Inhibition of Rat Liver Threonine Dehydratase by Tris(hydroxymethyl) aminomethane (Tris) and Reversal by Orthophosphate[1] (36090)

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Rat liver biodegradative threonine dehydratase [L-threonine hydro-lyase (deaminating) (EC 4.2.1.16)] has been purified 55-fold in the presence of 30% glycerol and 10 m*M* mercaptoethanol (1). Burns and Zarlengo (2) have shown that the activity of the purified Salmonella dehydratase was independent of added pyridoxal-5′-phosphate (PLP) but that dialysis against Tris resulted in significant inactivation of the enzyme. Furthermore, these authors found PLP to remain associated with the enzyme when dialyzed against phosphate buffer. The present communication reports the reactivation of Tris-dialyzed rat liver dehydratase by PLP or orthophosphate.

Materials and Methods. Pyridoxal-5′phosphate and tris(hydroxymethyl)aminomethane were purchased from Sigma and allo-free L-threonine from Calbiochem. Other chemicals and technical grade glycerol were obtained from several sources.

Dialysis against Tris. The purified enzyme preparation was rendered free of phosphate by dialysis at 4° against 0.1 *M* Tris–acetate buffer pH 8.3, 30% in glycerol and 10 m*M* in 2-mercaptoethanol. After 21 hr of dialysis, only 4% of the original enzyme activity was recovered. The standard assay procedure (1) was used with the Tris–acetate buffer replacing the phosphate buffer.

Effect of pyridoxal-5′-phosphate or orthophosphate. One-tenth milliliter aliquots of the dialyzed phosphate-free enzyme solution (12.3 mg of protein/ml) were mixed with an appropriate aliquot of 0.048 *M* pyridoxal phosphate or 0.1 *M* potassium phosphate solution at pH 8.3 and incubated at 43° for 30 min. The dehydratase reactions were initiated by the addition of 600 μmoles of L-threonine in 0.1 *M* Tris–acetate (pH 8.3) buffer. All solutions were 30% in glycerol and 10 m*M* in 2-mercaptoethanol. The final assay volume was 2.5 ml. Each assay tube had its own blank containing the appropriate amount of PLP or orthophosphate. The reactions were allowed to proceed for 10 min at 43° and were terminated by addition of trichloroacetic acid. The amount of 2-ketobutyrate formed was determined by Nishimura and Greenberg's modification (3) of the colorimetric method of Friedemann and Haugen (4).

Results and Discussion. Inhibition of L-threonine dehydratase by Tris and other amines has been reported by Nishimura and Greenberg (3) and others (2, 5–7). Partial reactivation of the amine-inhibited dehydratase by PLP has been demonstrated (2, 3, 5, 6, 8, 9). We have confirmed these observations for the purified rat liver dehydratase. Further, the effect is not due to the acetate or potassium ions since these were neither stimulatory nor inhibitory to enzyme activity (1).

In addition, we now report the reactivation of the Tris-dialyzed enzyme by either PLP or orthophosphate. Further, these factors were roughly equally effective and at concentrations calculated for the free amine concentration of the Tris buffer at pH 8.3. The extent of reactivation is presented in Fig. 1.

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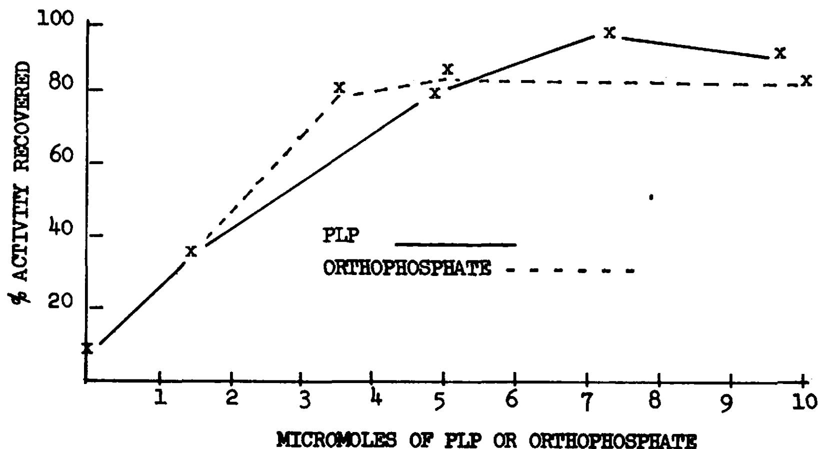

FIG. 1. Reactivation of Tris-inhibited rat liver threonine dehydratase by pyridoxal-5′-phosphate (PLP) or orthophosphate. The enzyme preparation was dialyzed for 21 hr at 4° against 0.1 *M* Tris–acetate buffer (pH 8.3) which was also 30% in glycerol and 10 m*M* in 2-mercaptoethanol. After dialysis, a 0.1 ml aliquot (1.23 mg of protein) was incubated with the indicated micromoles of PLP or orthophosphate for 30 min at 43° and pH 8.3 followed by the enzyme assay. The 0.1 ml aliquot of Tris buffer at pH 8.3 would contain 6.66 μmoles of Tris as the free amine.

Maximum recovery of activity occurs at about 7 μmoles of PLP or 5 μmoles of orthophosphate, which approximates the amount of Tris present as the free amine in the 0.1 ml aliquot of dialyzed enzyme initially incubated with PLP or orthophosphate. The reactivation effect was found to be time dependent requiring 30 min at 43° to be complete.

Since orthophosphate reactivates the Tris-inhibited enzyme, the postulate of Burns and Zarlengo (2) that Tris inactivates the enzyme by removal of the pyridoxal group seems an unlikely hypothesis. Indeed, several reports have already appeared (8, 9) suggesting that the reactivation of the serine-inhibited dehydratase by added PLP occurs by displacement of the serine from the enzyme bound PLP to the added PLP. Our finding that either PLP or orthophosphate reverses the Tris inactivation of rat liver dehydratase suggests that both the aldimine and the phosphate functions are involved in the possible formation of an aberrant Tris–PLP–enzyme complex. The previous observations of Burns and Zarlengo (2) that the nonphosphorylated pyridoxal or pyridoxamine were ineffective in reversing Tris inhibition corroborates our suggestion. We suggest that orthophosphate is effective in reversing Tris inhibition of the dehydratase enzyme by competing at the binding site between phosphate of PLP and the apoenzyme, thus weakening the Tris–PLP–enzyme complex resulting in dissociation of the Tris from the inhibited complex.

Summary. A 55-fold purified rat liver threonine dehydratase in phosphate buffer was found to retain only 4% of the original activity after dialysis against Tris buffer at pH 8.3. Addition of pyridoxal phosphate (PLP) to the Tris-inactivated dehydratase restored 96% of the original activity; addition of orthophosphate restored 83% of the activity. The concentration of pyridoxal phosphate or orthophosphate required for reactivation approximated the free amine concentration of Tris at pH 8.3.

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