INHIBITION OF TRANSKETOLASE BY ANALOGUES OF THE COENZYME

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Received April 26, 1971

SUMMARY

The effect of thiamine, thiamine monophosphate, pyrophosphate and thiazole pyrophosphate on the enzymatic activity of transketolase has been studied. All these compounds have been proved to inhibit the enzyme by competing with the coenzyme (thiamine pyrophosphate) for apotransketolase. The experimental data obtained indicate that the interaction of thiamine pyrophosphate with apoenzyme occurs at least in three points and at the expense of the thiamine moiety of the coenzyme molecule and its phosphate residues.

In the course of isolation of transketolase (TK; EC: 2.2.1.1.) from baker's yeast and its storage in the ammonium sulphate solution there occurs cleavage of the coenzyme (thiamine pyrophosphate, TPP) from the enzyme. Therefore, when the transketolase activity is measured, TPP should be added to the system. It has been shown in the previous work (1) that the measurable rate of the enzymatic reaction is constant in time only at the saturating concentration of the coenzyme. At lower concentrations the reaction rate is at first low, then it increases and becomes constant only after some time has elapsed. This phenomenon was accounted for by a low rate of interaction between the coenzyme and apoenzyme. In the same paper we have suggested a two - step mechanism of interaction of TPP with apoTK. It remained obscure, however, which groups of the coenzyme are responsible for its linking to the apoenzyme. The present paper reports the data pertaining to this question.

EXPERIMENTAL

TK was isolated from baker's yeast essentially as described by Racker et al. (2) and was stored in 50% saturated ammonium sulphate solution. TK activity was determined as described earlier (3). The specific activity of the enzyme preparations was 6 U/mg of protein. Practically no activity was observed without addition of TPP. Prior to being used the enzyme solution was passed through a Sephadex G-50 column equilibrated with 0.05 M glycyl-glycine buffer pH 7.6 to remove ammonium sulphate. Thiazole pyrophosphate was prepared from TPP (4) in the section of chemical synthesis of the Laboratory of Bioorganic Chemistry of Moscow State University*). Thiamine and thiamine monophosphate were purchased from Calbiochem, USA.

The experiments aiming at elucidation of the effect of TPP analogues on the enzymatic activity of TK were carried out as follows. At room temperature the following components were incubated: glycyl-glycine buffer - 0.05 M; bovine serum albumin (for stabilization of TK (5)) - 0.1%; TK - 30 μg; TPP and the TPP analogue (the concentrations of the two latter components are indicated in figure captions). Total volume - 0.35 ml, pH 7.6. Control - samples without inhibitor. In 90 minutes (the time necessary for the system: TK, TPP, TPP analogue to achieve equilibrium (1)) 0.06 ml aliquots were taken and TK activity was measured in the system: glycyl-glycine buffer - 2.8×10^{-2} M; a mixture of pentosephosphates - 1×10^{-2} M; NAD - 3.1×10^{-4} M; glyceraldehyde-phos-

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*) The authors wish to thank the staff of the section of chemical synthesis for preparing thiazole pyrophosphate.

phate dehydrogenase from rabbit muscle - 3 U; cystein - 3.2×10^{-3} M; arsenate - 1.1×10^{-3} M. Total volume 1.8 ml; pH 7.6. As no TPP was added to the system, the value of the activity determined characterized the quantity of the holoenzyme formed during the preincubation of apoTK with TPP (both in the presence of the inhibitor and without it)*).

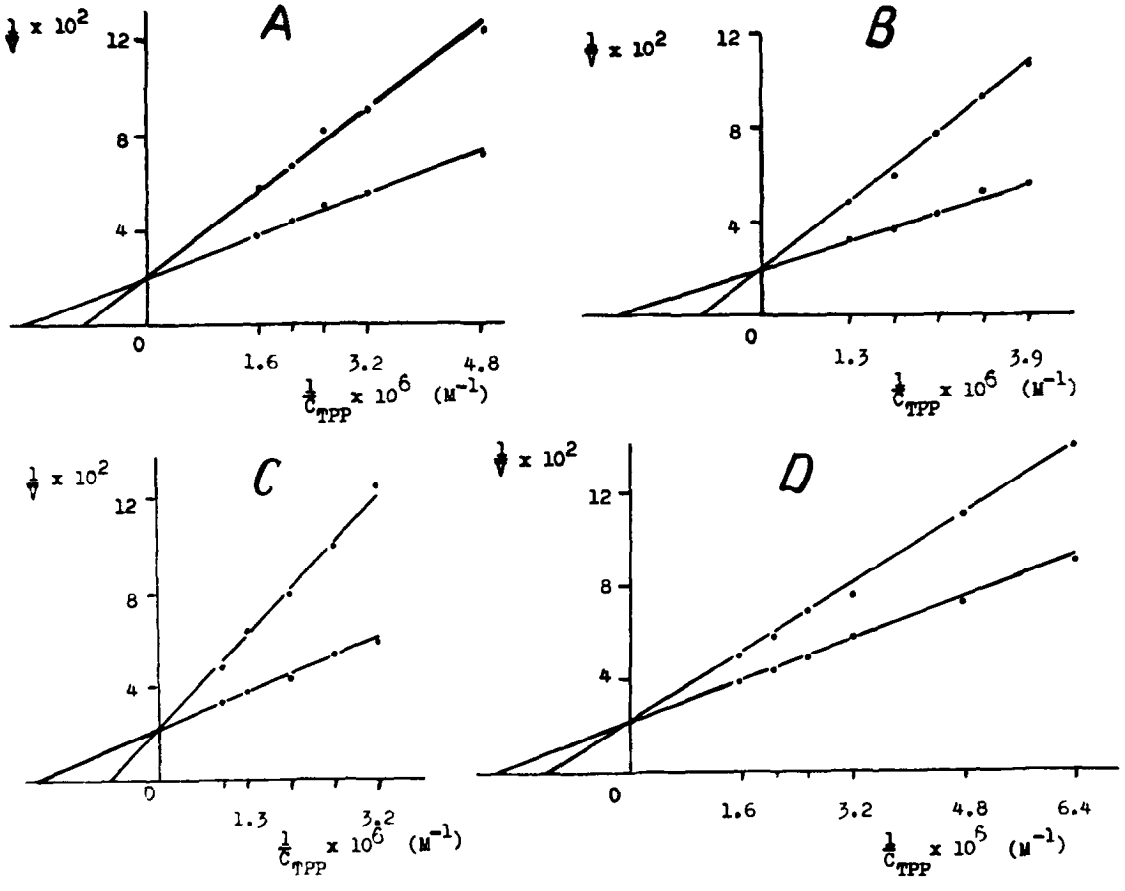

Fig. 1. Inhibition of the transketolase by thiamine (A), thiamine monophosphate (B), pyrophosphate (C) and thiazole pyrophosphate (D).
Inhibitor concentration: thiamine - 5×10^{-2} M, thiamine monophosphate - 1.8×10^{-3} M, pyrophosphate - 3.4×10^{-4} M, thiazole pyrophosphate - 3.1×10^{-6} M.

*) The holoenzyme is sufficiently stable and TPP does not cleave off it during the time of activity measurement (1).

RESULTS AND DISCUSSION

As is clear from the data shown in the Fig.1 all the TPP analogues studied have proved to be TK inhibitors and the mechanism of their action in relation to the coenzyme is purely competitive. (In other words, in the presence of TPP analogues the quantity of holoTK formed from the apoenzyme and coenzyme decreases). This indicates that the TPP analogues interact with the same sites of the enzyme's active centre as the coenzyme.

According to the degree of TK inhibition TPP analogues form the following series (see Table 1): thiamine < thiamine

Table 1. Values of Inhibitory Constants for TPP Analogues*).

Inhibitor	Thiamine	Thiamine monophosphate	Pyrophosphate	Thiazole pyrophosphate
Inhibitory constants (M)	3.4×10^{-2}	2.0×10^{-3}	2.8×10^{-4}	5.4×10^{-6}

Values of inhibitory constants (K_i) were calculated (from the data presented in Fig.1.) according to the formula

$$K_i = \frac{[i]}{\frac{K'_m}{K_m} - 1}$$

where $[i]$ - concentration of the inhibitor;
K_m - the Michaelis constant for TPP in the absence of the inhibitor;
K'_m - the effective Michaelis constant for TPP in the presence of the inhibitor.

*) Pyrophosphate as a fragment of the TPP molecule could be called a TPP analogue only tentatively.

monophosphate < pyrophosphate < thiazole pyrophosphate. This means that the interaction of TPP with apoTK occurs at least in three points and at the expense of the thiamine moiety of the coenzyme molecule and its two phosphate residues.

Free thiamine is characterized by a very low affinity to the apoenzyme (K_i=3.4 x 10^{-2} M). The affinity of thiamine monophosphate is also rather low (K_i=2.0 x 10^{-3} M). At the same time the affinity of TPP to apoTK is very high (the Michaelis constant*) is of the order of 6.0 x 10^{-7} M) and the respective constant for the coenzyme and the two above analogues differ by several orders. Thus, the pyrophosphate residue of TPP makes an important contribution to the interaction of the coenzyme with the apoenzyme (inorganic phosphate does not affect the equilibrium of the system: TPP + apoTK $\rightleftharpoons$ holoTK (1)).

On the other hand, the affinity of thiazole pyrophosphate is much higher than that of free pyrophosphate (see the Table 1) and differs negligibly from the TPP affinity to the enzyme. This indicates the participation of the thiazolium ring of TPP in the formation of the bond between the coenzyme and the protein portion of the TK molecule. The positively charged nitrogen atom of the thiazolium ring apparently makes an additional contribution to the interaction of the coenzyme with the apoenzyme (6). As to the pyrimidine ring of TPP, the question of its participation in linking together the coenzyme and apoenzyme is open to discussion.

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*) The value of the Michaelis constant for TPP was determined from the curves presented in the Fig.1.

ACKNOWLEDGEMENT

The authors to express their profound gratitude to Prof. S.E.Severin for valuable advice and discussion.

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