ACTIVATION OF THIAMINE PYROPHOSPHATE PHOSPHOHYDROLASE OF RAT LIVER BY ATP

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Crude extracts from animal tissues have been shown to decompose thiamine pyrophosphate (TPP). In fact, the enzyme thiamine pyrophosphate phosphohydrolase (TPPase), which catalyzes the conversion of TPP to thiamine monophosphate (TMP), was reported to occur in liver, kidney and brain (Tilander, et al., 1961; Horie, 1957), but none of these enzymes has been purified because of a marked loss of activity during the purification.

In the course of studies on the metabolism of thiamine phosphoric acid esters by cell-free extracts of rat liver, the author observed that the activity of TPPase was considerably enhanced by the addition of ATP to the reaction mixture. Studies on the mechanism of this activation reveal that this effect is due to the increased affinity of the enzyme towards the substrate in the presence of ATP.

MATERIALS AND METHODS

Enzyme purification: The enzyme was partially purified from acetone powder of rat liver by extraction, ammonium sulfate fractionation, passage through a CM-sephadex column, second ammonium sulfate fractionation and chromatography on DEAE-sephadex columns. The overall purification was 140-fold from the extract of acetone powder with the yield of about 18%.

Assay of enzyme activity: The activity of TPPase was assayed by

the estimation of TMP produced from TPP. TMP was determined fluorometrically according to the method of Rindi et al (1961), except that IRC-50 was used instead of Dowex-1 for the separation of TMP from TPP. The standard incubation mixture contained, in a volume of 1.0 ml, 75 μmoles of glycylglycine buffer (pH 8.5), 4 μmoles of $MgCl_2$, 2 μmoles of TPP, 0.3 μmole of ATP and the enzyme. The reaction was carried out at 37° and stopped by heating at 100° for 1 minute.

Determinations: TPP was determined fluorometrically by a modification of the method of Rindi et al, and manometrically by its cocarboxylase activity for yeast apocarboxylase (Kaziro, 1957). Inorganic phosphate was determined colorimetrically as phosphomolybdic acid after extracting into iso-butyl alcohol (Takahashi, 1955).

RESULTS

Stoichiometry of the reaction: When TPP was incubated with the purified enzyme under the conditions described in Table I, stoichiometric formation of TMP and inorganic phosphate from TPP was observed both in the presence and absence of ATP. ATP stimulated TPPase activity approximately 5-fold under these conditions. Liberation of inorganic phosphate

Table I

Stoichiometry of TPPase reaction, effect of ATP on the enzyme activity

	TPP	TMP	Pi
complete	-2.48	+2.57	+2.51
-ATP	-0.38	+0.45	+0.48
-TPP			0.05

Complete system, in a volume of 3.0 ml, contained: 250 μmoles glycylglycine buffer (pH 8.5), 12 μmoles $MgCl_2$, 6 μmoles TPP, 0.9 μmole ATP and the enzyme (0.2 mg protein).
The reactions were initiated by the addition of enzyme and carried out for 6 minutes at 37°. Values are expressed in μmoles.

from ATP was almost negligible. Formation of thiamine was negligible although it was determined by the method of Rindi et al.

Effects of ATP: ATP was found to stimulate the TPPase reaction at concentrations as low as 10^{-5}M, and the greatest amount of stimulation occurred at an ATP concentration of about 3 x 10^{-4}M in the standard reaction mixture. ADP, 3'-AMP, 5'-AMP and 3'.5'-cyclic AMP could not replace ATP although ITP and GTP had the same degree of activating effect as ATP. Deoxy-ATP gave approximately 50% of the activity of ATP. Other nucleoside di- and triphosphates were almost inactive. Inorganic pyro- and triphosphate strongly inhibited the reaction at a concentration of 10^{-4}M.

No decrease of the concentration of ATP was observed during the reaction, when it was determined by the $NADPH_2$ formation with the hexokinase-glucose 6- phosphate dehydrogenase system (Kornberg, 1950). Thus, ATP seems to stimulate the reaction in catalytic rather than in stoichiometric amounts.

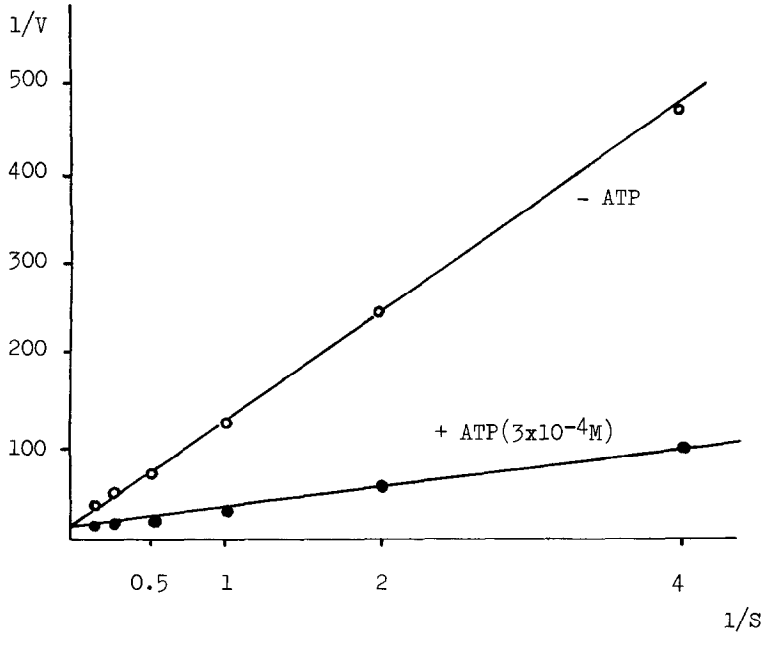

Fig. 1. Velocity as a function of TPP concentration in the presence and absence of ATP. The reciprocal of velocity (V, TMP formed μmole/min.) is plotted against the reciprocal of TPP concentrations (S, M x 10^3). The standard incubation conditions were used.

Effects of TPP concentration: The one factor that markedly affected the ATP effect was the concentration of TPP present in the incubation mixture. The effect of ATP was much more pronounced at low concentrations of TPP than at high concentrations. If the reciprocal of TPP concentration between 2.5 x 10^{-4} and 8 x 10^{-3}M are plotted against the reciprocal of velocity, a linear curve is obtained both with and without ATP, as shown in Fig. 1. The stimulation by ATP can be attributed mainly to a marked decrease in the apparent Michaelis constant (Km) for TPP. The following Km values were calculated: with ATP, 2.3 x 10^{-3}M; without ATP, 1.2 x 10^{-2}M. The fact that the lines converge at the ordinate suggests that V_{max} might be about the same either with or without ATP.

pH response: A further effect of ATP was to cause an apparent shift of the pH optimum. As shown in Fig. 2, when TPP concentration was relatively low (2 x 10^{-3}M), the maximal activity was attained at pH 9.3 or higher in the absence of ATP, but the pH optimum shifted to pH 8.8 in the presence of 3 x 10^{-4}M ATP. A similar shift in pH optimum was obtained in the absence of ATP by increasing the TPP concentration 10-fold.

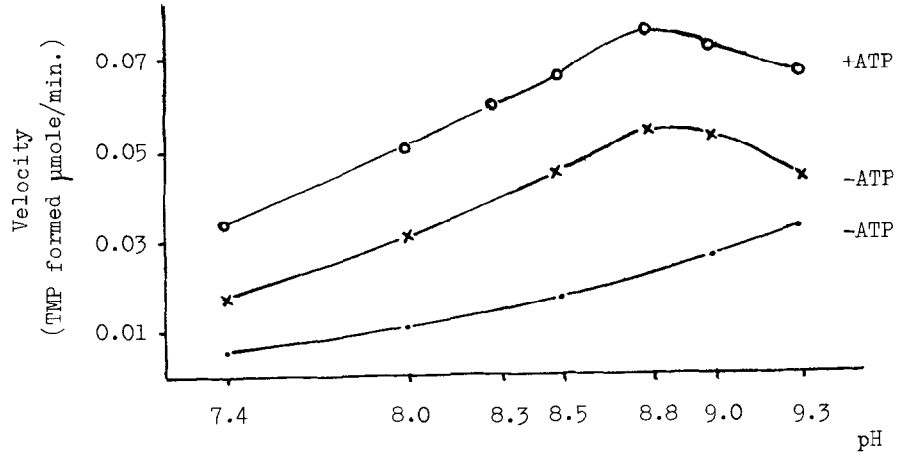

Fig. 2. Effect of ATP and TPP concentration on pH optimum. Each reaction mixture of 1.0 ml contained 75 umoles of glycylglycine buffer, 4 umoles of $MgCl_2$, the enzyme. TPP and ATP varied as follows: o——o, 2 x 10^{-3}M TPP and 3 x 10^{-4}M ATP; x——x, 2 x 10^{-2}M TPP and no ATP; ·——·, 2 x 10^{-3}M TPP and no ATP.

Cation Requirement: Enzyme activity was completely dependent on the presence of a bivalent cation as an activator. Mg^{++} was used routinely, but Mn^{++}, Ca^{++} were also effective. Zn^{++}, Co^{++} had very little effect. The effect of ATP was also observed with Mn^{++} and Ca^{++}.

DISCUSSION

In the present experiment the decomposition of TPP was observed to be stimulated by catalytic amounts of ATP. ATP did not participate directly in the reaction but markedly increased the affinity of the enzyme for its substrate, thus ATP may be called an "allosteric effector" (Monod, et al., 1963).

Although highly speculative at this time, a possibility of the physiological significance of this phenomenon may be that the effect of ATP might amount to a negative feedback control by ATP through TPPase activity, described as follows:

TPP is well known as a cofactor of pyruvate decarboxlase and of oxoglutarate dehydrogenase in the citric acid cycle. If ATP accumulates in living cells as a result of over-production, it would accelerate the hydrolysis of TPP. The decrease in concentration of TPP would subsequently affect the flow rate of citric acid cycle, a major source of electrons for ATP generation. In this way, ATP levels in living cells might be controlled through TPPase activity.

Similar phenomena have been reported in the activation of NAD-linked isocitrate dehydrogenase by ADP in bovine heart (Chen, et al., 1963) and by AMP in yeast (Hathaway, et al., 1963).

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