CALCIUM: COFACTOR OF TRANSKETOLASE FROM BAKER'S YEAST

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Transketolase from baker's yeast contains two atoms of calcium per molecule of protein. Removal of one atom is accompanied by a decrease in enzymatic activity while the removal of the second atom does not affect the value of activity determined without the addition of the metal. Thiamine pyrophosphate is capable of binding with transketolase to form a catalytically active complex without the participation of the metal.

In the previous studies of transketolase from baker's yeast (EC 2.2.1.1.) magnesium was used as a one of cofactors (1-3). We have shown that some other divalent metals also can activate the enzyme, manganese being even more effective than magnesium (4). It was not clear which metal is present in native transketolase if it present at all. The present paper describes the investigation carries out to elucidate the above question.

Isolation of transketolase and determination of its enzymatic activity was carried out as described earlier (5) with some modifications. Specific activity of the preparation obtained was 10 U per mg. The amount of protein was determined spectrophotometrically (by the optical density at 280 and 260 nm and subsequent estimation from the nomogramm) and also according the Lowry method. Qualitative analysis of transketolase for content of metals was done in a ISP-22 quartz spectrograph; quantitative analysis - in a Hitachi-207 atomic absorption spectrophotometer. Prior to the analysis the enzyme solution

was passed once or twice through a G-50 Sephadex column to remove free ions. All reagents were prepared with distilled water which had been passed through ion-exchange resins.

Qualitative analysis of transketolase (specific activity 3 U per mg) as to the content of metals revealed the presence of Mg, Ca, Fe, and Cu. But at the last stage of purification of the enzyme (Table 1) the quantity of Mg, Fe and Cu did not exceed 0.2 g·atom per mole of protein; at the same time the content of Ca reached 1.7 g·atom per mole of protein. (Transketolase was prepared as holoenzyme because the value of activity both in the presence and in the absence of cofactors was equal).

Table 1

Determination of Metal in Holotransketolase at Different Stages of Purification

Enzyme purification stage	Specific activity (U/mg)	Content of metals							
		Ca		Mg		Fe		Cu	
		μg/mg	g·atom/mol	μg/mg	g·atom/mol	μg/mg	g·atom/mol	μg/mg	g·atom/mol
Dialysis	1	0.13	-	0.28	-	0.05	-	0.14	-
Thermal treatment	1	0.17	-	0.27	-	0.06	-	0.24	-
Alcohol fractionation	1	0.17	-	0.95	-	0.07	-	0.03	-
Purification on DEAE-cellulose	3	1.05	-	0.22	-	0.06	-	0.02	-
Ammonium sulfate fraction (59-65%)	6	0.38	1.3	0.04	0.2	0.06	0.2	0.02	0.1
Resedimentation	10	0.48	1.7	0.03	0.2	0.06	0.2	0.02	0.1

Racker et al. showed that after long storage of transketolase in $(NH_4)_2SO_4$ solution, pH 7.6, the value of activity determined without added metal amounted to the half of that with magnesium and TPP* (3); this value decreases to approximately 20% on dialysis against EDTA** (1). We carried out parallel measurements of activity and content of calcium both in holoenzyme and after transketolase has been treated with $(NH_4)_2SO_4$ and versene (Table II).

When the enzyme was stored in $(NH_4)_2SO_4$ solution the content of calcium decreases from 1.7 to 0.9 g·atom per mole of protein which may be interpreted as the removal of one metal atom from a molecule of transketolase. At the same time there was observed a decrease in the value of activity deter-

Table II

Determination of the Content of Calcium in the Holoenzyme and after Treatment of Transketolase with $(NH_4)_2SO_4$ and EDTA

Preparation	Activity (%)		Quantity of Ca (g·atom/mol)
	without cofactors	with TPP only	
Holoenzyme	100	100	1.7
Enzyme stored in $(NH_4)_2SO_4$	10	60–70	0.9
Enzyme dialysed against EDTA solution (without subsequent passing through Sephadex)	10	10–20	-
Enzyme dialysed against EDTA and then passed through Sephadex	10	60–70	0.3

* TPP - thiamine pyrophosphate
**EDTA - ethylenediaminetetraacetate

mined without the added metal. Further decrease in the calcium content in the course of dialysis against EDTA from 0.9 to 0.3 g·atom per mole of protein (i.e. the removal of the second metal atom) did not affect the value of enzyme activity determined with TPP only. Hence TPP is capable of binding with transketolase to form a catalytically active complex without the participation of the metal.

It is not excluded that calcium may be a part of other TPP enzymes whose activity is usually determined with magnesium; however, no direct determination of the metal present in the holoenzyme has been done.

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