ACETYL PHOSPHATE UTILIZATION WITH ANIMAL AND BACTERIAL ENZYMES*

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During recent studies with highly purified carbamate kinase (Grisolia et al, 1962), an enzyme that catalyzes reaction 1:

$$1)\quad NH_2COO^- + ATP \rightleftharpoons CP + ADP,$$

it has become evident that marked activity with acetyl phosphate, AP, replacing carbamyl phosphate, CP, is probably not due to acetokinase contamination but more likely to the identity of carbamate kinase and acetokinase, since the activities for CP and AP are about equal and remain constant with purification, as does the yield for both activities from Strain D_{10} Group D streptococci after arginine induction (Slade and Slamp, 1952). Competitive substrate inhibition experiments also indicate that carbamate kinase is indeed acetokinase. These findings led us to test other reactions in which AP could perhaps replace CP.

Synthesis of ATP from CP and ADP catalyzed by CP synthetase requires acetyl glutamate, AG. This has been the basis for postulating (Metzenberg et al, 1958) two enzymatic sites accounting for the partial reversibility of CP synthesis according to reactions 2 and 3:

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2) $ATP + CO_2 \xrightarrow{AG} ADP + Pi + \text{"active } CO_2\text{"}$

3) $ATP + \text{"active } CO_2\text{"} + NH_3 \xrightleftharpoons{AG} ADP + CP$

Carbamyl-P synthetase catalyzes the interaction of acetyl phosphate and ADP to form ATP and in this case, as with CP, it requires acetyl glutamate; this is illustrated in Table I. The enzyme uses AP about 3 times faster than CP, as calculated from the date shown in Table I and from that in the literature (Caravaca

TABLE I

ACETYL GLUTAMATE REQUIREMENT FOR ACETYL PHOSPHATE UTILIZATION WITH FROG LIVER CARBAMYL PHOSPHATE SYNTHETASE

Additions		μmoles AP transferred	
Enzyme Protein	Acetyl Glutamate	Exp. 1	Exp. 2
mg	μmoles		
0.4	0	0	-
0.4	10	0.9	-
0.8	0	0	0
0.8	10	2.4	1.6
1.6	0	0	0
1.6	10	4.0	2.2

Each tube contained the following in μmoles: Tris-Cl^- pH 7.4, 100; $MgSO_4$, 20; acetyl phosphate, 10; ADP, 10 and the indicated amount of carbamyl phosphate synthetase (Raijman and Grisolia, 1961). Tubes from experiment #1 contained also 50 units of hexokinase and 50 μmoles of glucose (Mokrasch et al, 1960). 10 min incubation at 38°. 1.0 ml final volume. The above findings were confirmed in other experiments by measuring ATP formation (Mokrasch et al, 1960), and with rat liver CP synthetase. There was no demonstrable AP formation with the above system when AP was replaced by 100 μmoles of K acetate and the ADP by ATP, even when including 100 μmoles of NH_2OH.

and Grisolia, 1960; Metzenberg et al, 1958). It appears that the present findings may serve to further the understanding of the mechanism of action of CP synthetase.

As illustrated in Table II, AP is a substrate with preparations containing ornithine transcarbamylase when they are supplemented with ornithine. However, parallel experiments not documented here show that AP is used at about 12, 3 and 0.6% the rate with CP for rat liver, frog liver and bacteria respectively, indicating that AP utilization is due to an enzyme other than the transcarbamylase present in the preparations.

TABLE II

ORNITHINE REQUIREMENT FOR ACETYL PHOSPHATE UTILIZATION WITH ANIMAL AND BACTERIAL PREPARATIONS

μmoles Ornithine Added	Enzyme source and μmoles AP used		
	Rat Liver	Frog Liver	Bacteria
0	0	0	0
10	4.1	7.4	5.4

Each tube contained the following in μmoles: Tris-Cl^- pH 7.4, 50 (100 for the experiments with frog liver enzyme); acetyl phosphate, 10; $MgSO_4$, 20 and L-ornithine as indicated. 0.8 mg protein from frog liver (Raijman and Grisolia, 1961), 0.9 mg rat liver mitochondria (Grisolia and Cohen, 1951), and 1.0 mg of bacterial ornithine transcarbamylase (Caravaca and Grisolia, 1960) was used when indicated. The incubation volume was 1.0 ml. 10 min at 38°. Acetyl phosphate utilization was measured as indicated in Table I.

The present findings demonstrate for the first time the utilization of AP for synthetic purposes with animal enzymes. The possibility that aminoacids other than ornithine may be acetylated with AP by animal enzymes remains open. As indicated, our preliminary evidence is negative regarding synthesis of AP by CP synthetase. However, it is attractive to speculate that the demonstration of AP synthesis in animal tissues may await only the recognition of an unsuspected cofactor, as has been the case for AG and CP synthesis. Indeed, the presence of acetyl aspartate in brain suggests there may be metabolic roles, perhaps catalytic, for other acetyl aminoacids.

REFERENCES

Caravaca, J., and Grisolia, S., J. Biol. Chem., 235, 684 (1960).
Grisolia, S., and Cohen, P. P., J. Biol. Chem., 191, 189 (1951).
Grisolia, S., Caravaca, J., and Joyce, B. K., Biochim. et Biophys. Acta, 29, 432 (1958).
Grisolia, S., Harmon, P., and Raijman, L., Biochim. et Biophys. Acta, in press (1962).
Lipmann, F., and Tuttle, L. C., J. Biol. Chem., 159, 21 (1945).
Metzenberg, R. L., Marshall, M., and Cohen, P. P., J. Biol. Chem., 233, 1560 (1958).
Mokrasch, L. C., Caravaca, J., and Grisolia, S., Biochim. et Biophys. Acta, 37, 422, (1960).
Raijman, L., and Grisolia, S., Biochem. and Biophys. Research Communs., 4, 262 (1961).
Slade, H. D., and Slamp, W. C., J. Bact. 64, 455 (1952).