ENERGY-LINKED REGULATION OF THREONINE METABOLISM BY ADP*

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Received September 25, 1963

Although the allosteric effect of isoleucine on the so-called biosynthetic L-threonine dehydrase has recently been investigated (Umbarger and Brown, 1957; Changeux, 1961), much less attention has been paid to the other enzyme, degradative L-threonine dehydrase. We have recently described a specific stimulatory effect of ADP on the latter enzyme from Clostridium tetanomorphum. At a threonine concentration of 10^{-3} M, for example, the activity is enhanced about 10 fold in the presence of 10^{-4} M ADP. This effect appears to be due to the increased affinity of the enzyme towards the substrate in the presence of the nucleotide (Hayaishi et al., 1963).

Further experiments in our laboratory now provide evidence that the product of the reaction, α-ketobutyrate is further metabo-

* This investigation was supported in part by research grants from the National Institutes of Health (C-4222), the Rockefeller Foundation, the Jane Coffin Childs Memorial Fund for Medical Research, the Squibb Institute for Medical Research, and the Scientific Research Fund of the Ministry of Education of Japan.

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lized by the cell-free extract to propionyl phosphate, CO_2 and H_2. The reaction is analogous to the conversion of pyruvate to acetyl phosphate, CO_2 and H_2 described by previous investigators in cell-free extracts of similar clostridia (Koepsell and Johnson, 1942; Wolfe and O'Kane, 1953). Propionyl phosphate thus formed is then converted by acyl kinase to propionate and ATP in the presence of ADP. It appears to be a reasonable conjecture, therefore, that ADP is acting as a specific metabolic regulator in the threonine catabolism, being linked to the ATP synthesis as shown in Fig. 1.

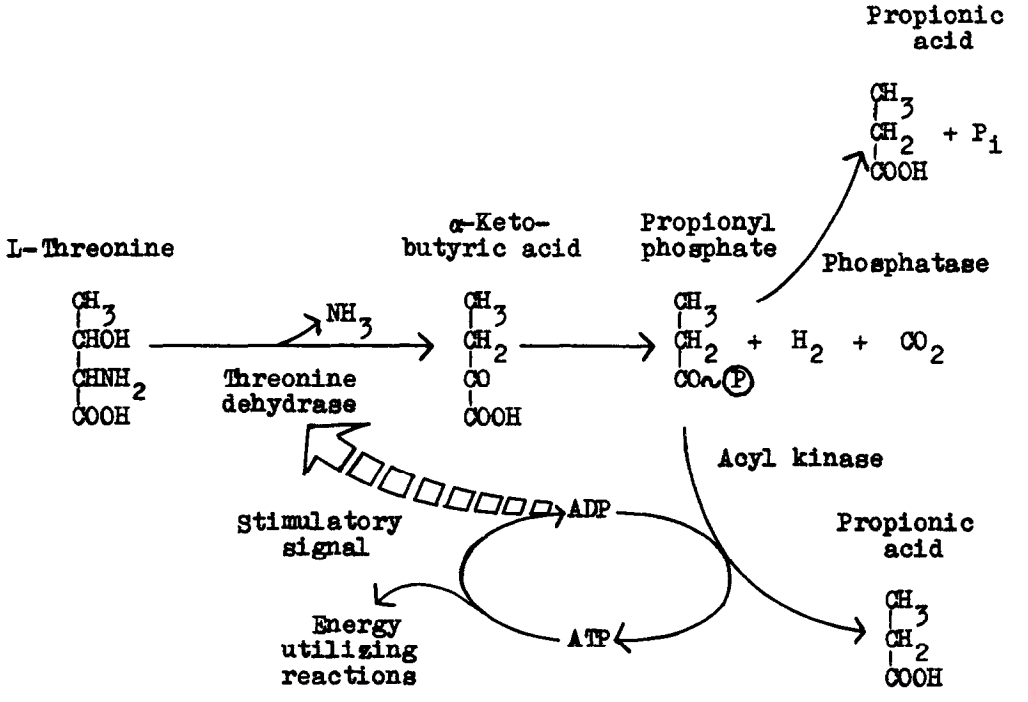

Fig. 1. Regulatory mechanism of L-threonine metabolism by ADP.

C. tetanomorphum (ATCC 3606) was grown overnight at 37° in 5 liter Erlenmeyer flasks in a 4 liter medium containing 0.5 % yeast extract, 1.5 % Takeda polypeptone, 0.1 % L-threonine, 0.5 %

sodium glutamate, 0.03 % thioglycollic acid and 0.25 % K_2HPO_4. Cells were suspended in water, disrupted by a 10 KC Raytheon sonic oscillator for 30 minutes, then the residue was removed by centrifugation at 20,000 x g for 30 minutes at 0°. The clear supernatant fraction thus obtained was used for the following experiments throughout.

When L-threonine was incubated with the extract under the conditions specified in Table I, the stoichiometric formation of α-ketobutyric acid, H_2, CO_2 and propionic acid from L-threonine was observed.

Table I.

Stoichiometry of threonine metabolism

Threonine	α-Ketobutyric acid	Hydrogen	Carbon dioxide	Propionic acid
-3.9	+0.6	+3.2	+3.1	+3.3

A reaction mixture (2.0 ml) in a Warburg flask contained the crude extract (30 mg protein), 20 μmoles U-C14-threonine (20,000 c.p.m.), 0.4 μmole CoA, 100 μmoles potassium phosphate, pH 6.6, and 40 μmoles reduced glutathione. Incubation was carried out for 30 minutes at 37° under pure nitrogen. Numbers are expressed by μmoles.

For the determination of threonine, one ml of the deproteinized reaction mixture (pH 2) was passed through a Dowex-50(H^+) column (1/2 x 2 cm), the column was washed with 1 ml of water, then the eluate from the column with 0.1 N ammonia was used for the measurement of radioactivity (Hayaishi et al., 1963). α-Ketobutyric acid was determined as its 2,4-dinitrophenylhydrazone at

413 mμ with a Beckman DU spectrophotometer (Katsuki et al., 1961). H_2 was determined manometrically. $C^{14}O_2$ was trapped in KOH in the center well of a Warburg flask and was determined as $BaCO_3$ suspended in Cab-O-Sil using a Packard Tri-Carb liquid scintillation spectrometer (Feigelson and Feigelson, 1963). Propionic acid was determined by the radioactivity of volatile acid upon lyophilization of the reaction mixture under acidic condition (pH below 2). The acid was identified by silicic acid column chromatography using water-saturated chloroform as solvent (Peterson and Johnson, 1948).

When a reaction mixture (2.0 ml) containing 20 μmoles of sodium α-ketobutyrate, 40 μmoles of reduced glutathione, 100 μmoles of phosphate buffer, pH 6.6, and the extract (about 30 mg protein) was incubated for 30 minutes at 37°, 1.2 μmoles of propionohydroxamate were formed upon addition of hydroxylamine. Propionohydroxamate thus formed was identified by paper chromatography with water-saturated n-butanol as solvent (Rf 0.65) (Stadtman and Barker, 1950). During the incubation described above, 8.5 μmoles of α-ketobutyrate disappeared. This discrepancy was probably due to some enzymatic hydrolysis of the propionic acid ester.

When 2.7 μmoles of propionyl phosphate was incubated for 20 minutes with 2.5 μmoles of ADP and the extract (340 μg protein) in a total volume of 1.0 ml, 1.7 μmoles of ATP were formed with concomitant disappearance of 1.9 μmoles of propionyl phosphate. The amount of ATP was determined by the $NADPH_2$ formation with the hexokinase-glucose 6-phosphate dehydrogenase system (Kornberg, 1950).

In contrast with the above results, the disappearance of propionyl phosphate as well as the formation of ATP was negligible either in the control or the presence of AMP as seen in Fig. 2.

The ADP dependent degradation of propionyl phosphate is reversible. When a reaction mixture (1.0 ml) containing 800 μmoles

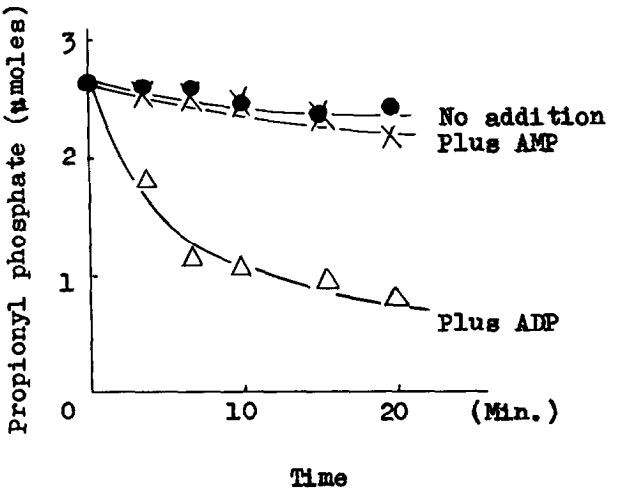

Fig. 2. Acyl kinase activity in C. tetanomorphum extract. Reaction mixture (1.0 ml) contained 340 μg extract, 2.7 μmoles propionyl phosphate, 50 μmoles Tris-HCl buffer, pH 7.4, 10 μmoles $MgCl_2$, 10 μmoles cysteine and 2.5 μmoles ADP or AMP. Incubation at 37°.

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of sodium propionate, 10 μmoles of ATP, 10 μmoles of $MgCl_2$, 50 μmoles of Tris-HCl buffer pH 7.4, and 200 μmoles of hydroxylamine was incubated with the extract (about 3 mg protein), 8.9 μmoles of propionohydroxamate accumulated in 60 minutes.

The above results are consistent with the interpretation that the degradative pathway of threonine in this organism is linked to the ATP synthesis; as ATP is utilized, an increase in the ADP level transmits a signal to the first enzyme, threonine dehydrase, to accelerate the initial reaction in order to provide necessary energy for the additional ATP synthesis. Experimental results presented in this communication provide for the first time evidence that adenine nucleotide acts as a metabolic regulator stimulating the initial reaction in this particular metabolic pathway.

References

Changeux, J. P., Cold Spr. Harb. Symp. Quant. Biol., (1961) 26, 313.

Feigelson, P., and Feigelson, M., J. Biol. Chem., (1963) 238, 1073.

Hayaishi, O., Gefter, M., and Weissbach, H., J. Biol. Chem., (1963) 238, 2040.

Katsuki, H., Kawano, C., Yoshida, T., Kanayuki, H., and Tanaka, S., Anal. Biochem., (1961) 2, 433.

Koepsell, H. J., and Johnson, M. J., J. Biol. Chem., (1942) 145, 379.

Kornberg, A., J. Biol. Chem., (1950) 182, 779.

Lipmann, F., and Tuttle, L. C., J. Biol. Chem., (1945) 159, 21.

Peterson, M. H., and Johnson, M. J., J. Biol. Chem., (1948) 174, 775.

Rose, I. A., Grunberg-Manago, M., Korey, S. R., and Ochoa, S., J. Biol. Chem., (1954) 211, 737.

Stadtman, E. R., and Barker, H. A., J. Biol. Chem., (1950) 184, 769.

Umbarger, H. E., and Brown, B., J. Bact., (1957) 73, 105.

Wolfe, R. S., and O'Kane, D., J. Biol. Chem., (1953) 205, 755.