ISOLATION AND PROPERTIES OF A CARBOXYLESTERASE FROM PIG KIDNEY

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In 1963 we reported on the isolation, properties, and substrate specificity of a carboxylesterase (EC 3.1.1.1) from pig liver microsomes (Krisch, 1963). Studies with other organs of the pig revealed the presence of similar carboxylesterase activities in the kidney, and also, to a less extent, in the duodenum and brain. As in liver, the highest specific activities were found in the microsomal fraction of the kidney (Benöhr et al., 1966). In the following we report briefly on the isolation and properties of a carboxylesterase from pig kidney.

1. Isolation: The preparation follows essentially the prescription given previously for pig liver esterase (Krisch, 1963). Solubilization of the activity from large-scale prepared kidney microsomes is achieved easily by extraction with glycerol. Further purification is accomplished by fractionation with ammonium sulfate and column chromatography on DEAE-Sephadex A 50. The precipitation with acetic acid at pH 4.2 (step III of the liver preparation) is omitted because of the greater instability of the kidney enzyme at acid pH-values. Instead, the enzyme was purified by additional gel filtration on Sephadex G 200 and by a final rechromatography on DEAE-Sephadex A 50. In Table I the isolation procedure, purification and yield of pig kidney carboxylesterase are summarized:

TABLE I. PURIFICATION OF PIG KIDNEY CARBOXYLESTERASE

Step		Specific activity+)	Yield (%)	Purification
I	Pig kidney microsomes	13	100	1
II	Supernatant of glycerol extract after 50% AS saturation	74	72	5.7
III	AS fraction 50-80% after dialysis	125	55	9.6
IV	Combined eluates after column chromatography on DEAE-Sephadex A 50	446	43	34.3
V	Combined eluates after column chromatography on Sephadex G 200	970	36	74.5
VI	AS fraction after rechromatography on DEAE-Sephadex A 50, precipitated at 64% saturation	977	26	75.0

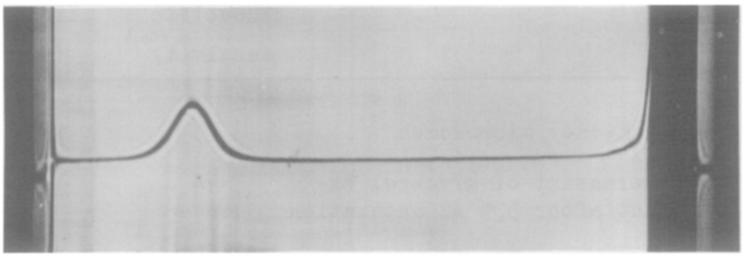
AS = ammonium sulfate

+) Acetanilide test (Krisch, 1963). Activities expressed as nmoles aniline formed/min . mg protein.

2. Properties: The final enzyme preparation is colorless and, after lyophilization, pure white. It shows a typical protein absorption spectrum in the UV region with a maximum at 280 nm. The ratio of the optical densities 280/260 nm is 1.62. In paper electrophoresis experiments (veronal-acetate buffer pH 8.6) only one band migrating anodically is detected. In high-voltage electrophoresis on starch gel, also, one band is only visible after staining with amido black 10 B. In some preparations, however, one additional weak band was observed which moved more slowly. Substrate staining with α-naphthyl acetate revealed that this minor band also shows esterase activity.-

In the analytical ultracentrifuge the enzyme behaves as a homogeneous symmetrical boundary. One example of the sedimentation pattern is given in Fig. 1:

FIG. 1. VELOCITY SEDIMENTATION DIAGRAM OF PIG KIDNEY ESTERASE



Protein concentration c_o = 5,56 mg/ml, dissolved in 0.2 M NaCl in 0.005 M tris buffer pH 8.6. Sedimentation from left to right. The photograph was taken 50 minutes after the final speed of 45 000 r.p.m. was reached. Phase plate angle 80°.

From these experiments the sedimentation coefficient was calculated as $s^{o}_{20,w} = 8.25 \pm 0.25$ and the molecular weight was found to be 170 000. Independently, the molecular weight was also estimated by gel filtration on Sephadex G 200. Within the limits of experimental error the same value was obtained as in the ultracentrifugation experiments. Similar to the carboxylesterase from pig liver (Krisch, 1966) the kidney enzyme reacts stoichiometrically and rapidly with the organophosphorus inhibitor E 600 (diethyl-p-nitrophenyl phosphate). In the presence of an excess of E 600, one mole of p-nitrophenol is liberated by about 80 000 g of esterase protein. Since this corresponds of about one half of the molecular weight it can be assumed that one esterase molecule contains two active sites.

In the column chromatography experiments on DEAE-Sephadex occasionally one small additional activity peak was observed eluting at lower NaCl concentrations (0.06 M) than the main fraction. This phenomenon occurred particularly if the protein concentration of the applied enzyme solution was low. Since this small peak was also found

sometimes when the main peak was rechromatographed it obviously is derived from the main enzyme fraction. This is very probably explained by partial dissociation of the enzyme molecule into two subunits. Analogous results were also obtained recently in our laboratory with pig and beef liver carboxylesterase preparations (Benöhr and Krisch, unpublished observations).- The pH optimum of the kidney carboxylesterase is rather broad with a maximum between pH 8 and 9.-

3. Substrate specificity: A marked similarity with pig liver carboxylesterase was also observed in the substrate specificity. As the liver enzyme, the kidney preparation catalyzes two different types of reaction, i.e. hydrolysis of carboxylic ester bonds and hydrolysis of amide bonds of certain acylated aromatic amines. The various substrates split by pig kidney esterase and their respective turnover numbers are listed in Table II. Obviously, the turnover numbers of pig kidney esterase towards amide substrates are very low as compared to the corresponding reaction rates towards carboxylic esters. With the exception of procaine these are 2-3 orders greater of magnitude.

The substrates listed in Table II are identical with those reported earlier for pig liver esterase (Krisch, 1963; Bernhammer and Krisch, 1965, 1966). There are, however, some differences in the turnover numbers. The kidney enzyme hydrolyses tributyrin about 10 times slower whereas its activity towards acetanilide is about twofold higher as compared with microsomal liver esterase. Neither proteolytic activity (substrate hemoglobin) nor dipeptidase activity (substrates glycyl-L-tyrosine, glycyl-L-phenylalanine amide acetate, L-leucyl-L-tyrosine, L-leucyl-glycine and L-seryl-glycine) was detected. Other substrates not attacked by pig kidney esterase are p-nitrophenyl phosphate, α-glycerophosphate, p-nitrophenyl sulfate, p-nitrophenyl-ß,D-glucopyranoside and acetylcholine.

4. Inhibitors: The enzymatic activity is blocked completely by

TABLE II. SUBSTRATE SPECIFICITY OF PIG KIDNEY CARBOXYLESTERASE

Substrates	Turnover number
1. Amide bonds	
Acetanilide (8)	192
Phenacetin (8)	12
L-Leucyl-ß-naphthylamide (6)	7
L-Leucyl-p-nitroanilide (4)	3
2. Carboxylic ester bonds	
Procaine (8)	104
Tributyrin +) (8)	3040
L-Tyrosine ethyl ester ++) (4)	6100
p-Nitrophenyl acetate ++) (4)	6500

The activities are expressed as moles substrate hydrolysed/min · 170 000 g of enzyme protein ($t = 37^{\circ}$).

+) Activity given as equivalents acid liberated/min · mole enzyme.

++) In these tests the temperature was 25°.

The data represent means obtained with different enzyme preparations, the number of experiments being indicated in brackets behind the substrates.

10^{-5}M E 600 and DFP (diisopropylphosphofluoridate). Other inhibitors are sodium fluoride, quinine and atoxyl. With these compounds, however, higher concentrations (10^{-2} - 10^{-3}M) are required for inhibition than with the organophosphorus compounds. Physostigmine sulfate is a less effective inhibitor of the kidney preparation (50 % inhibition at a concentration of $0.5 \cdot 10^{-4}$M) than of liver esterase. On the other hand, the kidney enzyme is more sensitive towards organic solvents such as methanol and acetonitrile. Addition of various inorganic ions (Mg^{++}, Zn^{++}, Fe^{++}, Ca^{++}, Co^{++}; 10^{-3}-10^{-4}M) did not significantly alter the enzymatic activity. EDTA

(ethylenediaminetetraacetic acid) and SH-blocking agents do not inhibit kidney carboxylesterase.-

Part of this work, presented here in a preliminary form, will be reported in detail elsewhere.

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