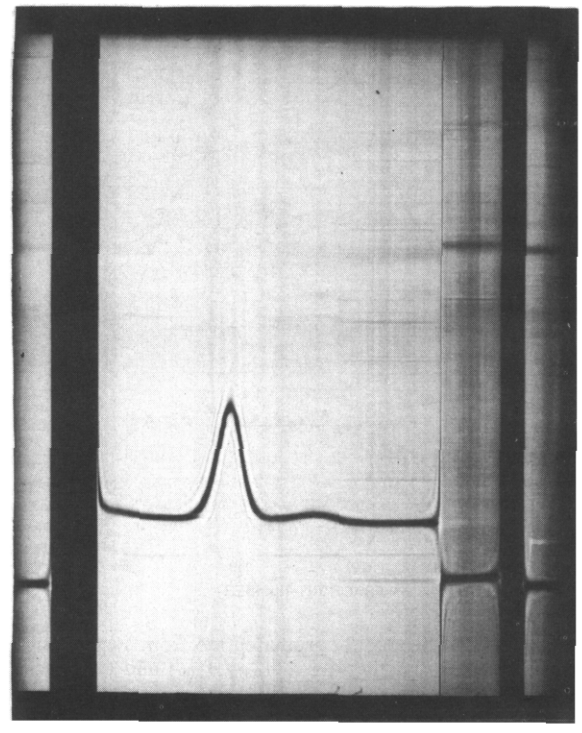

FIGURE 3: Schlieren diagram of aged pig liver carboxylesterase (see text). The photograph was taken 63 min after reaching speed (59,780 rpm).

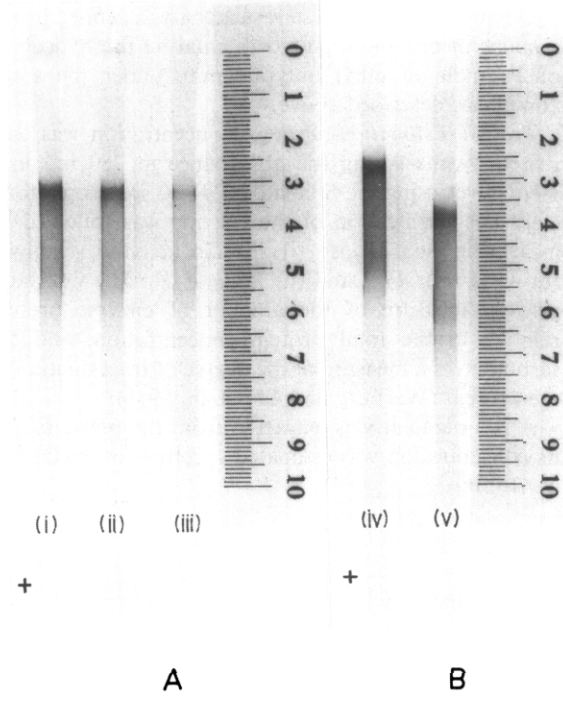

FIGURE 4: Polyacrylamide gel electrophoretograms of pig liver carboxylesterase stained for enzyme activity. (A) Enzyme of specific activity 570; 7% gel: (i and ii) 0.2 mg/ml; (iii) 0.13 mg/ml. (B) Enzyme of specific activity 566: (iv) 0.27 mg/ml, 8% gel; (v) 0.27 mg/ml, 7% gel.

In our hands, this material has failed to yield satisfactory results. Chromatography of a sample with specific activity of 400 and an A_{280}/A_{260} ratio of 1.64 on DEAE-cellulose (pH 7.5, 0.05 M Tris, 0–0.4 M NaCl linear gradient) raised the specific activity to only 427 and resulted in a lowering of the A_{280}/A_{260} ratio to 1.58.

The procedures are suitable for obtaining large amounts of highly purified enzyme. From 800 g of chloroform–acetone powder, the yield is ~250 mg of enzyme with specific activity of 500. The methods yield purer preparations of pig liver carboxylesterase than those previously reported. The elution profile from CM-Sephadex (Figure 1), from which 320 mg of enzyme of constant specific activity (500) and constant A_{280}/A_{260} ratio (1.70) was isolated, is good evidence for a significantly purified enzyme. While the absorbance profile (Figure 1) might suggest some inhomogeneity of the protein, none was apparent on polyacrylamide gel electrophoresis, apart from the usual proportion of faster moving isoenzymes in a sample of this specific activity.

Starch gel electrophoresis of the purified enzyme at pH 5.5 and 8.6 showed only a single band when the gel was stained for both protein and esterase activity. Polyacrylamide gel electrophoresis, however, has revealed that the enzyme does not behave as a single species. While the protein stain shows only a single band, the far more sensitive esterase stain reveals the presence of up to four faster moving minor components which together in a freshly prepared sample of specific activity of 500–520 constitute possibly 5–10% of the total activity (*cf.* Figure 4).

While the highest specific activity obtained from the procedures in Tables I and II is 530 (A_{280}/A_{410} = 360), pig liver carboxylesterase of specific activity 550–570 appears to be very nearly pure enzyme. Specific activities of this magnitude have been reproducibly obtained in peak tubes from enzyme, prepared by the second procedure (Table II), which had lost activity on storage. For example, the particular batch of enzyme whose preparation is described in Table II had fallen in specific activity to 465 after 16 days at pH 5.0 (A_{280} = 2.3–2.4). After concentration (ammonium sulfate), dialysis, and storage in 0.024 M phosphate (pH 7.0) for 5 weeks at 4° (A_{280} = 14.3), the enzyme was further concentrated and stored in the same buffer (A_{280} = 36.4); 15 days after concentration, the analytical ultracentrifuge showed two components whose sedimentation coefficients were consistent with molecular weights of ~80,000 and ~160,000 (Figure 3). The enzyme was stored for 5 months at 4°. The specific activity after this time was 446, and the enzyme solution was cloudy and very pale yellow. Gel filtration on Sephadex G-200 of this sample in 0.046 M phosphate (pH 7.0) raised the specific activity of the enzyme to 550 across the peak tubes, and the pooled peak had specific activity of 520. The elution profile from Sephadex G-200 showed the separation of inactive protein at the void volume of the column as well as the material of mol wt ~80,000. After storage of the pooled peak for a further 7 months at 5° (A_{280} = 3.67), the specific activity had fallen to 490, the solution was slightly yellow, and it contained a small amount of white precipitate. Gel filtration on Sephadex G-200 (as above) again raised the specific activity of the enzyme to 550–570 (A_{280}/A_{410} ~ 460) in the peak tubes. Almost all the contamination was eluted at the void volume of the column. Polyacrylamide gel electrophoresis of this enzyme (specific activity ~ 570) is shown in Figure 4. The enzyme still contains faint traces of faster moving components, but is obviously a respectable protein. Moreover, the results of the analytical ultracentrifuge study on enzyme of specific activity 503 (Horgan *et al.*, 1966b) are consistent with these data. While this Sephadex G-200 step has not been applied to enzyme obtained by the first procedure (Table I), there is no reason to believe it would not be equally effective.

Finally, attention should be drawn to the fact that there are considerable variations within the group of esterases covered by the single entry EC 3.1.1.1. This entry was based originally on the properties of a preparation from horse liver with a high activity toward ethyl butyrate as substrate. The pig liver enzyme and the ox liver enzyme (Runnegar *et al.*, 1969), while similar to the horse liver enzyme, are distinctly different proteins. The problem is further complicated by the existence of isoenzymes which have not, as yet, been completely resolved.

Acknowledgments

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Carboxylesterases (EC 3.1.1). The Molecular Weight and Equivalent Weight of Pig Liver Carboxylesterase*

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ABSTRACT: The molecular weight of pig liver carboxylesterase (~88% pure) has been determined as 163,000 (±15,000). The enzyme has two active sites per molecular weight of 163,000 as shown by titration with *p*-nitrophenyl dimethylcarbamate and *p*-nitrophenyl diethyl phosphate. The enzyme undergoes slow irreversible inactivation at pH 5, but on dilution at pH 7.5, the enzyme dissociates apparently into half-molecules which are active.

No evidence has been obtained for the dissociation of the enzyme into species of mol wt ~40,000, either at pH 8 or in 8 M urea.

The physicochemical properties of mammalian liver carboxylesterases have not been extensively investigated. Moreover, only a few enzymes from different animal sources have been studied. This is a direct consequence of the lack of large quantities of these enzymes in highly purified form.

Adler and Kistiakowsky (1961) estimated the molecular weight of pig liver carboxylesterase purified from an acetone powder as 150,000–200,000, using only sedimentation coefficients as the basis for their estimate. The molecular weight of pig liver "microsomal" carboxylesterase as measured by sedimentation and diffusion, by approach to sedimentation equilibrium (Archibald, 1947), and by gel filtration has been given as 174,000 ± 9,000 (Boguth *et al.*, 1965) with reasonable agreement between the three methods used. These authors assumed a partial specific volume of 0.75. Deviations from this value would considerably affect the calculated values of the molecular weight. Further, their experiments using the Archibald method indicated some degree of heterogeneity, as the molecular weights calculated at the air–solution meniscus were invariably less than those calculated at the bottom of the centrifuge cell. No attempt to evaluate the extent of this heterogeneity or to demonstrate the homogeneity of the major component was made. More recently, Barker and Jencks (1967) have reported a molecular weight of 168,000 at pH 7.4, measured by sedimentation equilibrium, for pig liver carboxylesterase purified apparently by the procedure of Adler and Kistiakowsky (1961). Their figure is based on a partial specific volume calculated from amino acid analysis of 0.740.

In contrast to the above results, molecular weights of 180,000 at pH 7.2 and 45,000 at pH 8.0 have been reported for pig liver carboxylesterase (Kibardin, 1962).

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