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The activity of K^+-activated yeast aldehyde dehydrogenase following rapid changes in cation environment

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SUMMARY

The activity of aldehyde dehydrogenase decreases following dilution of activating K^+, or addition of Li^+ which is competitive with K^+, at a rate which is considerably smaller than the likely rate of K^+ dissociation from the enzyme. These results are interpreted to mean that the active site is complete in the absence of K^+.

A number of enzymes show an absolute requirement for a univalent cation[1]. The majority of these enzymes show maximal activity in the presence of K^+, while activity is often zero in the presence of Li^+ or Tris.

There is no evidence that K^+ binding to substrate is important in the activation process, even when such binding is likely on structural grounds[2]. Two general models have been proposed on the basis that K^+ binds to the enzyme; (i) that K^+ binds to, and forms an essential part of the catalytic structures at the active site[3], (ii) that K^+ induces or stabilises an active conformation[1].

In support of the latter hypothesis, conformational differences between such enzymes in the presence of activating and non-activating cations have been detected by fluorescence spectroscopy[4], sucrose gradient centrifugation[5], resistance to proteolytic digestion[6], and immunoelectrophoresis[7]. In addition, the stability of a number of such enzymes in denaturing environments is profoundly influenced by the nature of the univalent cation present[8–10]. However, such changes are not necessarily related to activity. For instance, differences in ultraviolet spectra have been demonstrated between pyruvate kinase in Tris, a non-activator, and K^+, an activator, but differences are also seen between pyruvate kinase in two non-activators, Li^+ and Tris[11].

We have approached this problem by proposing that if K^+ is needed only to maintain an active conformation, then the rate of decay of that active conformation upon removal of K^+ may be slow relative to the catalytic turnover of the enzyme. In this case, activity should be observable for a period following the removal of K^+. Existing techniques do not permit the instantaneous removal of K^+ from a protein solution. However, two methods have been used which result in the rapid lowering of the effective K^+ concentration. These are (i) diluting out the K^+ in a small enzyme sample with a large reaction mixture containing no other K^+, and (ii) adding an enzyme sample containing K^+ to a reaction mixture containing a large concentration of Li^+ which is competitive with K^+.

K^+-activated aldehyde dehydrogenase (EC 1.2.1.5) from yeast (N.G. and S.F., British Fermentation Products Ltd) was prepared by the method of Black[12] with minor modifications. Enzyme preparation, stored at −18 °C, was thawed as required and equilibrated with the desired buffer by chromatography on Sephadex G-25 at 0 °C, then used immediately.

The first type of experiment, illustrated in Fig. 1, is that in which the K^+ concentration of 0.05 M in an enzyme sample of 0.05 ml is diluted to 0.001 M by addition to 2.45 ml of reaction mixture containing no other K^+. (The K_a for K^+ in this system is 0.007M.) Mixing was by rapid inversion of the cuvette following enzyme addition. Product formation was monitored by the increase in absorbance at 340 nm using a Unicam SP 1800 spectrophotometer. The time between adding the enzyme and the start of the recorder trace is approximately 10 s. In Fig. 1 (Line A) the time course has been interpolated between the start of the recorder trace and the zero time absorbance, determined by omitting enzyme or NAD^+, neither of which absorb at 340 nm. It can be seen that the rate does not immediately reflect the low concentration of K^+ in the total reaction mixture. Line B in Fig. 1 shows a control in which the K^+ concentration of 0.05 M present in the enzyme preparation is maintained during the reaction by the inclusion of more K^+ in the remainder of the assay mixture.

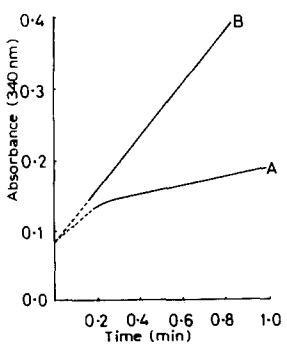

Fig. 1. Activity of aldehyde dehydrogenase following rapid dilution of K^+ activator. Reaction was started by adding 0.05 ml of enzyme in 0.1 M Tris–HCl, pH 8.0, 0.05 M KCl, and 10^{-3} M mercaptoethanol to 2.45 ml of a solution which contained all other reagents. Final reaction concentrations were for Line A, 0.1 M Tris–HCl, pH 8.0, 10^{-3} M KCl, 10^{-3} M mercaptoethanol, $5 \cdot 10^{-4}$ M NAD^+, $2 \cdot 10^{-3}$ M acetaldehyde, 0.01 mg protein; Line B as for A except KCl 0.05 M. The graph has been interpolated (dotted line) between the absorbance at zero time and the start of the recorder trace. All assays were conducted at 25 °C.

In order to observe the first few seconds of the reaction, use was made of a stopped-flow apparatus (Durrum 13 000, Durrum Instruments, Palo Alto, Calif., U.S.A.). This sort of rapid mixing system cannot be used successfully to mix volumes in the ratio 1:50 as was done in the experiment of Fig. 1. Instead, enzyme in one syringe was rapidly mixed with the contents of the second syringe which included substrates and 0. 2 M Li^+. Fig. 2 shows the adjustment of enzyme activity to this change in cation environment. The rate constant for the reversible decay of active to inactive enzyme is 0.7 s^{-1} and this appears to be similar to the decay of activity on dilution shown in Fig. 1.

When an enzyme is freed from K^+ by chromatography on Sephadex G-25, the recovery of activity on replacing K^+ also takes several seconds (see Fig. 3). This is quite separate from the much slower recovery process which can be observed if activating cation is restored to enzyme which has been incubated for long periods in its absence.

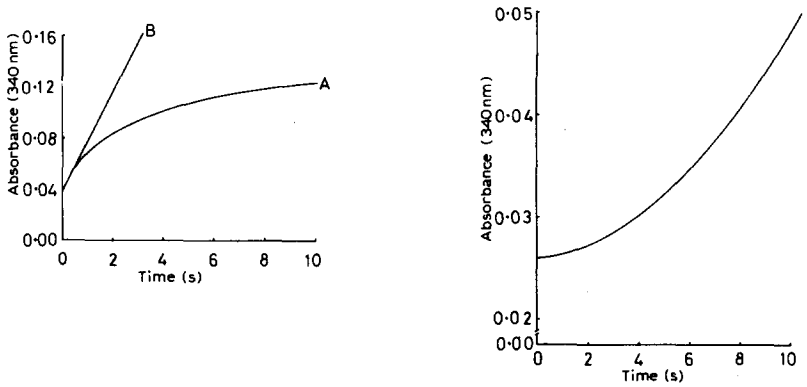

Fig. 2. Activity of aldehyde dehydrogenase following rapid mixing into different cation environments. Enzyme in 0.1 M Tris–HCl, pH 8.0, 0.05 M KCl, 10^{-3} M mercaptoethanol in one syringe is mixed with the contents of the second syringe to give reaction concentrations of A as for Fig. 1 (A) except 0.025 M KCl, 0.1 M LiCl; B as for Fig. 1 (B).

Fig. 3. Recovery of activity of aldehyde dehydrogenase on restoring K^+. Enzyme in 0.1 M Tris–HCl, pH 8.0, 10^{-3} M mercaptoethanol in one syringe is mixed with the contents of the second syringe to give reaction concentrations identical to Fig. 1 (B).

This work shows that the activity of aldehyde dehydrogenase takes several seconds to respond to a change in the cation environment. The persistence of relatively high levels of activity on diluting out K^+ or adding a competitive inhibitor such as Li^+, could mean that K^+ dissociates from the enzyme with a rate constant as small as 0.7 s^{-1}. However, K^+ is a highly mobile ion which dissociates rapidly from ligands. For example, rate constants for the release of K^+ from valinomycin and various crown ethers are of the order of 10^6 s^{-1} (refs 13 and 14). With univalent cation activated β-galactosidase hydrolysing *o*-nitrophenyl β-galactoside in experiments equivalent to those of Figs 2 and 3, we have found that the period of adjustment of enzyme activity to a new cation environment is so small as to be undetectable, even with a time base expanded 50-fold. Thus, if the mechanism of activation of this enzyme involves cation binding to protein, in this case at least the

cation must dissociate at a rate greater than 10^2 s^{-1}. Hence, the transient high rates of activity following an unfavourable change in cation environment demonstrated in Figs 1 and 2 suggest that catalytic activity persists after K^+ has left its binding site. In this case, the role of K^+ must be to maintain an active conformation rather than to form an essential part of the active site.

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