PRODUCT ACTIVATION OF YEAST THREONINE DEHYDRATASE BY AMMONIA*

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Threonine dehydratase (ThrDH)(EC 4.2.1.16: L-threonine hydro-lyase (deaminating)) catalyzes the reaction L-threonine ⟶ NH_3 + α-ketobutyrate. The activity of the enzyme can be measured by following the reaction of the α-ketobutyrate formed with lactate dehydrogenase and DPNH in an optical test according to Warburg (Holzer et al. 1963). Experiments by Umbarger (1956) have revealed a specific inhibition of ThrDH from E.coli by low concentrations of L-isoleucine. As α-ketobutyrate is the compound from which the biosynthesis of isoleucine starts, this finding represents a classical example for a feedback mechanism. Also in yeast ThrDH activity is inhibited by L-isoleucine (Holzer et al. 1963). Not only the activity but also the biosynthesis of ThrDH is regulated in yeast (Holzer et al. 1963): ammonia, one of the products of the ThrDH reaction, represses the biosynthesis of the enzyme.

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This mechanism provides a control of ammonia formation by ammonia consumption in biosynthetic reactions. In this paper we report another effect of ammonia on ThrDH, namely the activation of the enzyme by ammonia to an extent of about 600 %. This type of activation may be called product activation.

In Table 1, the activation of a ThrDH-containing extract from yeast by different cations is shown. The effect of NH_4^+ is far above that given by all other cations. Mg^{++} and Ca^{++} are ineffective. Na^+ exerts only a small effect. With 3.7×10^{-2}M Na^+ the enzymatic activity was 11, this is a slight but significant activation. 14.8×10^{-3}M K^+ or Na^+, together with 3.7×10^{-3}M NH_4^+, result in the same activity as does 3.7×10^{-3}M NH_4^+ alone. The anion of $(NH_4)_2SO_4$ is not responsible for the activation; NH_4Cl gives the same effect. Both the NH_4^+ and the K^+ activated enzyme have the same pH optimum of about 8.1 to 8.3 .

In Fig.1, the saturation curves of ThrDH activity with NH_4^+ and K^+ are shown. The resulting Michaelis constants are 2×10^{-4} and 1×10^{-3}M for NH_4^+ and K^+ respectively. Thus the enzyme is more sensitive to NH_4^+ than to K^+.

The activation of a reaction by one of its products leads to the characteristic kinetics of autocatalytic processes. As demonstrated in Fig.2, in an incubation mixture containing sufficient NH_4^+ for maximal activity (curve 1), the reaction proceeds in a linear manner, whereas a mixture without addition of NH_4^+ shows a time dependent increase of the reaction rate (curve 2). In the experiment without added NH_4^+, the reaction was followed

Table 1

Effect of different cations on the activity of threonine dehydratase.

	$-\Delta$O.D. x 1000/min	
	5.8×10^{-4}M cation	3.67×10^{-3}M cation
No addition	7	
$(NH_4)_2SO_4$	31	41
KCl	18	32
RbCl	16	31
Li_2CO_3	14	27
Cs_2CO_3	12	26
NaCl	8	9
$MgSO_4$	-	5
$CaCl_2$	-	5

Preparation of extract. Saccharomyces cerevisiae R 59 was grown in minimal medium (Tavlitzki 1949) with DL-threonine as the sole nitrogen source. The harvested and washed cells were ground with the 3 fold weight of alumina powder Alcoa A 305, extracted with the 3 fold volume of 0.05 M sodium phosphate buffer pH 7.6 (containing 10^{-3}M EDTA) and centrifuged for 1 hour at 34 000 x g. The supernatant was stabilized by addition of 10^{-3}M pyridoxal-5-phosphate. All operations were carried out at 0° - 4°C.

Optical assay of threonine dehydratase. λ= 366 mμ, d = 1 cm, total volume 3.00 ml, 22°C. 2.72 ml 0.1 M triethanolamine-HCl buffer pH 7.9 + 0.1 ml DPNH (10 mg/ml) + 0.04 ml lactate dehydrogenase (5 mg/ml LDH Boehringer in 2.2 M $(NH_4)_2SO_4$, dialyzed 12 hours against 10^{-3}M NH_4OH and then 2 hours against H_2O) + 0.02 ml 0.01 M pyridoxal-5-phosphate + 0.1 ml 0.5 M L-threonine. Start with 0.02 ml extract (ca. 0.3 mg protein).

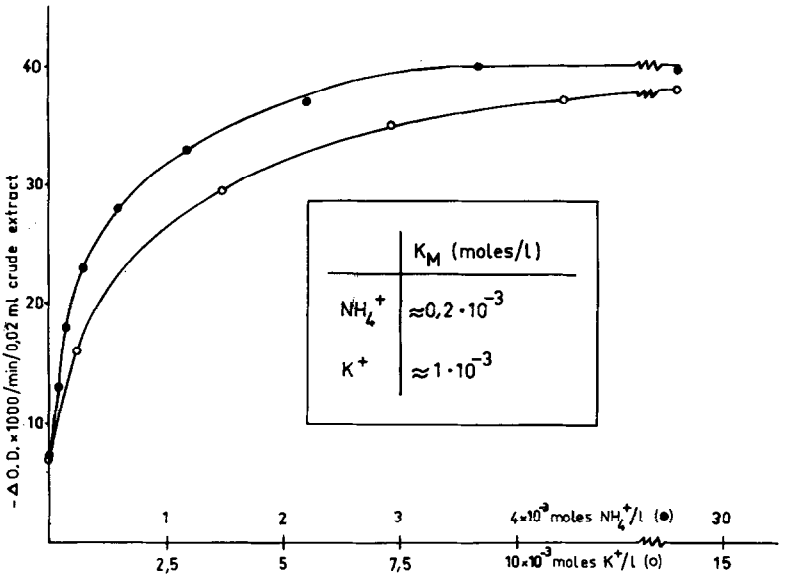

Fig.1 Activity of ThrDH with different Concentrations of NH_4^+ and K^+. (Assay conditions see Tab.1)

by determination of α-ketobutyrate as well as of ammonia. In accordance with the equation of the ThrDH reaction both substances are formed in equimolar amounts.

At present the biological significance of the activation of ThrDH by NH_4^+ is unknown.

SUMMARY

Threonine dehydratase from yeast is activated by NH_4^+ up to 6 fold. The Michaelis constant with NH_4^+ is about 2 x 10^{-4}M. K^+, Rb^+, Li^+ and Cs^+ at higher concentrations also activate the enzyme. Na^+ is only slightly effective, Mg^{++} and Ca^{++} have no effect.

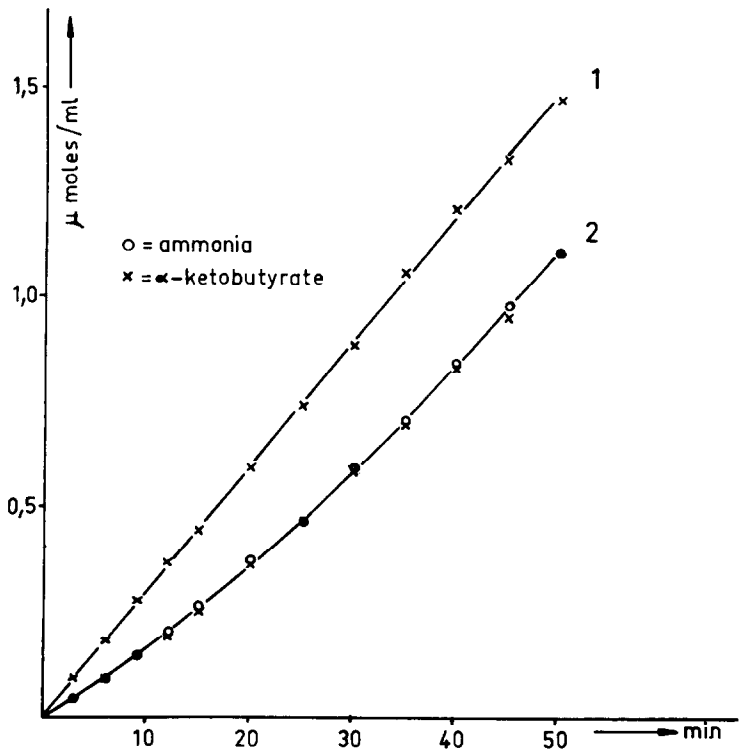

Fig.2 Kinetics of the Activation of Threonine dehydratase by Ammonia.

Final concentrations in the reaction mixtures: 1.67 x 10^{-2}M L-threonine, 0.66 x 10^{-4}M pyridoxal-5-phosphate, 0.67 x 10^{-3}M L-valine, 0.09 M triethanolamine-HCl buffer pH 7.9 . Start with 0.02 ml extract per ml incubation mixture. Temp. 22°C. Curve 1, with 3.4 x 10^{-3}M NH_4^+; curve 2, without added NH_4^+. L-valine was added to protect ThrDH from inactivation when diluted (Holzer et al. 1963). At the indicated times 3 ml aliquots were taken, deproteinized by addition of 0.5 ml of 25 % perchloric acid and neutralized with 2 N KOH. After removal of $KClO_4$ by centrifugation, the supernatants were analysed for α-ketobutyrate and for ammonia by optical methods: λ = 366 mµ, d = 1 cm, T = 22°C, total volume including sample 3.0 ml.

Conditions for α-ketobutyrate assay: 0.3 mg DPNH/ml, 0.09 M tris(hydroxymethyl)aminomethan-HCl buffer pH 7.6 . Start with 0.1 mg lactate dehydrogenase (Boehringer) suspended in 0.02 ml 2.2 M $(NH_4)_2SO_4$.

Conditions for ammonia assay: 0.3 mg DPNH/ml, 1.3 x 10^{-3}M EDTA, 1.7 x 10^{-2}M α-ketoglutaric acid, 0.09 M triethanolamine-HCl buffer pH 8.3 . Start with 0.4 mg glutamic acid dehydrogenase (free of NH_4^+)(Boehringer) in 0.02 ml.
Both reactions are completed in 10 - 15 min.

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