addition, the ability of the drug to alter water movement appears not to be related to its action on the passive leakage of ions.

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A note on the purification procedure used to isolate canine liver lipase for kinetic studies with BCME*

(*Received* 6 *June* 1963; *accepted* 11 *June* 1963)

IT HAS been previously reported (1) that the kinetics of tributyrin hydrolysis observed with crude aqueous extracts of acetone powders of dog liver were different from the kinetics observed with a partially purified preparation of the same enzyme. This difference was due to a binding phenomenon between tributyrin and an unknown component in the crude extract which rendered a constant concentration of tributyrin unavailable to the enzyme. Upon partial purification this component was removed, thereby resulting in a change in kinetics. The purification procedure used to partially purify the canine liver lipase for these studies follows.

METHODS

Enzyme assays using the tributyrin "clearing" method have been previously described;[1] in this an enzyme unit is defined as that amount of enzyme inducing an optical density change of 0·001/min under the conditions of assay. Protein concentrations were measured with the following micro biuret method of Dr. V. W. Rodwell. To a 2-ml sample containing 0·2 to 2·0 mg of protein is added 1 ml of biuret reagent. The contents of the tube are mixed, and readings are made in a Beckman DU spectrophotometer set at 540 Mμ against a reagent blank. Results are expressed in terms of a standard of crystalline bovine serum albumin (Armour).

Step 1, *extraction.* Five grams of dog liver actone powder prepared as described[1] were extracted with 50 ml of cold water for 10 min in ice. The mixture was then centrifuged and the supernatant fluid was decanted and saved. The residue was re-extracted with 25 ml of cold water as before, followed by centrifugation. The second extract was combined with the first. This is designated the "crude extract" in Table 1.

Step 2, *lead precipitation.* (Note that all volumes of reagents added refer to the volume of enzyme solution at the beginning of the step.) To the crude extract add 0·02 volume of 1 M $KHCO_3$. Then add 0·15 volume of 0·1 M lead acetate solution by drops with thorough mixing. The precipitate that forms is centrifuged down and discarded. To the supernatant fluid is added 0·06 volume of 0·25 M sodium ethylene diamine tetraacetic acid. This is called "lead fraction I."

Step 3, *first ammonium sulfate precipitation.* To EPb_1 add 0·56 volume of cold saturated ammonium sulfate solution. Centrifuge and discard precipitate. To the supernatant fluid add the same volume of ammonium sulfate solution. The precipitate is collected by centrifugation and made up to 0·5 volume in water. This fraction is designated "ammonium sulfate fraction I."

* Butyl carbamic acid methyl ester.

Step 4, *heating*. In a water bath at 55° the enzyme solution is brought to 54° as rapidly as possible and held at this temperature for 5 min. Immediately thereafter the solution is cooled, and the precipitate that forms is centrifuged down and discarded. The supernatant fluid is denoted the "heat-treated fraction" in Table 1.

Step 5, *dialysis*. The heat-treated fraction is dialyzed overnight against approximately 225 volumes of cold deionized water. This fraction is called the "dialyzed fraction" in Table 1.

Step 6, *calcium phosphate gel treatment*. To the dialyzed enzyme is added 0·15 volume of calcium phosphate gel containing 33 mg of calcium phosphate/ml. After thorough mixing, the preparation is held in ice for 5 min before centrifugation. The supernatant fluid is then decanted and discarded, and the calcium phosphate residue is washed once in 1 volume of 0·01 M NaH_2PO_4. During each elution, the gel is dispersed in the eluting medium with the aid of a Branson Sonifier, turned up to 10 amps for 15 sec. The mixture is then gently stirred in ice for 5 min, centrifuged, and the supernatant fluid discarded. The gel then undergoes two more similar elutions with 0·5 volume of 0·25 M NaH_2PO_4. These eluants are combined and are designated "gel fraction I" in the table.

The results obtained with three different preparations taken to this stage have been averaged and are shown in Table 1. Step 7 combines two ammonium sulfate fractions and has also been repeated three times.

TABLE 1. RESUMÉ OF THE FIRST SIX STEPS OF CANINE LIVER LIPASE PURIFICATION

Step no.	Fraction	Total volume (ml)	Units/ml	Total units	Protein (mg/ml)	Specific activity	Yield (%)
1	Crude extract	65·1	8,000	515,326	38·4	208·1	100
2	Lead fraction I	72·0	6,086	438,240	19·8	307·0	84·3
3	Ammonium sulfate fraction I	36·0	8,880	334,446	11·5	802·5	64·7
4	Heat-treated fraction	34·1	7,598	259,432	5·8	1,376·0	50·2
5	Dialyzed fraction	40·6	6,533	263,446	4·0	1,757·0	52·06
6	Gel fraction I	40·6	4,201	171,483	1·5	2,933·0	33·1

To gel fraction I is added 0·5 volume of cold saturated ammonium sulfate solution. The precipitate is centrifuged off and discarded. To the supernatant fluid is added 0·7 volume of cold saturated ammonium sulfate solution. The precipitate is collected by centrifugation and is made up in a minimal amount (0·75 to 0·8 ml) of water. A light flocculent material may form, and this is centrifuged off and discarded.

To the clear, straw-colored solution is added saturated ammonium sulfate in 0·05-ml increments with agitation, until a marked precipitate forms; this is collected by centrifugation and saved. In like fashion two more such fractions are precipitated and collected by centrifugation. All fractions are made up in 0·5 to 0·75 ml of water for assay. Those fractions with a specific activity above 4,500 (usually the last two) were saved from three separate isolations and combined. The composite enzyme had 90,825 units/ml, with a specific activity of 5,500. It contained 16·5 mg of protein/ml. This represents a 26-fold purification over the initial crude extract. While not an optimal procedure (each step in the purification does not result in a 2-fold purification with 80% recovery of enzyme from the preceding step), less than 2% of the initial protein present in the crude extract remained. The kinetic studies reported[1] on the purified canine liver lipase were conducted with this preparation.

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