change in the enzyme thereby exposing buried active sites. For it is obvious that sites for substrate binding are available whether an activator univalent cation is present or not. Thus, a proposed enzyme conformation[5] induced by K^+ and essential for phosphoryl transfer, could be attained either before or after substrate binding but must be important at some later stage in the overall mechanism. One can imagine that any conformational change involved need be only small, perhaps explaining why so little success has been obtained from most of the existing methods of detecting conformational variations.

This investigation was supported in part by Research Grant AM08123 from the U.S. Public Health Service. The authors are indebted to Dr R. R. BECKER for both theoretical and practical help.

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Received April 29th, 1968

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BBA 63340

The inhibition of univalent cation activated enzymes by tris(hydroxymethyl) aminomethane

Many enzyme systems are stimulated by or show an absolute requirement for a univalent cation[1]. K^+ is commonly the most effective ion, and in general, enzymes requiring univalent cations are not activated by Li^+. Various organic cations such as $Tris^+$ and $tetramethylammonium^+$ also fail to activate and have been used widely, therefore, in buffering systems where univalent cation requirements are being studied. In addition to failing to substitute for K^+ as a univalent cation activator, Li^+ frequently has been shown to inhibit certain enzymes in environments containing K^+ (refs. 2–6). The type of inhibition has rarely been studied adequately, but occasionally, has been shown to be competitive with K^+ (refs. 2, 6). The question then arises whether non-activators of enzymes such as Tris can also inhibit competitively, in which case

saturation curves for activator cations could vary simply as a result of differing concentrations of background buffer. Tris inhibition has been considered previously and stated to be negligible at usual concentrations[3,4]. A reexamination of the effect of Tris on the activity of K^+-activated enzymes was prompted by the finding in this laboratory[7] that the effect of various activating and non-activating univalent cations on ultraviolet difference spectra of pyruvate kinase was markedly affected by the presence of Tris in the enzyme environment.

Rabbit muscle pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) (Calif. Biochem. Co. A grade) was suitably diluted with 0.01 M Tris–HCl (pH 7.4) and freed from contaminating NH^+_4 by chromatography on Bio-gel P-2 columns (Bio-Rad laboratories), equilibrated with the same buffer.

Pyruvate kinase was assayed as by MILLER AND EVANS[8] except that Mg^{2+} was added at 0.008 M (ref. 9) and Tris concentration was as indicated in legends of Figs. 1–3. The monocyclohexylammonium salt of phosphoenolpyruvate and Tris–ADP were adjusted to pH 7.4 with Tris. The concentration of Tris added to any reaction mixture other than that in the buffer totaled 0.022 M. This is always considered in calculations of total Tris concentration.

KACHMAR AND BOYER[4] have noted previously that there is an inhibition of pyruvate kinase at high ionic strengths even when the salt added is KCl. COHN[2] has demonstrated an inhibition at high salt concentration (presumable tetramethyl ammonium chloride) and also a competitive relationship between K^+ and Mg^{2+}. It was necessary therefore to establish optimal concentrations of cation activators under the conditions used here and to establish the extent of non-specific inhibition by high salt concentration for comparison with the effect of Tris. Fig. 1 demonstrates the effect of increasing K^+ concentration up to and above the optimal level. The inhibition at high K^+ concentration seems to be due in part to a competition of K^+ with Mg^{2+}, since increasing the level of Mg^{2+} from 0.008 to 0.016 M partially reverses the inhibition. Increasing the level of Mg^{2+} still further, fails to regain maximal activity, and thus this inhibition seems to be due to high salt concentration rather than an unfavourable balance between K^+ and Mg^{2+}.

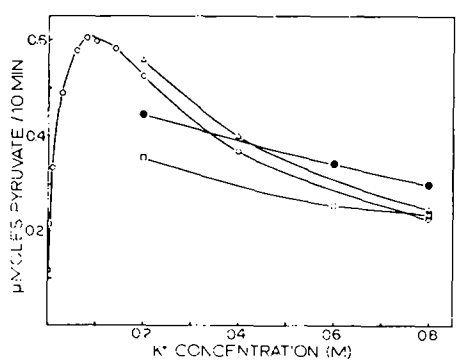
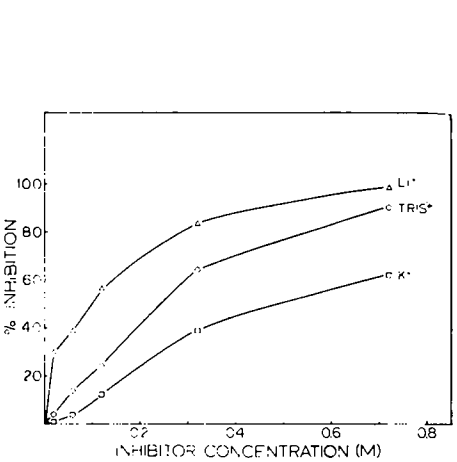


Fig. 1. The effect of high concentrations of K^+ and Mg^{2+} on the activity of pyruvate kinase. Each reaction mixture contains in a volume of 1 ml: $2.25 \cdot 10^{-3}$ M ADP; $1.5 \cdot 10^{-3}$ M phosphoenolpyruvate; 0.022 M Tris HCl (pH 7.4); 0.05 μg protein; KCl and $MgCl_2$ as shown. The reaction temperature was 37° and the reaction time was 10 min. Mg^{2+} molarity: ○, 0.008; △, 0.016; ●, 0.032; □, 0.096.

Any specific inhibitory effect of Tris must be contrasted therefore with the effect of K^+ at high concentration. Fig. 2 shows the results of adding levels of KCl, Tris–HCl at pH 7.4 or LiCl to reaction mixtures containing the optimal cation concentrations established in Fig. 1. It is seen that under these conditions both Tris and Li^+ inhibit more strongly than do equivalent supra-optimal concentrations of K^+.

To investigate the type of inhibition by $Tris^+$, low levels of this cation were used in an attempt to avoid the complicating effects of high salt concentration. Fig. 3



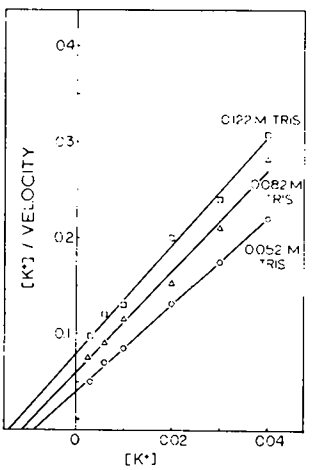


Fig. 2. Inhibition of pyruvate kinase by univalent cation chlorides. Conditions were as in Fig. 1 except that the reaction mixtures contained standard concentrations of 0.008 M $MgCl_2$; 0.08 M KCl. To these reaction mixtures were added KCl, Tris-HCl (pH 7.4) or LiCl as shown. The uninhibited system produced 0.4 μmole pyruvate per 10 min.

Fig. 3. The effect of Tris on the activation of pyruvate kinase by K^+. Reaction conditions were as in Fig. 1 except for the following: 0.008 M $MgCl_2$; 0.035 μg protein; KCl and Tris–HCl (pH 7.4) as shown. Plots are given as K^+ concentration/velocity *versus* K^+ concentration with velocity expressed as μmoles pyruvate formed per 10 min. Apparent K_a for K^+ at the various Tris levels are as follows: 0.052 M Tris, 0.009 M; 0.082 M Tris ,0.0155 M; 0.122 M Tris, 0.014 M.

presents plots of K^+ concentration/velocity *versus* K^+ concentration at three different levels of Tris–HCl. The results show that the inhibition by Tris is due largely to a competition with K^+. The plots however do not have a common slope and therefore the inhibition by Tris exhibits both competitive and non-competitive characteristics.

When Tris is being used to adjust and maintain the pH of the reaction mixture it is impossible to obtain an accurate estimate of K_a from such plots as Fig. 3. If $K_p = K_a$ in the presence of inhibitor I, then plotting the various K_p's against their corresponding I's and extrapolating to zero inhibitor concentration for the data in Fig. 3, a tentative value of 0.005 M is obtained for the K_a of K^+ when no Tris is present in the pyruvate kinase reaction mixture.

Similar investigations to those in Fig. 3 have been conducted using K^+-activated yeast acetaldehyde dehydrogenase which does not require a divalent cation in addition to a monovalent cation for activity. Essentially similar results are obtained as those for pyruvate kinase. While not offering a complete explanation of the effect of Tris, it would seem that its action in the pyruvate kinase and acetaldehyde dehydrogenase

reactions is in large part due to a competition with activating K^+. When Tris is used as the buffer in studies of these enzymes a K_a for K^+ will be obtained which can vary simply as a result of varying buffer concentration. Thus in the pyruvate kinase system, a K_a for K^+ of 0.011 M has been reported[4] using a Tris buffer concentration of 0.04 M. As can be seen from Fig. 3, a K_a for K^+ both higher and lower than 0.011 M has been obtained simply by varying the Tris concentration.

In order to assess the possible physiological significance of enzyme activation by univalent cations it is essential to obtain accurate data for saturation characteristics of these enzymes with K^+. It is clear that in obtaining such data for pyruvate kinase, acetaldehyde dehydrogenase and perhaps other K^+-activated enzymes, the buffer system used for assays must be accurately defined, and actively considered in the interpretation of results.

This investigation was supported in part by Research Grant AMo 8123 from the U.S. Public Health Service.

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Received April 29th, 1968

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* This is technical paper No. 2391 of the Oregon Agricultural Experiment Station.

BBA 63329

Rat liver glycine acyltransferase: Partial purification and some properties

Glycine acyltransferase (acyl-CoA:glycine *N*-acyltransferase, EC 2.3.1.13) catalyzes the third and final step in one of the detoxication pathways for benzoic acid and similar substances[1]. Its specificity is indicated by its systematic name, and it is found in the mitochondrial fraction of liver and kidney[2]. In the course of studying the activity of the enzyme in developing rat liver[3], partial purification and characterization were performed.

Adult rats of the Sprague–Dawley strain were killed by decapitation and exsanguination. The livers were homogenized in 9 vol. of cold 0.25 M sucrose, and the mitochondria were isolated by differential centrifugation, washed 4 times and made