AD No. 31401
ASTIA FILE COPY

[Reprinted from the Journal of the American Chemical Society, 76, 1718 (1954).]
Copyright 1954 by the American Chemical Society and reprinted by permission of the copyright owner.

PARTICIPATION OF THIOCTIC ACID IN THE ACETATE-ACTIVATING REACTION[1,2]

Sir:

The well-known role of thioctic acid as an acyl carrier in the oxidation of α-keto acids[3,4] suggests that this cofactor may participate in other acyl transfer reactions. Accordingly, extracts of pigeon liver acetone powder were examined for a thioctic acid requirement in the acetate-activating reaction[5,6]

$$\text{Acetate} + \text{ATP}^{7} + \text{Co A} \rightleftharpoons \text{Acetyl-Co A} + \text{AMP} + \text{PP}$$

Extracts were prepared by grinding 1 g. of powder with 10 ml. of 0.2 *M* $NaHCO_3$ and the insoluble material was removed by centrifuging at 2,000 × *g* for 30 min. Co A was removed from the supernatant by treatment with Dowex-1.[8] The crude extracts respond to the alumina procedure[9] for the removal of significant quantities of bound thioctic acid from the enzymes. Although assay[9] shows that only about 50% of the thioctic acid is removed by the alumina procedure, treated enzymes possess significantly less acetate activating activity, as measured by the hydroxamic acid method,[10] than do untreated enzymes (Table I). This indicates that although the cofactor has not been completely removed, the concentration has been decreased to below enzyme saturation level. Activity of the acetate-activating system may be restored to alumina treated preparations by the addition of 7.5 Mμg. of synthetic thioctic acid.[11]

Two fractions of the pigeon liver extracts have been separated by ammonium sulfate precipitation; the fractions were collected by centrifugation at 18,000 × *g* for 15 min. Fraction I (0–35% saturated) contains only very slight acetate activating activity. Fraction II (35–70% saturation) contains almost the entire activity contained in the crude extract; but it does not respond to alumina treatment. Removal of thioctic acid by the alumina procedure is achieved upon combination of the two fractions. Heating Fraction I for 10 min. at 65° completely destroys the activity. It thus appears that Fraction I functions by splitting protein-bound thioctic acid from the enzyme; liberated thioctic acid is then adsorbed and removed by the alumina.

As anticipated[12] with a reaction requiring thioctic acid, the acetate-activating system is inhibited by arsenite, but not by arsenate. The arsenite sensitivity of systems containing alumina-treated enzyme plus added thioctic acid is much greater than is the sensitivity in mixtures with untreated enzymes containing an excess of the cofactor. The inhibition is reversed by BAL, but not by such monothiols as cysteine or glutathione.

TABLE I

Incubation mixture contained, per 1.4 ml.: 100 μM. Na acetate; 20 units Co A; 200 μM. TRIS buffer, pH 8.2; 10 μM. glutathione; 200 μM. hydroxylamine; 10 μM. Na-ATP; 10 μM. $MgCl_2$; 60 μM. NaF; enzyme solution. Incubated 90 min. at 32°. Glutathione, Na-ATP and hydroxylamine were adjusted to pH 8 with 1 *M* TRIS before use. Thioctic acid content of crude untreated material was 20 Mμg. per mg. protein; after alumina treatment the content was 11 Mμg. per mg. Fractions I and II contained 17 Mμg. thioctic acid per mg. protein before alumina treatment and 9 Mμg. per mg. after treatment. Alumina treatment of Fraction II alone resulted in no change in thioctic acid content.

Additions and treatment	μM. hydroxamic acid formed			
	Crude (0.8 ml.)	Fraction I (0.8 ml.)	Fraction II (0.8 ml.)	Fractions I + II (0.1 ml.) (0.8 ml.)
None	0.96	0.02	0.52	0.54
7.5 Mμg. thioctic acid	.95	.03	.50	.53
Alumina treated	.42	..	.53	.34
Alumina treated + 7.5 Mμg. thioctic acid	.80	..	..	.47
2 μM. arsenite	.88	..	.48	.49
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite	.83	..	..	.38
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. cysteine	.44	..	..	.40
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. addnl. glutathione	.50	..	..	.39
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 10 μM. BAL	.84	..	..	.50

(1) Aided by grants from the National Institutes of Health, United States Public Health Service, from the National Vitamin Foundation, and by a contract between the Office of Naval Research and the University of Texas Medical Branch.

(2) Appreciation is expressed to Dr. Mary Ellen Jones for the courtesies extended the author during a recent visit to the Biochemical Research Laboratory, Massachusetts General Hospital, at which time several of the results reported here were reaffirmed.

(3) G. R. Seaman, *Proc. Soc. Exper. Biol. Med.*, 88, 184 (1955).

(4) L. J. Reed and B. G. DeBusk, THIS JOURNAL, 75, 1261 (1953).

(5) F. Lipmann, *et al.*, *ibid.*, 74, 2334 (1952).

(6) H. Baisert, *et al.*, *J. Biol. Chem.*, 200, 35 (1953).

(7) The following abbreviations are used: ATP, adenosine triphosphate; Co A, coenzyme A; AMP, adenosine monophosphate; PP, pyrophosphate; TRIS, tris-(hydroxymethyl)-aminomethane.

(8) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, 191, 365 (1951).

(9) F. C. Gunsalus, M. I. Dolin and L. Struglia, *ibid.*, 194, 849 (1952).

(10) T. C. Chou and F. Lipmann, *ibid.*, 196, 60 (1952).

(11) Kindly supplied by Dr. E. L. R. Stokstad.

(12) I. C. Gunsalus, *J. Cell. Comp. Physiol.*, 41 (Suppl. 1), 113 (1953).

CARTER PHYSIOLOGY LABORATORY
UNIVERSITY OF TEXAS MEDICAL BRANCH
GALVESTON, TEXAS
GERALD R. SEAMAN

RECEIVED JANUARY 20, 1954