Yeast Pyruvate Kinase

II. KINETIC PROPERTIES

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SUMMARY

The kinetic properties of purified yeast pyruvate kinase were investigated. The enzyme showed cooperative kinetics toward the essential activating monovalent cations K⁺ and NH₄⁺, Mg²⁺, and phosphoenolpyruvate. Fructose 1,6-diphosphate, which yielded homotropic cooperative kinetics and did not affect maximal velocity, transformed the sigmoidal kinetics of K⁺ and NH₄⁺, Mg²⁺, and phosphoenolpyruvate to hyperbolic and lowered the apparent Kₘ for each variable. Adenosine diphosphate, however, exhibited no cooperativity and was relatively unaffected by fructose 1,6-diphosphate. The enzyme displayed a complex velocity dependence on pH.

Pyruvate kinases from a variety of sources including brewers' yeast, rat and mouse liver, and rat adipose tissue (1-4) have been shown to exhibit sigmoidal kinetics and activation by fructose 1,6-diphosphate. The homogeneous bakers' yeast pyruvate kinase preparation of Hunsley and Suelter (5) in the preceding paper of this series was examined kinetically.

EXPERIMENTAL PROCEDURE

Materials—Yeast pyruvate kinase of a minimum specific activity of 150 μmoles per min per mg at 30°C was used in the kinetic experiments reported in this paper and was prepared according to the method of Hunsley and Suelter (5). Crystalline rabbit muscle aldolase, tetracyclohexylammonium FDP,¹ and barium FDP were from Sigma, and rabbit muscle or-glycerophosphate dehydrogenase-triose phosphate isomerase mixed crystals were Calbiochem products. (CH₃)₄NCl from Aldrich was recrystallized from absolute ethanol. All other reagents were purchased as described previously (5). Barium FDP was converted to the (CH₃)₄N⁺ salt with [(CH₃)₄N]₂SO₄ prepared from (CH₃)₄NOH. ADP and PEP concentrations were estimated by a modification of the Buecher and Pfleiderer (6) pyruvate kinase assay in the presence of excess pyruvate kinase isolated from frozen rabbit muscle (Pel-Freeze Biologicals) by a modification (7) of the Tietz and Ochoa (8) procedure. FDP was estimated in the presence of excess aldolase as modified from the assay of Rutter et al. (9).

Methods—Yeast pyruvate kinase solutions were assayed for protein concentration by the extinction coefficient, E₂₈₀ = 0.653 at 280 nm, and linked activity assays of the enzyme were performed with lactic dehydrogenase free of (NH₄)₂SO₄ as previously described (5) on enzyme dilutions in 50% (v/v) aqueous glycerol containing 10 mM Na phosphate, pH 6.5. Extraneous alkali metal and ammonium ions in pyruvate kinase assay mixtures were estimated to be 300 μM in Na⁺ from NADH and less than 100 μM in NH₄⁺ from enzyme additions. The reaction was initiated in all experiments by addition of enzyme to 1.00-ml total volume of reaction mixture at 30°C. Concentrations of reagents are given in each appropriate diagram. In all kinetic experiments except in those with FDP as a variable, the substrate or activator under examination was added to a system in which all other catalytic variables were at saturating or near saturating levels. In studies involving pH changes, measurements were made directly on reaction mixtures immediately after assay with a Sargent model LS pH meter fitted with a Sargent 8-30070-10 unit electrode.

Kinetic data were treated as Hill plots (10), plotting on the abscissa total concentrations of added substrate or activator. Lines through the points were drawn by eye and the value of the slopes noted are the actual slopes and do not indicate the limits of accuracy of the experiment. The apparent Kₘ is defined as that concentration of substrate or activator where v = ½ maximal observed velocity.

RESULTS

Yeast pyruvate kinase exhibited a requirement for alkali metal and ammonium ions. In Table I the monovalent cation activating effects are illustrated. K⁺ and NH₄⁺ were activators both in the presence and absence of FDP. Na⁺, however, was effective only in the presence of FDP. Enzyme with FDP alone or with high levels of (CH₃)₄NCl exhibited no activity that could not be accounted for by cationic contaminants.

The effects of activating monovalent cation concentrations on

¹ The abbreviations used are: PEP, phosphoenolpyruvate; FDP, fructose 1,6-diphosphate.
TABLE I

Requirement of yeast pyruvate kinase activity for alkali metal or ammonium ions

The assay mixture (1.0 ml) contained 100 μmoles of (CH₃)₄N cacodylate, pH 6.2; 24 μmoles of MgCl₂; 5 μmoles of PEP; 10 μmoles of ADP; 33 μg of lactic dehydrogenase; and 0.15 μmole of NADH. FDP was added as the (CH₃)₄N⁺ salt.

<table>
<thead>
<tr>
<th>Reagents added</th>
<th>Final concentration</th>
<th>Initial velocities</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDP</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>(CH₃)₄NCl</td>
<td>50</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>(CH₃)₂NCl</td>
<td>200</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>(CH₃)₂NCl, FDP</td>
<td>50, 1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>50</td>
<td>0.82</td>
</tr>
<tr>
<td>NaCl</td>
<td>500</td>
<td>0.48</td>
</tr>
<tr>
<td>NaCl, FDP</td>
<td>170, 1.0</td>
<td>155</td>
</tr>
<tr>
<td>KCl</td>
<td>170</td>
<td>72</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>NH₄Cl, FDP</td>
<td>30, 1.0</td>
<td></td>
</tr>
</tbody>
</table>

The initial velocity are given in Fig. 1. With FDP, Na⁺, K⁺, and NH₄⁺ cooperativity was greatly reduced with Hill slopes, n, of 1.40, 1.08, and 1.30, respectively. Without FDP, n for K⁺ and NH₄⁺ was 2.85 and 3.80. The pattern of FDP reducing both Hill slopes and apparent Kₘ values will be noted in all remaining kinetic variables except ADP.

K⁺- and NH₄⁺-dependent FDP activation of the enzyme (Fig. 2) was cooperative with n equal to 2.33 and 2.55, respectively, both at necessarily low concentrations of monovalent cation. Na⁺-dependent FDP activation gave a Hill slope for FDP near unity. Lowering the concentration of Na⁺ did not increase n for FDP, thus demonstrating a second dissimilarity in Na⁺ dependence (the first was the lack of activation without FDP) and emphasizing the necessity for stringently defining monovalent ion species in kinetic studies of this enzyme.

Fig. 3 gives Hill plots with PEP as a variable. Similarly to monovalent cation kinetics, n was lowered from 2.85 (K⁺) and 2.94 (NH₄⁺) to 0.94 (K⁺-FDP) and 1.09 (NH₄⁺-FDP). FDP effectively abolished all cooperativity toward PEP and lowered the apparent Kₘ by an order of magnitude or more. Fig. 4 presents the relationship between Mg²⁺ concentration and ac-
FIG. 4. Relationship between initial velocity of yeast pyruvate kinase and total MgCl₂ concentration. The assay mixture (1.00 ml) contained 100 μmoles of \((\text{CH}_2)_2\text{N}\) cacodylate, pH 6.2; 24 μmoles of MgCl₂; 9.0 μmoles of PEP; 1.0 μmole of \((\text{CH}_2)_2\text{N}\) FDP (when present); 33 μg of lactic dehydrogenase; 0.15 μmole of NADH; left: ●●●●, 120 μmoles of KCl; ○○○○, 100 μmoles of KCl; and □□□□, 170 μmoles of NaCl; and right, ●●●●, 50 μmoles of NH₄Cl and ○○○○, 30 μmoles of NH₄Cl. Hill slopes for the curves are shown in parentheses.

FIG. 5. Relationship between initial velocity of yeast pyruvate kinase and total ADP concentration. The assay mixture (1.00 ml) contained 100 μmoles of \((\text{CH}_2)_2\text{N}\) cacodylate, pH 6.2; 24 μmoles of MgCl₂; 9.0 μmoles of PEP; 1.0 μmole of \((\text{CH}_2)_2\text{N}\) FDP (when present); 33 μg of lactic dehydrogenase; 0.15 μmole of NADH; left: ●●●●, 120 μmoles of KCl; ○○○○, 100 μmoles of KCl; and □□□□, 170 μmoles of NaCl; and right, ●●●●, 50 μmoles of NH₄Cl and ○○○○, 30 μmoles of NH₄Cl. Hill slopes for the curves are shown in parentheses.

FIG. 7. The effect of pH and PEP concentration on the activity of yeast pyruvate kinase. The assay mixture (1.00 ml) contained 100 μmoles of Tris-acetate buffer; 24 μmoles of MgCl₂; 10 μmoles of ADP; 33 μg of lactic dehydrogenase; and 0.15 μmole of NADH. In addition, the top curves contained 170 μmoles of NaCl; 1 μmole of tetracyclohexylammonium FDP, the middle curves 100 μmoles of KCl and 1 μmole of tetracyclohexylammonium FDP, and the bottom curves 180 μmoles of KCl; ○○○○, 10 μmoles of PEP; ●●●●, 0.10 μmole of PEP; ○○○○, 0.50 μmole of PEP; □□□□, 0.10 μmole of PEP; and ▽▽▽▽, 0.30 μmole of PEP.
tivity. Again, both $n$ and the apparent $K_m$ were decreased by the inclusion of FDP with the Na+-dependent system yielding an intermediate cooperativity.

ADP, however, displayed linear or nearly linear kinetics (Fig. 5) regardless of the presence of FDP at saturating levels of Na$^+$, K$^+$, or NH$_4^+$. Also, only small differences were seen in the apparent $K_m$ for this substrate.

Under fully saturating conditions, FDP was shown to have no effect within experimental error on the observed maximal velocity of the reaction with K$^+$ and NH$_4^+$ as activating cations (Fig. 6). The case for Na$^+$ alone is pointed out in Table I.

Finally, in Fig. 7 are plotted the pH profiles of the K$^+$, K$^+$-FDP, and Na$^+$-FDP systems at varying levels of PEP. Activity fell off rapidly in all three systems on the acidic sides of the curves. The basic sides displayed complex results with several reproducible discernible shoulders. In addition, the inclusion of FDP at low concentrations of PEP broadened the maxima in the basic pH range.

**DISCUSSION**

Like rabbit muscle pyruvate kinase (11) and other pyruvate kinase preparations (12), this yeast enzyme required an activating monovalent cation for activity, a property observed of an earlier unstable preparation from bakers' yeast (13). In contrast to the muscle enzyme, which Na$^+$ weakly activates (11), this yeast enzyme responds only to the combination of FDP and Na$^+$. Hess and Haeckel (14) claimed FDP activation in the absence of activating monovalent cations, but the levels of cations in their experiments were not clearly defined. A probable explanation of the difference in these results is the presence of Na$^+$ from ADP additions in their experiments. However, this and other differences may reflect the source, Saccharomyces cerevisiae, as against the S. cerevisiae enzyme used in the experiments presented in this paper. Maeba and Sanwal (15) have reported that Escherichia coli pyruvate kinase is unaffected by K$^+$.

The sigmoidal to hyperbolic transition toward K$^+$ and NH$_4^+$ due to FDP was noted previously (14), but maximal velocity differences were found only for the K$^+$ system and not for the NH$_4^+$ system. We found no $V_{max}$ differences induced by FDP under saturating conditions of substrate and Mg$^{2+}$ and suggest that this enzyme is best classified as a K system in the nomenclature of Monod, Wyman, and Changeux (16).

Pogson (4) demonstrated that FDP did not change the cooperativity of K$^+$ or Mg$^{2+}$ kinetics in an FDP-activated form of rat adipose tissue pyruvate kinase. The rabbit muscle enzyme displays normal kinetics toward cations, but Suelter et al. (17) found the binding of both monovalent and divalent cations, studied exclusively of each other, to be cooperative.

The effect of FDP on PEP kinetics in pyruvate kinase from several sources including rat and mouse liver, rat adipose tissue, brewers' yeast, and E. coli (14, 2, 18, 3, 19, 15) has been shown to be similar to the effect with this preparation. The Hill slopes and apparent $K_m$ values for PEP with the K$^+$ and K$^+$-FDP activated enzyme agree closely with the data for the brewers' yeast preparation of Haeckel et al. (1). The apparent $K_m$ values for Mg$^{2+}$ kinetics are similar also, but this bakers' yeast enzyme displayed higher cooperativity. ADP at saturating levels of substrates and cations appeared to be only weakly controlled by FDP, an observation made by Pogson (4) with rat adipose pyruvate kinase.

An ionizable group near the active center in the PEP-enzyme complex has been hypothesized (1) as an explanation of the sharp pH profile at low concentrations of PEP with FDP absent. The effect of this group was abolished when FDP was bound, resulting in a broad pH profile.

The kinetic studies presented in this paper involve dilutions of the enzyme by at least a factor of 10$^6$ over the concentration in whole yeast cells. The note of Srere (20) cautioning extrapolation of kinetic observations in vitro to function in vivo may be reasonably dismissed since the control characteristics of yeast pyruvate kinase have been observed in glycerolizing yeast suspensions and were first noted by Hommes (21).

**REFERENCES**
