A LIPID COFACTOR FOR THE ACID LIPASE OF THE CASTOR BEAN[1]

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The acid lipase of the castor bean, Ricinus communis, catalyzes the hydrolysis of triglycerides without requiring added emulsifiers (Ory et al., 1960). In this respect it differs from pancreatic lipase which acts only on emulsified fat (Desnuelle, 1961). One explanation for not requiring emulsifiers might be the existence of cofactor(s) in the particulate preparation, which mediate between the enzyme and the triglyceride substrate. This interpretation seems to hold for phospholipases, which have been shown to require cardiolipin, inositol phosphatides, or certain synthetic emulsifiers as cofactors (Weiss et al., 1959; Bangham and Dawson, 1960). In this communication we present some of the properties of a lipid cofactor extracted from the castor lipase with butanol following the procedure of Morton (1955).

The fatty layer obtained by centrifuging an extract of castor beans (Baker 296[3] variety kindly supplied by D. S. Bolley and W. E. Domingo of the Baker Castor Oil Company) in phosphate buffer, pH 7.0, contains the enzyme substantially free of most of the protein and the cell debris. Further extraction of this fatty layer with ether and saturated NaCl removes neutral fats and salt-soluble proteins. The particulate matter remaining is dialyzed and lyophilized to yield a lipase preparation which is stable in the cold for at least six months (Ory et al., 1962).

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3/ Trade names are given in reporting exact experimental conditions; no recommendations of the products over others of similar manufacture are implied.

This enzyme preparation may be split into two components: a particulate fraction with greatly reduced lipolytic activity (apoenzyme) and a viscous oil (cofactor) which, when added back to the apoenzyme, increases its lipase activity. In a typical preparation, 200 mg. of lipase were extracted with three 30 ml. portions of n-butanol in a tissue homogenizer set in ice. The butanol extract was clarified by centrifugation, the residue (apoenzyme) was extracted with acetone to remove excess butanol, and then dried *in vacuo*. Whereas 2.6 mg. of lipase in an excess of cottonseed oil produced 129 μmoles of fatty acid in 10 min., an equivalent amount of the apoenzyme produced 33 μmoles under the same conditions.

When the lipase is prepared from relatively fresh castor beans, the above-mentioned procedure removes most of the cofactor. In some experiments we used 4-year old seeds as the source of lipase. Although yield and activity of the enzyme were unaffected, it was no longer possible to extract the cofactor with butanol. This difficulty was overcome by freeze-drying the lipase from a suspension containing 0.05 M cysteine prior to extraction with butanol or by adding 2,3-dimercaptopropanol (BAL) to the butanol (0.05 moles/liter). The preferred procedure was to homogenize the freeze-dried lipase with butanol containing 0.05 M BAL, let stand for 17 hours in a refrigerator, centrifuge, re-extract the residue once more with the same solvent, then acetone extraction, and vacuum drying.

Approximately 50% of the weight of the original lipase preparation is recovered as the apoenzyme. It contains 12-14% nitrogen, is not wetted by water, and retains its activity for over a month when kept at room temperature. It was possible in some instances to remove all of the cofactor (*e.g.*, B, Table 1), but this was accompanied by destruction of *ca.* 75% of the apoenzyme. In no instance was it possible to remove cofactor without some loss in activity, hence, the wide variation in activity of various apoenzyme preparations on a single substrate.

The clear, thick, oily extract darkens readily on exposure to air, is water-insoluble, but dissolves easily in ether or ethyl alcohol. After removal

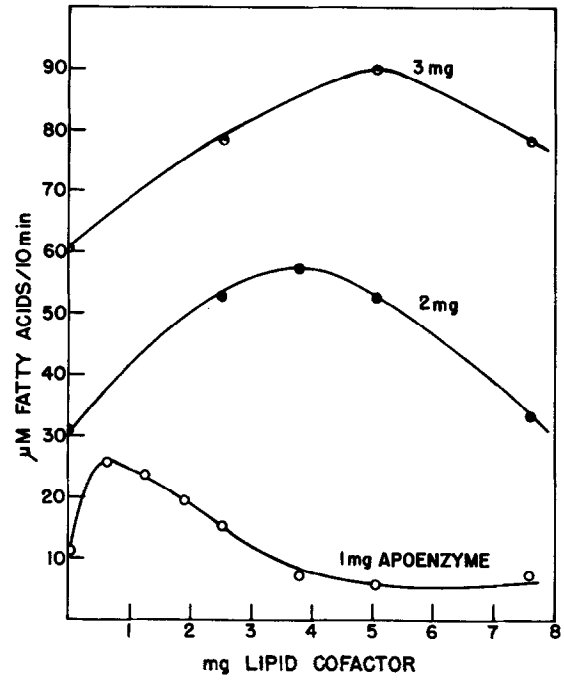

Figure 1. The effect of apoenzyme and cofactor concentrations on lipolysis.

Apoenzyme, cofactor, 1000 μmoles cottonseed oil, 0.05 ml. of 0.1 M Tris buffer, pH 8, and water to a total of 4.9 ml. were homogenized, then placed in a reaction vessel at 24°, and stirred. The pH was lowered to 4.2 by adding 0.1 M acetic acid, initiating lipolysis. After 10 minutes, 5 ml. of ethanol were added and the fatty acids titrated in a pH-Stat to pH 8.5 with 0.1 N NaOH.

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of the butanol by evaporation under nitrogen, the oil is taken up in ethanol and stored under nitrogen in the refrigerator, where it is stable for several weeks. The alcohol is evaporated under nitrogen prior to testing.

The effect of various amounts of lipid cofactor at three levels of apoenzyme is shown in Figure 1. A summary of the results of addition of cofactor to apoenzyme for various preparations and substrates is given in Table 1.

Under the conditions of the test, the requirement for cofactor is related to the concentration of apoenzyme; there is an optimum concentration for any given set of conditions. It is not simply a matter of providing an emulsifier, since emulsification of cottonseed oil with synthetic detergents will not eliminate the requirement for cofactor. Castor oil itself is not the cofactor since the effect of cofactor is clearly demonstrated with castor oil as the substrate.

Table 1.

Effect of cofactor on lipolysis of various substrates by apoenzyme preparations.

Apoenzyme Prep.	Apoenzyme Conc. mg./5ml.	Cottonseed Oil		Cottonseed Oil Emulsion		Tributyrin	
			Cofactor Added		Cofactor Added		Cofactor Added
		μmoles fatty acid hydrolyzed per 10 min.					
A	1	5.5	17.5	0	2.5	30.1	34.4
A	3	48.7	72.2	11.7	32.0	85.3	95.8
B	2	2.2	34.0	--	--	86.8	95.3
C	2	23.0	61.1	--	--	100.2	128.4
						Castor Oil	
D	2	--	--	--	--	24.1	63.1
E	2	22.3	71.3	--	--	34.3	61.6

Method of assay as described for Figure 1; 2.5 mg. of cofactor were added as indicated. Cottonseed oil was a commercial refined and bleached oil (Wesson oil); tributyrin was obtained from Eastman Chemicals; castor oil was a commercial refined oil obtained from Baker Castor Oil Co. The emulsion consisting of 15% cottonseed oil, 1.25% Drumulse, 0.2% TEM, and 0.2% Pluronic was supplied by W. S. Singleton (for constituents, see Singleton et al., 1958). Apoenzyme preparations A, B, C, and D were made by extraction with butanol; E was prepared by extraction with butanol containing BAL.

Hydrolysis of tributyrin is only slightly affected by the addition of the cofactor to the residue. On the basis of other evidence Ory et al. (1962) suggested that there were two enzymes in the acid lipase preparation: one for triglycerides of short-chain saturated fatty acids and the other primarily for the triglycerides of unsaturated fatty acids. The evidence in Table 1 would seem to support this contention.

The castor endosperm contains close to 70% of its dry weight as lipid. It is of interest to contemplate that the requirement for cofactor shown by the acid lipase might be typical of lipases found in tissues of high lipid content, even of adipose tissue.

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