THALLIUM ACTIVATION AND INHIBITION OF YEAST ALDEHYDE DEHYDROGENASE

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Received 1 September 1975

1. Introduction

Tl^+ has been shown to replace the physiological ion K^+ in the activation of several monovalent cation activated enzymes [1–6]. This provides the opportunity to employ Tl^+ as a probe for K^+ in n.m.r. and fluorescence studies [6–8]. Preliminary to such studies on yeast aldehyde dehydrogenase (EC 1.2.1.3.) Tl^+ activation of this enzyme was investigated and compared to the previously demonstrated activation by K^+ [9]. A general mechanism for this activation has been proposed whereby an activating monovalent cation induces and maintains an enzyme conformation suitable for catalytic activity [10].

2. Materials and methods

Partially purified aldehyde dehydrogenase was prepared from N.G. and S.F. yeast (British Fermentation Products Ltd.) obtained from a local baker. The method was essentially that of Black [9] except for the inclusion of 1.2×10^{-3} M phenylmethylsulphonyl fluoride (PMSF) throughout the procedure. The buffer composition at the final stage contained 0.1 M K_2HPO_4, pH 7.8, 10^{-3} M mercaptoethanol, and 1.2×10^{-3} M PMSF. Enzyme activity was between 7–10 units/ml when assayed with optimal K^+ (0.1 M) with a specific activity of 1. Samples were stored frozen at −18°C, and thawed out immediately prior to use.

Enzyme was equilibrated with 0.1 M Tris–NO_3 pH 8.0, 10^{-3} M mercaptoethanol and activating salt where indicated, by passage through a Sephadex G-25 column at 4°C. Because of the instability of the enzyme in the absence of an activating monovalent cation, the enzyme was stored on ice following chromatography and all assays in a given run were completed within 5 min.

Enzyme activity was measured by following the rate of reduction of NAD^+ by observing the increase in absorbance at 340 nm in a Pye Unicam SP1800 spectrophotometer using 1 cm quartz cells. Reaction mixtures contained in a final volume of 2.5 ml: 2×10^{-3} M acetaldehyde, 5×10^{-4} M NAD^+, 0.1 M Tris–NO_3 buffer, pH 8.0, 10^{-3} M mercaptoethanol, and an appropriate concentration of monovalent cation activator as its nitrate salt. Reaction was begun by the addition of 0.1 ml of enzyme to an otherwise complete reaction mixture maintained at 25°C.

Tl^+ and K^+ concentrations in the final reaction mixture were determined by atomic absorption (Hilger and Watts Atomspek). NAD^+ (grade III), 2-mercaptoethanol, Tris and PMSF were purchased from Sigma Chem. Co. Acetaldehyde was redistilled and stored at −18°C as 2 M solution. All other reagents were analar grade.

3. Results

Fig.1 is a semilog plot of $TlNO_3$ concentration against enzyme activity. It clearly demonstrates that Tl^+ is an activator of aldehyde dehydrogenase. Lineweaver–Burk plots are linear up to 10^{-3} M Tl^+ (fig.2) and statistical analysis by the method of Cleland [11] yields a K_s of 3.4×10^{-3} M and V_{max} of 0.762 absorbance units/min. This compares with a K_s of

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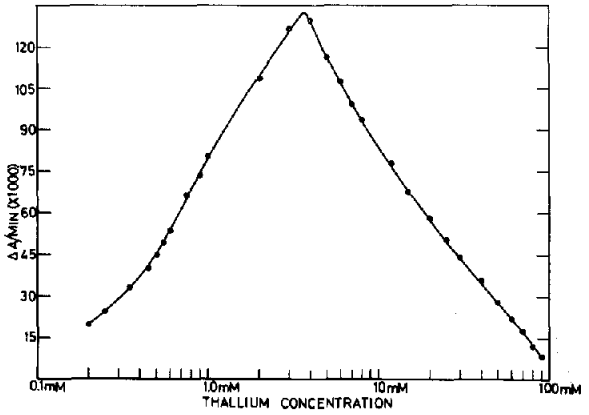

Fig.1. Semilog plot of activation and inhibition of aldehyde dehydrogenase by Tl^+. Assay conditions as in text with $TlNO_3$ concentrations as indicated.

1.33×10^{-2} M and V_{max} of 0.969 absorbance unit/min for KNO_3 as activator. It is worthy of note that in contrast to the situation in other enzymes activated by Tl^+ (for example pyruvate kinase [1] adenosine triphosphatase [5]), the apparent affinity for Tl^+ in aldehyde dehydrogenase is of the same magnitude as K^+

Optimal concentration for activating Tl^+ is seen to be 3.8×10^{-3} M (fig.1). At higher levels, inhibition by Tl^+ offsets any increase in activity due to greater saturation of the activator site. However inhibition is apparent at Tl^+ concentration in excess of 10^{-3} M as evidenced by non-linearity of the Lineweaver–Burk plot. This is

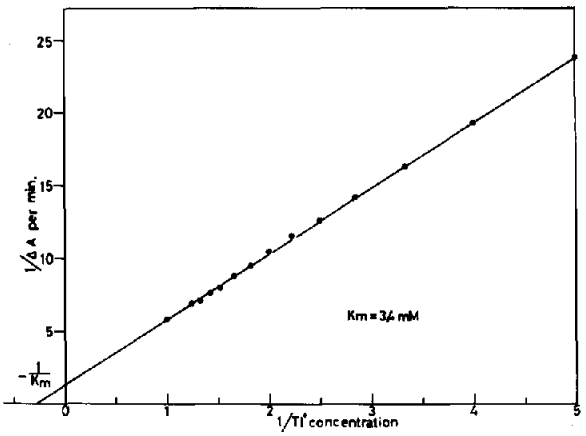

Fig.2. Double reciprocal plot showing linear relationship between aldehyde dehydrogenase activity and Tl^+ at concentrations below 1 mM.

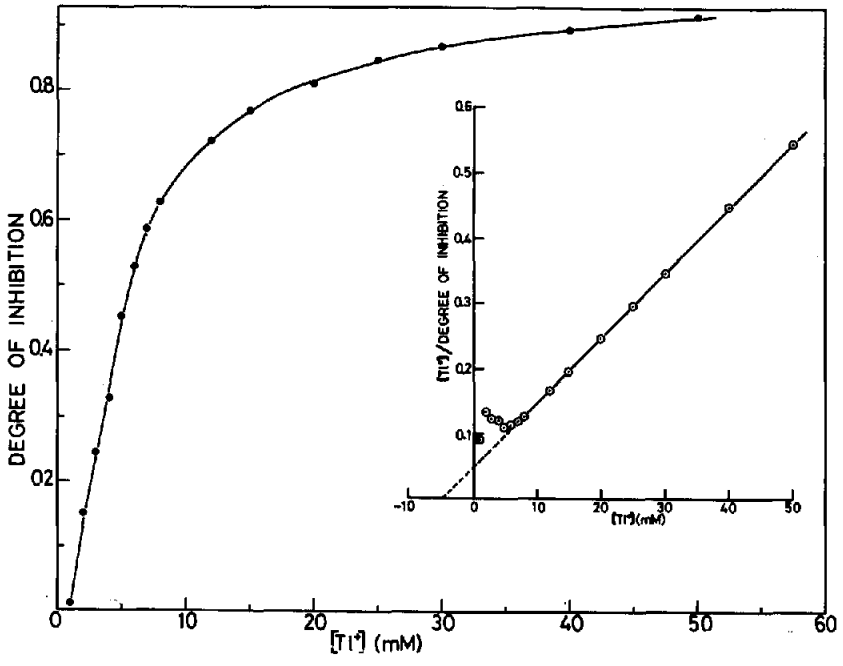

Fig.3. Inhibition of aldehyde dehydrogenase by Tl^+ at concentration above 1 mM.

in accordance with other enzymes shown to be activated by Tl^+ [1,6]. The nature of the inhibition is unclear. Extrapolation of the linear portion of fig.2 to higher Tl^+ concentrations allows calculation of theoretical velocities in the absence of inhibition. These have been compared with the experimental velocities at the same Tl^+ concentrations to obtain degree of inhibition. These are shown in fig.3. The reciprocal plot in the insert to fig.3 should be linear if inhibition is noncompetitive. At concentrations of Tl^+ above 0.05 M the line begins to curve downwards. It was noticed that after several minutes incubation in 0.1 M Tris HNO_3 pH 8.0 plus 10^{-3} M mercaptoethanol plus 0.06 M $TlNO_3$ appreciable protein precipitation was observed. In addition, the data for inhibition provides a poor fit to the equation for double binding at the activator site (cf. substrate inhibition).

In experiments with mixtures of cation activators, theoretical levels of activity were calculated on the basis of known activities of Tl^+ or K^+ acting separately. With Tl^+ below 7.5×10^{-4} M plus K^+ below 1.5×10^{-2} M measured activities agreed with these calculations. However, when either the Tl^+ or the K^+ concentrations were increased, inhibition was observed from the theoretically calculated activities.

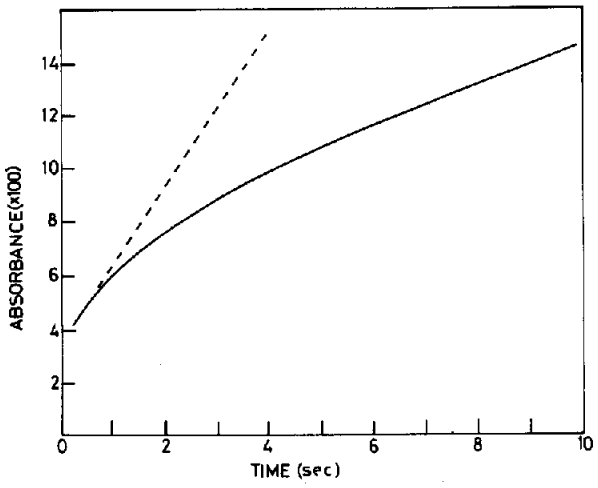

Fig.4. Activity of aldehyde dehydrogenase following rapid mixing into different cation environments: enzyme in 0.1 M Tris–NO_3 pH 8.0, 10^{-3} M mercaptoethanol. 3.5 mM $TlNO_3$ in one syringe is mixed with the contents of the second syringe to give normal assay concentrations except that, (A) 3.5 mM $TlNO_3$ (B) 1.75 mM $TlNO_3$ plus 0.1 M $LiNO_3$.

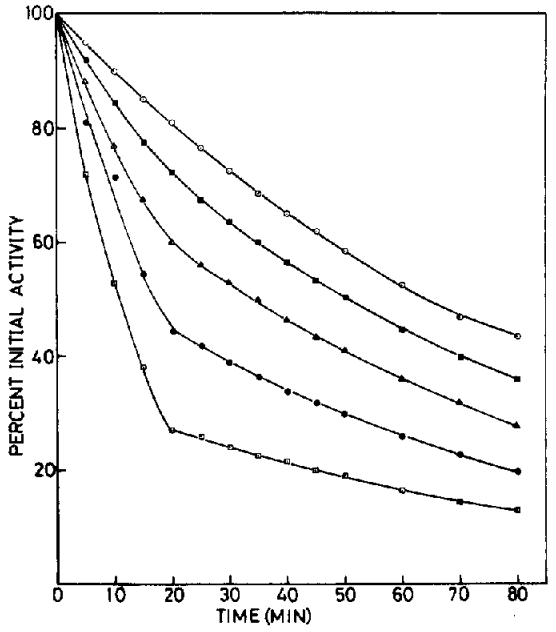

Fig.5. Plot of percent initial enzyme activity vs. time at different $TlNO_3$ concentrations: (○) 50 mM, (■) 10 mM, (△) 4 mM, (●) 1 mM, (□) 0.5 mM. Enzyme was equilibrated in 0.1 M Tris, pH 8.0, 10^{-3} M mercaptoethanol at an appropriate $TlNO_3$ concentration. Enzyme was adjusted to 25°C and at various times 0.1 ml fractions were removed and assayed for activity in a standard reaction mixture at a final $TlNO_3$ concentration of 4 mM.

Rapid dilution of activating Tl^+ (3.5×10^{-3} M) and replacement with inhibitory Li^+ (10^{-1} M) was achieved by use of rapid mixing and stopped flow apparatus (Durram instruments). The rate constant for the decay of enzyme activity demonstrated in fig.4 is 1.5 sec^{-1}. This is much slower than the likely rate of dissociation of Tl^+ from the enzyme [12]. Such behaviour has previously been demonstrated when K^+ instead of Tl^+ was the activating cation [13], and has been interpreted in terms of a conformation change induced by activating cation.

Fig.5 demonstrates the stability of aldehyde dehydrogenase in various levels of $TlNO_3$.

4. Discussion

The potential usefulness of Tl^+ as a probe for the monovalent cation activation process of yeast aldehyde

dehydrogenase is supported by these findings. However, difficulties arise when employing Tl^+ n.m.r. Tl^+ n.m.r. techniques at the present require at least 50 mM Tl^+ to supply sufficient signal for longitudinal and transverse relaxation measurements. At this concentration of Tl^+ the activation of yeast aldehyde dehydrogenase is offset by an additional inhibitory effect which lowers activity by 55%. This inhibition cannot be reversed by K^+. Thus it seems just as likely that at such concentrations structural probes may be detecting signals from Tl^+ interacting at a separate inhibitor site rather than at the activator site (see for example Tl^+ n.m.r. studies on pyruvate kinase [7] and adenosine triphosphatase [6]).

To avoid levels of Tl^+ which demonstrate this inhibitory effect the concentration must be kept below 1 mM. At these lower Tl^+ concentrations, structural probes at the end of a 30 min period (see fig.5) will be investigating an essentially denatured enzyme.

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