PARTICIPATION OF TOCOPHEROL DERIVATIVES IN LIPOLYSIS

By Robert L. Ory and Aaron M. Altschul
Seed Protein Pioneering Research Laboratory[1]
New Orleans, Louisiana

Received February 19, 1962

In an accompanying paper (Ory and Altschul, this issue) we described the extraction with n-butanol of an oily material which is required as a cofactor for the hydrolysis of long-chain unsaturated glycerides by the lipase of the castor bean, Ricinus communis (Ory, St. Angelo, and Altschul, 1962). The sensitivity of the cofactor to air oxidation, which caused discoloration and loss of activity, suggested that it might possibly have some properties in common with tocopherols. Consequently, a number of tocopherol derivatives as well as several other lipids were tested as replacements for the lipid cofactor.

The acid lipase was extracted with butanol containing 2,3-dimercapto-propanol (BAL), washed with acetone, and dried in vacuo as described by Ory and Altschul (this issue). Several preparations of the apoenzyme were tested on cottonseed oil, triolein, and tributyrin.

The results with cottonseed oil are shown in Table 1. α-Tocopherol and two derivatives were effective in boosting the hydrolytic activity of the apoenzyme; tocopherol phosphate, however, was ineffective. Neither Coenzyme-Q 10 nor a preparation of soybean lecithin in concentrations comparable to those of the tocopherols increased activity. Addition of a synthetic antioxidant, Ionol,[2] did not increase the activity of the apoenzyme; in fact, there was a slight inhibition of lipolysis. We might therefore conclude that the increase in activity resulting from addition of tocopherols is not the general effect of

---

1/ One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

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

adding back fat or fat derivatives, nor does it seem to be a general antioxidant effect.

Table 1.

Effect of additives on hydrolysis of cottonseed oil by apoenzyme preparations.

Apoenzyme Prep. No.	Additive	Amt. mg.	Hydrolysis μmoles fatty acids/10 min.
1	None	--	1.8
	Cofactor	2	42.7
	α-Tocopherol	2	25.6
	"	4	2.6
	α-Tocopherol succinate	2	22.4
	α-Tocopherol palmitate	2	11.6
	α-Tocopherol phosphate	2	0
	Coenzyme Q-10 (MK-128)	2	0
	Soybean lecithin	2	0
2	None	--	16.8
	α-Tocopherol succinate	1	56.9
	" "	2	69.9
	" "	3	73.4
3	None	--	32.7
	α-Tocopherol succinate	2	83.0
	Ionol	2	23.2

See Figure 1, Ory and Altschul (this issue) for assay procedure. Cottonseed oil was a commercial refined and bleached oil (Wesson oil). The tocopherol derivatives were obtained from Distillation Products Industries; Coenzyme Q-10 from Merck Sharp and Dohme Research Laboratories was a gift of Dr. James Hamilton, Tulane University School of Medicine; lecithin was from the Glidden Company; and Ionol (2,6-di-tert-butyl-4-methyl phenol), an antioxidant, was from Shell Chemical Corp.

A comparison of tocopherol succinate and the natural cofactor on triolein is given in Table 2; similar effects are obtained by addition of either one. There is even a boost in activity when these are added to the original unextracted enzyme, but the percentage increase is very much less than for the apoenzyme. The triolein sample did not contain any tocopherol, therefore the residual activity would be the result of incomplete extraction of cofactor. In natural oils the presence of varying amounts of tocopherol might be responsible in part for some of the residual activity.

Table 2.

Effect of cofactor and tocopherol succinate on hydrolysis of triolein by lipase and apoenzyme.

Enzyme	Additive	Hydrolysis μmoles fatty acids/10 min.
Lipase	None	129.0
	Cofactor	149.2
	α-Tocopherol succinate	136.6
Apoenzyme No.3	None	33.1
	Cofactor	71.3
	α-Tocopherol succinate	80.1

Assay as described previously. Equivalent quantities of lipase (2.6 mg.) and apoenzyme (2.0 mg.) and 2.0 mg. of additive were used as indicated. Triolein was obtained from California Biochemicals Corp.

Ory et al. (1962) suggested that the acid lipase preparation contained two enzymes, one with maximum activity on glycerides of short-chain saturated fatty acids and the other acting primarily on glycerides of long-chain unsaturated fatty acids. More evidence for this point was presented in the companion paper (Ory and Altschul, this issue). It was also noted that some lipase activity is destroyed during preparation of the apoenzyme. Both of these points are further illustrated in Table 3 which shows the activity of three preparations of apoenzyme on cottonseed oil and tributyrin. Even when all activity on cottonseed oil is destroyed there still remains considerable hydrolysis of tributyrin, but this cannot be increased by addition of tocopherol succinate. When there is minimum destruction of lipase (preparation 4) there is a significant increase in the amount of tributyrin hydrolyzed upon addition of tocopherol. This might indicate that the enzyme which catalyzes hydrolysis of long-chain fatty acids might also hydrolyze tributyrin, but at a much lower rate. The enzyme which has maximum activity on glycerides of short-chain fatty acids apparently does not require cofactor. The former is more sensitive to destruction and when destroyed completely, as in preparation 6, there is no further increase in rate of hydrolysis of tributyrin by addition of tocopherol succinate.

Table 3.

Hydrolysis of cottonseed oil and tributyrin by apoenzyme preparations.

Substrate	Additive	Apoenzyme, Prep. No.		
		4	5	6
		Hydrolysis μmoles fatty acids/10 min.		
Cottonseed oil	None	18.7	0	0
	α-Tocopherol succinate	60.7	24.0	0
Tributyrin	None	121.7	91.7	44.9
	α-Tocopherol succinate	145.7	92.3	44.8

Assay as described previously. Tributyrin was obtained from Distillation Products Industries. Two mg. of apoenzyme and one mg. of tocopherol succinate were used per test, as indicated. The apoenzyme preparation was as follows: 4, extraction with butanol containing BAL for 17 hrs. at 5° C.; 5, extraction with same solvent for 17 hrs. at 25° C.; 6, extraction for 48 hrs. at 25° C.

There are many similarities in behavior in this system between the natural cofactor and the tocopherol derivatives. The magnitude of the increases are the same, the behavior toward different substrates is similar, and α-tocopherol exhibits inhibition at a higher level similar to the cofactor. It will therefore be of interest to determine whether the natural cofactor is a tocopherol derivative.

Even so, the fact that a lipase system is sensitive to the presence of tocopherols is interesting in itself. Heretofore, it has not been possible to find a role for tocopherols in any known enzyme reactions (Boyer, 1960; Bro-Rasmussen and Hjarde, 1961). In some instances where such a role has been reported, it has later turned out to be a general effect of lipid addition and not specific for tocopherols (Redfearn, et al., 1960). Here is an example of a function of tocopherols which need not necessarily be related to its anti-oxidant properties. It certainly would seem that the search for biochemical functions of tocopherols should include lipolysis of glycerides of long-chain fatty acids and, perhaps more generally, acyl transfer reactions involving such fatty acids.

References

Boyer, P. D., in P. D. Boyer, H. Lardy, and K. Myrbäck, eds., The Enzymes, Vol. 2A, Academic Press, New York, 1960, p. 353.

Bro-Rasmussen, F. and Hjarde, W., Ann. Rev. Biochem., 30, 459 (1961).

Ory, R. L., St. Angelo, A. J., and Altschul, A. M., J. Lipid Res., 3, 99 (1962).

Ory, R. L. and Altschul, A. M., Biochem. Biophys. Res. Comm., this issue (1962).

Redfearn, E. R., Pumphrey, A. M., and Fynn, G. H., Biochem. Biophys. Acta, 44, 404 (1960).