THE FUNCTION OF CALCIUM – COFACTOR OF TRANSKETOLASE FROM BAKER'S YEAST

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Since baker's yeast transketolase has been isolated magnesium was used as the cofactor of this enzyme (besides TPP*) [1–4]. Recently we have shown the presence of two atoms of calcium per molecule of native transketolase. The removal of one atom of metal of the second atom had no effect on the activity measured without the added cation [5].

The present paper is the elucidation of the role of metal in the catalytic activity of transketolase.

Isolation of the enzyme and the measurement of its activity was carried out after the modified procedure of Racker et al. [3, 6]. Transketolase preparation (specific activity 6E per mg) stored in $(NH_4)_2SO_4$ solution was used. Practically no activity was observed without addition of cofactor or only with metal. In the presence of TPP 60–70% of the activity displayed with metal and coenzyme was observed. The relationship of the enzyme, metal and coenzyme with the saturated substrate was investigated. In fig. 1 the rate of transketolase reaction is plotted against TPP concentration both in the presence and in the absence of calcium. It is seen that calcium at the concentrations of 1.0×10^{-4} M acts as a competitive activator in relation to the coenzyme: the Michaelis constant of the coenzyme changes from 3.3×10^{-5} M, the maximum reaction rate being constant. Higher metal concentrations cause both a decrease in the Michaelis constant for TPP and an increase in the maximum reaction rate, i.e. mixed activation occurs. (At calcium concentrations of 5.0×10^{-3} M the K_m and $V_{max.}$ values are respectively 0.4×10^{-5} and 3.7.)

In the case of magnesium (fig. 2) at first the mixed effect is observed: the Michaelis constant for the coenzyme changes from 3.3×10^{-5} M in the absence of the metal to 2.0×10^{-5} M in its presence (0.7×10^{-4} M); the maximum rate increases from 2.3 to 2.7. Increased magnesium concentrations do not affect the Michaelis constant but cause further increase in the maximum reaction rate (in the presence of 2.0×10^{-3} M of magnesium $V_{max.}$ is 5.0).

The above picture of the effect of activating metals does not fit any "usual" mechanism: competitive, noncompetitive or mixed and is, in fact, their combination. The following hypothesis may be suggested to explain the above facts. In the transketolase molecule there are two sites responsible for binding with metals. The cation interaction with the first site leads to a decrease in the Michaelis constant for TPP and the interaction with the second site results in an increase in the maximum reaction rate. Let us call the former site K_m-site and the latter $V_{max.}$-site. Let us assume that the affinity of the metal to the K_m-site is much higher than that to the $V_{max.}$-site. In this case at low cation concentration the Michaelis constant for TPP would change whereas the maximum reaction rate would either change insignificantly (as for example, with 0.7×10^{-4} M of magnesium) or not at all (in the presence of 1.0×10^{-4} M of calcium). If under these conditions the K_m-site is saturated with metal, the increasing concentration of the latter would only cause an increase in the maximum rate without any increase in the value of the Michaelis constant (as is the case with magnesium). If the metal is still binding with the K_m-site, then the increase in the maximum rate would be accompanied by the further change in the Michaelis constant, but to a relatively lesser degree (as is the case with calcium).

* TPP, thiamine pyrophosphate.

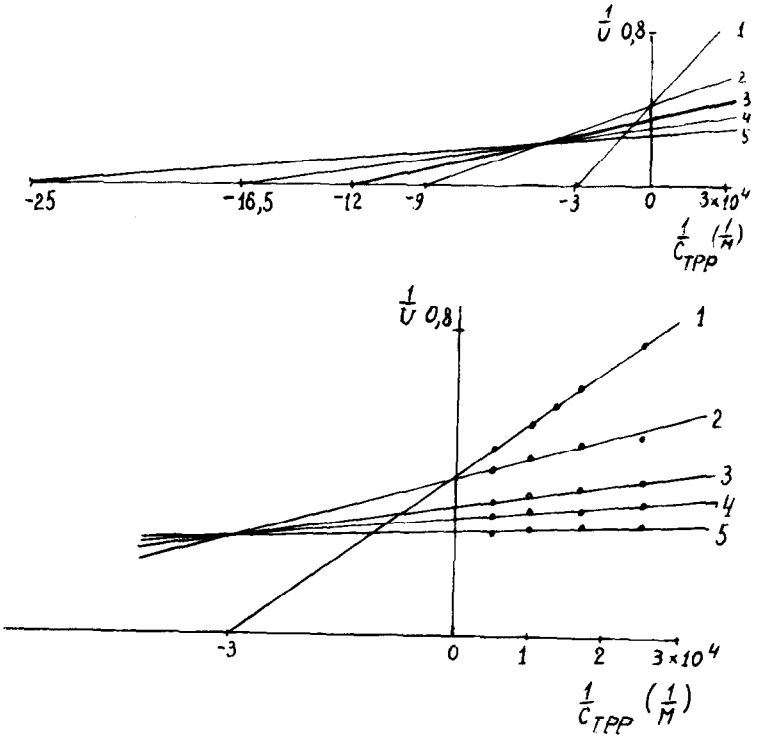

Fig. 1. The effect of calcium on the dependence of the rate of transketolase reaction upon thiamine pyrophosphate concentration. Concentrations of calcium ($CaCl_2$) used: (1) no metal; (2) 1×10^{-4} M; (3) 4×10^{-4} M; (4) 1×10^{-3} M; (5) 5×10^{-3} M.

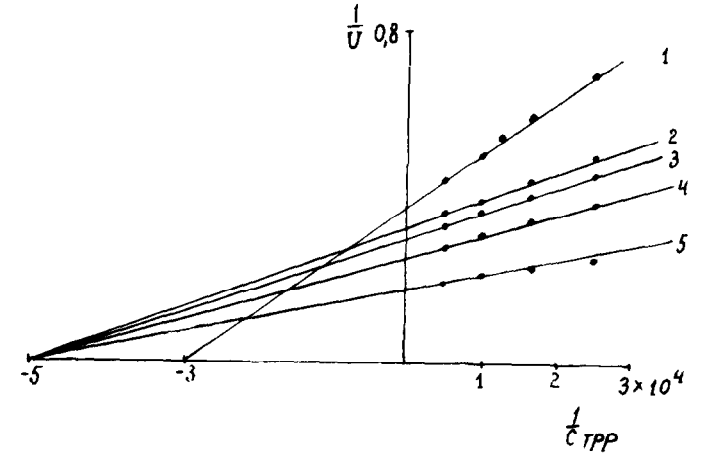

Fig. 2. The effect of magnesium on the dependence of the rate of transketolase reaction upon thiamine pyrophosphate concentration. Concentrations of magnesium ($MgCl_2$) used: (1) no metal; (2) 0.7×10^{-4} M; (3) 1×10^{-4} M; (4) 2×10^{-4} M; (5) 2×10^{-3} M.

Thus, metal has a double effect on transketolase: firstly, it diminishes the Michaelis constant for TPP, and secondly, it increases the maximum rate of the enzymatic reaction.

If the data on the determination of calcium in transketolase 5 are compared with our conclusion the following considerations will come to light. In the holotransketolase molecule there are two atoms of calcium. Removal of one of them entails a drop in activity which is displayed only in the presence of saturated TPP concentration (2.0×10^{-4} M). In other words, a decrease in the maximum reaction rate occurs. On the other hand, it is shown that the increase in the maximum rate observed on addition of metal to the enzyme is due to the binding of the cation with the $V_{max.}$-site. Hence, the calcium atom that is present in the holoenzyme and is the first to break off, is bound to the $V_{max.}$-site.

Removal of the second metal atom from transketolase does not affect the maximum reaction rate which is determined only with TPP. On the other hand, on binding of added calcium with the K_m -site of the apoenzyme the maximum rate is not affected. Consequently, it is possible to suggest that the metal atom which is the second to break off from transketolase is connected to the K_m-site.

Some metalloenzymes [7, 8] have been shown to lose their metal atoms without a loss of activity determined with saturated substrate concentrations. It is possible that in such cases the role of metals may be elucidated at sufficiently low concentrations of the substrate (coenzyme), as was done in the study described above.

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