COMMUNICATIONS TO THE EDITOR

PARTICIPATION OF ADENYL-ACETATE IN THE ACETATE-ACTIVATING SYSTEM[1]

Sir:

The exchange of inorganic pyrophosphate (PP)[2] with the terminal phosphates of ATP may be mediated by reversible pyrophosphorolysis of dinucleotide coenzymes[3] or by recently postulated mechanisms involving enzyme-intermediate compounds.[4,5] In an attempt to isolate and define more precisely the nature of these enzyme-intermediate compounds, an enzyme has been purified from yeast which catalyzes the exchange between $P^{32}P^{32}$ and ATP with acetate as an obligatory requirement (Table I).

TABLE I

REQUIREMENTS FOR $P^{32}P^{32}$ EXCHANGE WITH ATP

Reaction mixture: 100 μM potassium phosphate buffer, *p*H 7.5; 5 μM. $MgCl_2$; 2 μM. ATP; 2 μM. $P^{32}P^{32}$; 2 μM. potassium acetate; 1 unit purified enzyme; volume, 1.0 ml.; temperature 37° for 20 minutes; ATP separated from $P^{32}P^{32}$ by charcoal adsorption[6]; 1 unit of enzyme forms 1 μM. acetyl-CoA in 20 minutes.

		Minus			
Components	Compl.	ATP	Mg^{++}	Acetate	Enzyme
μM. $P^{32}P^{32}$ inc.	0.55	0.01	0.01	0.02	0.00

The enzyme also carries out the over-all synthesis of acetyl-CoA according to reaction 1.

$$\text{ATP} + \text{acetate} + \text{CoA} \rightleftharpoons \text{acetyl-coA} + \text{A5P} + \text{PP} \quad (1)$$

Acetate appears to act catalytically in the exchange reaction; thus 0.04 μM. of acetate catalyzed the incorporation of 0.15 μM. of $P^{32}P^{32}$ into ATP. That this exchange is associated with the acetyl-coA forming activity is supported by the fact that the ratio of acetyl-coA formation[7] to PP-ATP exchange increased with purification when acetate was omitted from the exchange assay, but remained essentially constant when acetate was present.

Evidence has now been obtained which supports the following mechanism for reaction 1.

$$\text{ATP} + \text{acetate} \rightleftharpoons \text{adenyl-acetate} + \text{PP} \quad (2)$$

$$\text{Adenyl-acetate} + \text{coA} \rightleftharpoons \text{acetyl-coA} + \text{A5P} \quad (3)$$

A compound with the properties of adenyl-acetate was prepared by the reaction of di-silver adenylate and acetyl chloride and partially purified by ion-exchange chromatography.[8] This preparation was enzymatically converted to ATP^{32} in the presence of $P^{32}P^{32}$ and the purified acetate-activating enzyme (Table II). In the absence of enzyme, there was no detectable formation of ATP and no significant disappearance of hydroxylamine reacting material. The ATP formed was characterized chromatographically, spectroscopically, and by its phosphorylation of glucose with hexokinase.[3]

TABLE II: STOICHIOMETRY OF CONVERSION OF ADENYL-ACETATE TO ATP

Reaction mixture: 50 μM. potassium phosphate buffer, *p*H 7.5; 5 μM. $MgCl_2$; 2.0 μM. $P^{32}P^{32}$; 0.75 μM. adenyl acetate (based on conversion to ATP); 1 unit of enzyme; volume, 1.0 ml.; temperature 37°.

	ATP[a]	PP	Adenyl acetate[b]
Δ 30 minutes	+0.28	....	−0.30
Δ 60 minutes	+0.42	−0.47	−0.43

[a] ATP separated from $P^{32}P^{32}$ by charcoal adsorption. [b] Determined by the method of Lipmann and Tuttle.[9]

Adenyl acetate was converted to ATP at approximately the same rate as the PP exchange with ATP. Under comparable conditions, 0.31 μM. $P^{32}P^{32}$ was incorporated into ATP and 0.23 μM. of adenyl acetate was converted to ATP.

The formation of acetyl-coA from adenyl acetate and coA is supported by the following evidence. The addition of 2 μM. of CoA to the reaction mixture (see Table I) caused an 80% inhibition of the exchange reaction and a 95% inhibition of the conversion of adenyl acetate to ATP. This is consistent with a competition of coA with PP for the adenyl acetate. Furthermore, the product, enzymatically formed from adenyl acetate and coA, disappeared in the presence of phosphotransacetylase and arsenate.[10] The formation of acetyl-coA was dependent on the presence of coA and the enzyme, and the arsenolysis was dependent on the phosphotransacetylase. Starting with 0.28 μM. of adenyl acetate, there was a formation of 0.19 μM. of acetyl-coA as measured by the disappearance of hydroxylamine reacting material in the presence of arsenate and phosphotransacetylase. Further experiments on the isolation and characterization of the acetyl-coA are in progress.[10a]

It seems likely, therefore, that the primary reaction in the activation of acetate and perhaps of the higher fatty acids[11] involves an acyl group cleavage of ATP, resulting in the liberation of pyrophosphate and in the formation of an acyl

(1) This work was carried out under the tenure of a Postdoctoral Fellowship and Scholarship of the American Cancer Society and was supported by funds from the U. S. Public Health Service and the National Science Foundation.

(2) The following abbreviations have been used: PP, inorganic pyrophosphate; ATP, adenosine triphosphate; CoA, coenzyme A; A5P, adenosine-5′-phosphate.

(3) A. Kornberg, *J. Biol. Chem.*, **182**, 779 (1950); A. W. Schrecker, and A. Kornberg, *ibid.*, **182**, 795 (1950); A. Munch-Petersen, H. M. Kalckar, E. Cutolo and E. E. B. Smith, *Nature*, **172**, 1036 (1953).

(4) M. E. Jones, F. Lipmann, H. Hilz, and F. Lynen, THIS JOURNAL, **75**, 3285 (1953).

(5) M. B. Hoagland, *Biochim. et Biophys. Acta*, **16**, 288 (1955).

(6) R. K. Crane, and F. Lipmann, *J. Biol. Chem.*, **201**, 235 (1953).

(7) M. E. Jones, S. Black, R. M. Flynn, and F. Lipmann, *Biochim. et Biophys. Acta*, **12**, 141 (1953).

(8) I am grateful to Dr. David Lipkin, Washington University, for suggesting the method of preparation of adenyl acetate.

(9) F. Lipmann, and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944).

(10) E. R. Stadtman, G. D. Novelli and F. Lipmann, *ibid.*, **191**, 365 (1951).

(10a) NOTE ADDED IN PROOF.—Further evidence for reaction (3) was the quantitative enzymatic conversion of adenyl-acetate to acetyl-CoA as measured by the malic dehydrogenase-condensing enzyme system (Stern, Shapiro, Stadtman and Ochoa, *J. Biol. Chem.*, **193**, 703 (1953)); I am deeply indebted to Dr. S. Ochoa for a gift of crystalline condensing enzyme). The acetyl-CoA formed was also characterized by the enzymatic acetylation of *p*-nitro aniline with a partially purified acetylating enzyme from pigeon liver (Tabor, Mehler and Stadtman, *ibid.*, **204**, 127 (1953).

(11) A. Kornberg, and W. E. Pricer, Jr., *ibid.*, **204**, 329 (1953); G. R. Drysdale, and H. A. Lardy, *ibid.*, **202**, 119 (1953); H. R. Mahler, S. J. Wakil, and R. M. Bock, *ibid.*, **204**, 454 (1953).

adenylate. This formulation is supported by the recent O^{18} exchange studies of Boyer[12] and by Jencks[13] who obtained a coA-independent activation of octanoic and similar fatty acids by pyrophosphate split of ATP. Definitive proof of this general mechanism must await experiments demonstrating the enzymatic synthesis of adenyl acetate from ATP and acetate (reaction 2), and the formation of adenyl acetate from acetyl-coA and A5P (reaction 3).

During the course of the above work a methionine requiring PP–ATP exchange system was also purified from yeast.[14] It is possible that this and the previously reported pantoic[15] and amino acid activated PP-ATP exchanges[5] may occur by the formation of the corresponding adenyl-acyl group derivatives. Thus, the pantothenate peptide bond formation may represent a variation of the acetate activation with the amino group of β-alanine serving as the acyl group acceptor in place of the sulfhydryl group of coA.

(12) P. D. Boyer, O. J. Koeppe, W. W. Luchsinger, and A. B. Falcone, *Fed. Proc.*, **14**, 185 (1955).
(13) W. P. Jencks, *ibid.*, **12**, 703 (1953).
(14) P. Berg, unpublished.
(15) W. K. Maas, quoted by F. Lipmann, *Science*, **120**, 855 (1954).

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REDUCTION OF ESTER AND OTHER DIFFICULTLY REDUCIBLE GROUPS BY SODIUM BOROHYDRIDE

Sir:

Since its original discovery[1] sodium borohydride has proven a very useful reagent for the selective reduction of aldehyde and ketone groups.[2] It has not been applicable to the reduction of ester and similar functional groups which are relatively difficult to reduce. Recently Kollonitsch, Fuchs and Gabor[3] reported that they had succeeded in reducing esters by the use of calcium borohydride[3a] and magnesium borohydride.[3b] We wish to report the rapid reduction at room temperatures of esters, carboxylic acids and nitriles by sodium borohydride in the presence of aluminum chloride. 1-Olefins are reduced at 75°; unconjugated 2-olefins are not affected by these conditions. Nitro and amide groups are not reduced by the reagent. Consequently the method offers a convenient procedure for the selective reduction of nitro esters.

The addition of aluminum chloride to a solution of sodium borohydride in diethylene glycol dimethyl ether ("diglyme") results in a clear solution of the reagent. Sodium chloride does not precipitate. Therefore it is improbable that aluminum borohydride is present in other than minor amounts. (We plan to investigate this point.) The reagent has been exposed to dry air with only minor loses in activity. It can be poured in air without difficulty.

Other polyvalent metal halides, such as gallium trichloride and titanium tetrachloride also bring about the reduction of esters. However, aluminum chloride possesses obvious advantages for general laboratory use so that our studies have been concentrated on the applicability of this reagent.

In a typical preparative procedure ethyl *p*-chlorobenzoate was reduced to *p*-chlorobenzyl-alcohol in 89% yield as follows. A stirred solution of 0.25 mole of sodium borohydride (99% purity) in 250 ml. of diglyme and 0.4 mole of ethyl *p*-chlorobenzoate was treated slowly with a total of 0.084 mole of anhydrous aluminum chloride (42.0 ml. of a freshly prepared 2 *M* solution of $AlCl_3$ in the same solvent). The reaction was vigorous in the initial stages and the rate of addition was controlled to maintain the temperature below 50°. The flask was then heated for a few minutes on the steam-bath to complete the reaction. The reaction mixture was poured onto crushed ice and dilute acid. The solid product (50.9 g., 89%) was recrystallized from hot water to give pure *p*-chlorobenzyl alcohol, m.p. 75°, in a yield of 84%.

We have examined the utilization of hydride from the reagent by various compounds usually at 25°. The first figure gives the time of reaction in hours and the second gives the moles of hydride per mole of compound (one mole of hydride utilized to form hydrogen in the case of carboxylic acids and amides is not included in the figure): ethyl acetate (0.5, 2.0); ethyl stearate (0.5, 2.0); ethyl *p*-chlorobenzoate (0.5, 2.0); ethyl oleate (0.5, 2.2); ethyl cinnamate (0.5, 3.0); ethyl *p*-nitrobenzoate (0.5, 2.0); benzoic acid (0.5, 2.0); benzamide (3.0, 0.0); benzoyl chloride (3.0, 2.0); benzonitrile (3.0, 2.0); acetonitrile (3.0, 2.0); nitrobenzene (3.0, 0.0); 1-nitropropane (3.0, 0.0); pyridine-N-oxide (3.0, 1.0); benzaldehyde (0.5, 1.0); benzophenone (0.5, 1.0); styrene (1.0, 1.0); 1-hexene (25°, 3.0, 0.8; 75°, 1.0, 1.0); cyclohexene (75°, 1.0, 0.0).

These results suggest that the reducing properties of sodium borohydride can be profoundly modified by addition of various polyvalent metal halides. We are continuing to explore the potentialities of these reducing systems.

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NON-SOLVATED ALUMINUM HYDRIDE[1]

Sir:

Previous preparations of aluminum hydride have resulted either in a very low yield of impure product[2] or in a solid solvated polymer from which it has been impossible to remove all the solvent without decomposition.[3]

We have now succeeded in preparing non-

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(2) S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).
(3) (a) J. Kollonitsch, O. Fuchs, V. Gabor, *Nature*, **175**, 346 (1955); (b) **173**, 125 (1954).

(1) The work reported herein was carried out under the auspices of the Office of Naval Research under Contract ONR-494(04).
(2) O. Stecher and E. Wiberg, *Ber.*, **75**, 2003 (1942).
(3) A. E. Finholt, A. C. Bond, Jr., and H. I. Schlesinger, THIS JOURNAL, **69**, 1199 (1947).