LACK OF LIPOIC ACID ACTIVATION OF ACETOTHIOKINASE PREPARATIONS

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Participation of lipoic acid in acetyl transfer catalyzed by acetothiokinase was suggested by Seaman,(1954a). His evidence was the observation that alumina treatment of crude acetothiokinase preparation from pigeon liver extracts resulted in about 40 per cent loss of both lipoic acid content and hydroxamate forming ability. Alumina treatment of purified enzyme however did not decrease hydroxamate forming activity. The use of alumina to remove lipoic acid was elegantly exploited by Reed, Leach and Koike, (1958) in elucidating the function of lipoic acid activating system in S. faecalis and in establishing the existence of a hydrolytic enzyme "lipoylhydrolase", responsible for the release of protein bound lipoic acid.

In in vivo studies with S^{35}-lipoic acid, Gal and Razevska (1958; 1960) showed acetothiokinase isolated from liver or brain to contain S^{35}-lipoic acid. This permitted further studies on the participation of lipoic acid in acetate activation. The present communication confirms that removal of lipoic acid from crude acetothiokinase of liver impairs hydroxamate formation,

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however, in this instance the effect of lipoic acid in overcoming this damage is not specific. Furthermore, acetothiokinase obtained from crude preparation by ammonium sulfate precipitation after alumina treatment is not restored to original enzyme activity by lipoic acid as compared to those precipitated with ammonium sulfate before the alumina treatment.

Pigeons and rats given 20 mg/kg I.P. DL-S^{35}-lipoic acid were sacrificed twenty-four hours later. Acetone dried powder of brain and of liver of several animals served as pools for the preparation of enzyme extracts. Crude pigeon liver acetone powder extracts were divided. One was subjected to fractionation by ammonium sulphate without prior alumina-treatment (Fractions F-I 35% and F-II 70% saturation) while the other was first treated with alumina and then fractionated (Fractions $F\text{-}I_{Al}$ and $F\text{-}II_{Al}$) according to the method of Seaman and Naschke (1955). Coenzyme A was removed by Dowex-1 treatment (Stadtman, Novelli and Lipmann, 1951). Table I shows that $F\text{-}II_{Al}$ from which lipoic acid was removed by alumina prior to fractionation did not respond to addition of lipoic acid. One would expect that $F\text{-}II_{Al}$ unlike F-II from which lipoic acid could not be removed without addition of F-I, should be reactivated in a fashion similar to the crude preparation. The lack of response by $F\text{-}II_{Al}$ was not due to incomplete removal of lipoic acid prior to fractionation since recombination of F-I with $F\text{-}II_{Al}$ followed by treatment with alumina did not decrease the hydroxamate formation.

Also apparent from Table I is the lack of specificity of the requirement of (+)-lipoic acid for acetate activation since

Table I

Effect of Lipoic Acid on the Activity of Pigeon Liver Acetothiokinase

Treatment and additions	Hydroxamate formed μmoles/h/10 mg of Protein					
	Crude	F-I[1]	F-II[1]	$F\text{-}II_{Al}$[2]	F-I:1 part F-II:2	F-I:1 part $F\text{-}II_{Al}$:2
None	1.18	.06	1.66	--	.75	.50
-Coenzyme A	.05	.04	.36	--	.22	.06
Al-Tr[3]	.68	--	1.61	.75	.48	.48
Al-Tr + (+)-lipoate[4]	.98	--	--	.68	.71	.47
Al-Tr + (-)-lipoate[4,5]	.99	--	--	.65	.65	.40

System contained in 1.65 ml: 200 μmoles Na acetate; 0.13 μmoles CoA; 200 μmoles TRIS buffer, pH 8.2; 150 μmoles KF; 20 μmoles $MgCl_2$; 30 μmoles Na-ATP; 20 μmoles glutathione; 200 μmoles hydroxylamine; 0.4 μmoles $NaBH_4$ and enzyme. Incubated 1 hour at 37° C.

[1] F_I and F_{II} fractions from crude extract prior to alumina treatment.

[2] $F_{II\text{-}Al}$ is fraction obtained from crude alumina treated extract.

[3] Al-Tr is alumina treatment.

[4] Lipoates added was 7.5 ng (ng = nanogram or 10^{-9} gm).

[5] Contained ca. 0.8 ng (+) lipoic acid.

similar reactivation is observable upon the addition of the unnatural (-)-lipoic acid.[1] The (-)-lipoic acid per total of 7.5 ng contained about 0.8 ng of the natural form; 1.0 ng of (+)-lipoic acid was shown not to increase alumina-treated acetothiokinase activity. Analysis of F-II for radioactivity after acid hydrolysis and extraction indicated the presence of 75.3 cpm/mg of protein in the untreated fraction.

Pigeon and rat brain acetothiokinase activity of the extract is decreased by alumina treatment but not restored by addition of lipoic acid, see Table II.

[1] A supply of the precursor (-)-6, 8-dichlorooctanoic acid (-)-ephedrine salt by W. J. Wayne of E. I. du Pont is gratefully acknowledged.

Table II

Lack of Activation of Brain Acetothiokinase by (+)-Lipoic Acid

Treatments and additions	Hydroxamate formed in μmoles/h/10 mg of Protein: Pigeon Brain Crude	Rat Brain Crude
None	0.67	0.26
-Coenzyme A	0.00	0.00
Al-Tr[1]	0.45	0.18
Al-Tr + 7.5 ng[1] (+)-lipoate	0.38	0.19

System contained per 1.4 ml: 200 μmoles K acetate; 24 μmoles TRIS Buffer pH 7.4; 1 μmole CoA; 10 μmoles NaATP; 50 μmoles KF; 6 μmoles KCl; 6 μmoles $MgSO_4$; 400 μmoles hydroxylamine; 4 μmoles $NaBH_4$; enzyme. Incubation 1 hour at 37° C.
[1] See Table I.

Highly purified acetothiokinase preparations from pigeon brain acetone powder extracts after Dowex-1 treatment, fractionation, dialysis and elution from calcium phosphate gel do not show detectable amounts of lipoic acid but were active in forming hydroxamate.

Seaman, (1954b) postulated that "lipoic acid-activating enzyme" is present in the crude enzyme preparations and is activated only by dihydrolipoic acid when the alumina treated preparations are placed in high concentrations of potassium ions. It is assumed that addition of 4 μmole of $NaBH_4$ to the system should reduce the 7.5 ng lipoic acid present and thus overcome the effect of potassium, Table II.

Incubation of purified acetothiokinase from pigeon liver with substrate amounts of radioactive DL-Lipoate S^{35} resulted in the incorporation of radioactivity, see Table III.

Table III

Hydroxamate Formation and S^{35} Incorporation by F-II_{A1} in Presence of Substrate Level Lipoic Acid-S^{35}

Additions[1]	Hydroxamate formed	Radioactivity of Protein	Recovered (+)-Lipoic Acid[3]
	μmoles	μmole/h/10 mg Prot.	cpm	ng
Acetate	200	0.51	--	
-CoA	0.13	0.19	--	
Lipoic Acid[2]	0.11	0.16	119	28
NaATP	--	0.00	52	11

[1] System given in Table I minus 200 μmoles Acetate.
[2] 22.4 μg lipoic acid (69680 cpm) was added to the system as substrate instead of acetate.
[3] Protein samples were BAL treated and acid hydrolyzed. Hydrolyzate was extracted with $CHCl_3$. After removal of $CHCl_3$ the residue was reextracted by $NaHCO_3$ solution and counted. (+)-Lipoic acid was identified by microbiological assay (Gunsalus and Razzell, 1957).

These data are interpreted to indicate that the presence of F-I is not essential for formation of protein bound lipoic acid.

On the basis of the experiments presented the participation of lipoic acid in acetate activation is seriously questioned.

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