Activators of Yeast Hexokinase*

DAVID P. KOSEW AND IRWIN A. ROSE‡

From the Institute for Cancer Research, 7701 Dunholme Avenue, Fox Chase, Philadelphia, Pennsylvania 19111

SUMMARY

The rate of the ATP:glucose transphosphorylase reaction catalyzed by yeast hexokinase, isozyme PII, is inhibited by lowering the pH of the reaction below 7.0, especially at suboptimal concentrations of ATP. This effect of acidity is largely overcome by activators such as orthophosphate, citrate, malate, 3-phosphoglycerate, and ribose triphosphates. Thus, in the acid range, ATP appears to serve both as an activator and a substrate with the result that l/v versus [ATP] plots are nonlinear. It appears that yeast hexokinase may exist as two conformational isomers, an inactive form which is favored in the acid range and an active form favored by various polyanions or by alkaline pH.

The conversion of inactive to active enzyme by citrate is slow, requiring about 1 min at 25 °C when citrate is added to the reaction mixture after the substrates. When citrate is added to the enzyme simultaneously with both substrates, the reaction begins at the fully activated rate indicating that only the ternary complex can undergo the conversion to inactive enzyme. Equilibrium isotope exchange experiments indicate that citrate activates the glucose 6-phosphate, ADP ⇌ ATP, and glucose-6-P ⇌ ATP exchanges equally. This is consistent with the idea that the ternary complex is undergoing the allosteric interconversions.

Native hexokinase assayed inside semipermeable yeast cells exhibits the same kinetic properties as the isolated native enzyme, indicating that the activation phenomena may be important in controlling the rate of hexokinase in vivo.

RESULTS

Effect of pH on MgATP Dependence of Yeast Hexokinase—Most previous reports on the MgATP dependence of yeast hexokinase have been limited to the alkaline range of pH where the activity is maximal and the initial velocity curves are strictly hyperbolic (1, 4–7). A recent report by Bohnensack and Hoffmann (13), in which measurements with commercially available enzyme were extended to pH 6.3, did not disclose any change from this pattern. However, as shown in Fig. 1A, there is a significant change to complex behavior when the pH is lowered in a study with the PII isozyme obtained from Dr. S. P. Colowick. Qualitatively similar results were obtained with the PI isozyme obtained from Dr. S. P. Colowick and with the enzyme obtained from Boehringer Mannheim. All experiments reported in this paper were performed with the PII isozyme. As seen in Fig. 1A, two "hyperbolic" regions were observed at pH 7.3 and below, presumably indicative of negative cooperativity (14). As expected, a double reciprocal plot of these data shown in Fig. 1B is nonlinear. It is evident from the double reciprocal plots shown in...
by further addition of MgATP. The question then arises which is indicative of a comparable effect of pH on the magnitude of the K_m of ATP and the V_max for the first hyperbolic region of the initial velocity plot (Fig. 1A). Thus, in this region, where there is no demonstrable cooperativity, the effect of pH on the extrapolated low ATP V_max values indicates the inhibition constant of H^+ as an uncompetitive inhibitor with respect to ATP as the variable substrate. A plot of such values, V_max', as a function of pH (Fig. 2A) over the range pH 6.6 to 8.0, indicates a rather sharp effect of pH. Fig. 2B is a replot of these data in order to examine the sensitivity to H^+ concentration, according to Footnote 1. The results clearly show that two protons are required for inhibition with a mean pK of 7.3. A working hypothesis would be that protonation of two sites on the enzyme causes a shift of one or more of the reaction intermediates to an inactive form and that this shift can be prevented by further addition of MgATP. The question then arises whether MgATP is acting as a substrate at a second reaction site, in which case the kinetics could be interpreted as an example of negative cooperativity, or whether the additional ATP functions as an activator. If the latter, then other activators might exist and a search for allosteric activators was undertaken.

**Effectors of Yeast Hexokinase**—Table I lists compounds which have been tested as effectors of yeast hexokinase at pH 8.0 and pH 6.6. Several observations are contained in this table. None of the compounds tested activate at pH 8.0. Several compounds, such as ADP, β,γ-methylene ATP, and GTP inhibit at pH 8.0, presumably in large part competitively with respect to ATP, but these are nevertheless potent activators at pH 6.6. In no case is the activated rate at pH 6.6 greater than the rate

<table>
<thead>
<tr>
<th>Effectors</th>
<th>pH 6.6</th>
<th>pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>33.3</td>
<td>85.3</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>60.6</td>
<td>94</td>
</tr>
<tr>
<td>P-enolpyruvate</td>
<td>21</td>
<td>104</td>
</tr>
<tr>
<td>L-Malate</td>
<td>54</td>
<td>104</td>
</tr>
<tr>
<td>d-Malate</td>
<td>44.6</td>
<td>91</td>
</tr>
<tr>
<td>Succinate</td>
<td>37.6</td>
<td>91</td>
</tr>
<tr>
<td>Citrate</td>
<td>33.3</td>
<td>91</td>
</tr>
<tr>
<td>Tricarbarylactate</td>
<td>16</td>
<td>75.5</td>
</tr>
<tr>
<td>AMP</td>
<td>16</td>
<td>75.5</td>
</tr>
<tr>
<td>GTP</td>
<td>48</td>
<td>52.5</td>
</tr>
<tr>
<td>CTP</td>
<td>60.8</td>
<td>87.1</td>
</tr>
<tr>
<td>UTP</td>
<td>23.9</td>
<td>93.5</td>
</tr>
<tr>
<td>β,γ-Methylene ATP</td>
<td>34.3</td>
<td>76.5</td>
</tr>
<tr>
<td>P_i</td>
<td>27</td>
<td>91</td>
</tr>
<tr>
<td>SO_4^-</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>EDTA</td>
<td>35</td>
<td>98</td>
</tr>
</tbody>
</table>
FIG. 3. Effect of pH on the activation of yeast hexokinase by Pi. Conditions were the same as those in Fig. 1, except that MgATP was held constant at 0.2 mM and the buffers were 2-(N-morpholino)ethanesulfonic acid, pH 6.6 (□), and H-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, pH 7.0 (○), pH 7.18 (■), and pH 7.4 (□).

obtained at pH 8.0 without additions. Although P1 activates, SO₄²⁻ does not; citrate activates but tricarboxylate does not; 3-phosphoglycerate is more effective than 2-phosphoglycerate or P-enolpyruvate; and succinate is not an activator, while malate is one of the better activators.

CTP, at best, is a very poor phosphoryl donor for yeast hexokinase under conditions where it stimulates ATP-dependent phosphorylation of glucose. Thus CTP must be acting as an activator. It is reasonable to propose that the effect seen with high levels of ATP (Fig. 1) is an allosteric one also.

Activation by P₁—The finding that P₁ activates yeast hexokinase is of interest, since P₁ has been shown to overcome glucose-6-P inhibition of mammalian hexokinases (15). In addition, Schulze and Colowick (10) have shown an effect of P₁ on the state of aggregation of the yeast enzyme. It was with these two facts in mind that an effect of P₁ on the pH dependence of the yeast enzyme was tested.

Fig. 3 shows the effect of pH on the activation of yeast hexokinase by P₁. The Kₐ for activation is dependent on the pH and increases from 1 mM to 4 mM as the pH decreases from 7.2 to 6.6, perhaps indicating that it is the dianion form of phosphate that binds to the activator site.

Activation by Citrate—Citrate differs from Pi in that its Kₐ for activation does not change with pH. The Kₐ for citrate is 0.06 mM at 0.4 mM ATP in the pH range of 6.0 to 7.0 (Fig. 4). Since a linear double reciprocal plot is obtained, it appears that only 1 eq of citrate activates the reaction between a proton and the citrate concentration. Malate and 3-phosphoglycerate also have Kₐ for activation of 0.06 mM over this pH range. Note that in Fig. 4 the maximum activation by citrate at a constant pH decreases below pH 6.6 where one of the carboxyl groups of citrate becomes protonated. Fig. 5 shows that at pH 6.5, a double reciprocal plot with MgATP as the variable substrate becomes less biphasic as more citrate is added. At 1 mM citrate, the plot is nearly linear and the Kₐ for MgATP is 0.22 mM, compared with the previously reported value of 0.29 mM at pH 8.0 (7).

Double reciprocal plots with glucose as the variable substrate are linear at all pH values that have been examined, that is, pH 6.0 to 8.0. Fig. 6 shows an experiment at pH 6.6 with and without 1 mM citrate. Almost no activation is seen at low glucose levels. This is consistent with the idea that the conversion to the inactive form with H⁺ requires that the enzyme be occupied by both substrates; thus, at glucose levels well below the Kₐ at pH 6.6 there is little activation by citrate because very little of the enzyme is in the inhibited state. The fact that citrate raises both V_max and Kₐ of glucose to the same extent (6-fold) indicates that citrate increases the concentration of both E and E·glucose to the same extent in the reaction path (16).

In order to test whether particular steps of the hexokinase reaction pathway were stimulated by activator, the isotope ex-
Fig. 5. Effect of citrate on yeast hexokinase. Conditions were the same as Fig. 1, except that buffer was 2-(N-morpholino)ethanesulfonic acid, pH 6.6, and citrate (cit.) was added as indicated.

Fig. 6. Velocity of yeast hexokinase as a function of glucose concentration. Conditions were the same as Fig. 1, except that MgATP was held constant at 0.05 mM and the buffer was 2-(N-morpholino)ethanesulfonic acid, pH 6.6.

earlier conclusion of alternate products release pathways in which the rates of release cannot be considered rapid compared with the phosphoryl transfer step. It will be noted that the exchange rates reported in Table II exceed the initial rate of forward reaction measured under the same conditions except for the absence of ADP and glucose-6-P. This result, which was repeatedly observed, is interpreted to indicate activation by the high concentration of glucose-6-P necessary to establish the equilibrium.

Activators as Metal Chelators—The activation by EDTA seen in Table I naturally suggests that the removal of an inhibitory metal ion may be responsible for the effect of the activators. On the other hand, EDTA may be acting as a poor analogue of malate or citrate by virtue of its polycarboxylic structure. A number of experiments were designed to evaluate the first possibility. Several metal ions at 0.1 mM were tested for inhibition of the assay at pH 6.6 in the presence of glucose (1 mM), ATP (0.05 mM), and MgCl₂ (5 mM). No inhibition greater than 20% was evident with Co²⁺, Ca²⁺, Cd²⁺, Ni²⁺, Zn²⁺, Cu²⁺, and Fe²⁺, while Mn²⁺ gave a 50% inhibition. None of these effects is sufficient to explain the over 3-fold stimulation of the hexokinase observed in the presence of citrate, 3-phosphoglycerate, malate, or GTP (Table I). A number of procedures lead to a doubling of the unactivated rate at pH 6.6 compared to the control but have no effect at pH 8.0; these include treatment of all the chemical reagents except MgCl₂ with Chelex-100 or with 8-hydroxyquinoline according to the procedure described under "Methods" or simply upon purification of the ATP on a DEAE-\(\text{HCO}_3\) column (18). However, in all these cases, further activation was obtained with 3-phosphoglycerate, citrate, or EDTA.
**Effect of 8-hydroxyquinoline on Activation**

All reagents, including the glucose-6-P dehydrogenase and MgCl₂, were treated with 8-hydroxyquinoline as described under "Materials and Methods." Where indicated, hexokinase was also treated. Each tube contained in a final volume of 1 ml, 2-(N-morpholino)ethanesulfonic acid, pH 6.5, 100 mM; glucose, 1 mM; ATP, 0.4 mM; MgCl₂, 5 mM; TPN, 0.5 mM; and glucose-6-P dehydrogenase, 0.5 unit. V₄ and V₅ are velocities in the presence and absence of activator, respectively. The decrease in velocity with the 8-hydroxyquinoline-treated enzyme is the result of shaking the hexokinase solution with chloroform.

<table>
<thead>
<tr>
<th>Hexokinase</th>
<th>(V₄ - V₅)/V₅</th>
<th>V₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>8-Hydroxyquinoline-treated</td>
<td>4.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

In the experiment summarized in Table III, where all reagents, including the enzymes, were treated with 8-hydroxyquinoline, there was good activation by citrate or EDTA. These results, the fact that neither o-phenanthroline nor tricarbalylate activate, and that such weakly complexing agents as 3-phosphoglycerate and malate have activation constants of $6 \times 10^{-5} \text{ M}$, indicate that the activation is not due to removal of a metal ion inhibitor by the activator.

**Time-dependence of Activation**—At acidic pH, the conversion of inactive to active enzyme by citrate is slow, requiring about 1 min to reach completion. However, if substrates are not present until citrate is added, then the reaction begins at the high fully activated rate as shown in Fig. 7. This suggests that the presence of substrates is required for the low pH effect.

That both substrates are required for the low pH effect was demonstrated by previously incubating the enzyme at pH 6.7 with either MgATP or glucose before the simultaneous addition of citrate and the second substrate. In both cases the reaction began at the fully activated rate without a detected lag. The addition of 15% glycerol increases the lag seen when citrate is added following the substrates to about 3 min (Fig. 7). Similarly, when inactive enzyme is converted to active enzyme by raising the pH from 6.7 to 8.0, there is a lag of about 2 min before optimal activity is obtained. Fifteen per cent glycerol increases this lag to over 8 min, as seen in Fig. 8A. In contrast to these results, when the pH is lowered from 8.0 to 6.5 in the presence of both substrates, the decrease in activity is too rapid to follow by the experimental method used (Fig. 8B). Inspection of Fig. 7 reveals that 15% glycerol inhibits the assay about 55% at pH 6.7 and that this inhibition is decreased to 20% by the addition of 0.5 mM saturating citrate. In studies performed at pH 8.0, 15% and 30% glycerol gave 20% and 50% inhibition, respectively, and this inhibition was not reversed by citrate in contrast to the results obtained at low pH. That the observed lags are due to a slow change in aggregation of the hexokinase is ruled out by the fact that enzyme activity with and without citrate is linear with respect to enzyme concentration at pH 5.9 to 8.0 and from 60 to 600 ng of hexokinase per ml (Fig. 9). Thus, it appears that a slow conformational change of the acidic, inactive form to an active form upon the addition of citrate or...
increase of pH is the cause of the observed lag in the activation response.

**Hexokinase Activity in Semipermeable Yeast Cells**—It was considered important to determine whether the kinetic properties demonstrated with the isolated yeast hexokinase isoenzyme might indicate the behavior of hexokinase at its concentration in the milieu of the yeast cell. This question was approached by use of yeast cell "ghost", prepared by the method of Schlenk and Zydek-Cwick (11) in such a way as to be freely permeable to substrate molecules. In this preparation the yeast proteins are retained within a cell wall envelope (19) in what is presumed to be a normal concentration and condition. Preliminary experiments indicated that there was free diffusion of hexokinase substrates and products in and out of the ghosts since no delay in establishing a linear assay rate in the glucose-6-P dehydrogenase-coupled system could be observed. At least half the total hexokinase of the preparation was assayable in this way, as indicated by assays before and after sonic oscillation. The failure to assay all the hexokinase results from incomplete conversion of cells to the "ghost," as shown with the phase contrast microscope. The hexokinase remained in the ghosts during the assay period (10-15 min); however, longer periods of incubation caused leakage of the enzyme. Most of the activators previously studied were tested with the semipermeable yeast preparation. As noted in Table IV, the behavior of the hexokinases of this preparation was very similar to the highly dilute enzyme studied in Table I; citrate and 3-phosphoglycerate were among the best activators; neither SO₄²⁻ nor tricarboxylate activated, and activation occurred only at acid pH. The concentration of hexokinase in the semipermeable yeast cells can be calculated from a determination of the total activity of enzyme per ml of packed cells and assuming an average specific activity of 500 units per mg of pure enzyme (10). A value of 44 μg per ml of cells was found. This is about 100 times the highest concentration used in the in vitro studies with purified enzyme.

An alternative explanation for the effect of activators such as citrate in the experiment with the semipermeable yeast might be that normally a portion of the glucose-6-P formed by hexokinase reacts with endogenous phosphoglucone isomerase and then with phosphofructokinase in competition with the external glucose-6-P dehydrogenase. The effects of citrate in inhibiting the phosphofructokinase would be seen as an increase in TPN⁺ reduction.

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**TABLE IV**

**Effectors of yeast "ghost" hexokinase activity**

Yeast "ghosts" (0.1 mg) were incubated at 25° in a final volume of 1 ml with glucose, 1 mM; ATP, 0.5 mM; MgCl₂, 5 mM; TPN, 0.5 mM; glucose-6-P dehydrogenase, 0.5 unit, and either 2-(N-morpholino)ethanesulfonic acid, pH 6.0 or 2,2',2"-nitrilotriethanol, pH 8.0, 100 mM.

<table>
<thead>
<tr>
<th>Additions (1 mM)</th>
<th>Relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6.0</td>
</tr>
<tr>
<td>None</td>
<td>29</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>48</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>56</td>
</tr>
<tr>
<td>P-enolpyruvate</td>
<td>38</td>
</tr>
<tr>
<td>L-Malate</td>
<td>41</td>
</tr>
<tr>
<td>Citrate</td>
<td>52</td>
</tr>
<tr>
<td>Tricarboxylicate</td>
<td>23</td>
</tr>
<tr>
<td>ADP</td>
<td>31</td>
</tr>
<tr>
<td>EDTA</td>
<td>36</td>
</tr>
<tr>
<td>β,γ-Methylene ATP</td>
<td>43</td>
</tr>
<tr>
<td>Pi</td>
<td>38</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>26</td>
</tr>
</tbody>
</table>

**TABLE V**

**Effect of citrate and 3-phosphoglycerate on phosphorylation of 2-deoxyglucose-3H by yeast ghost**

Each tube contained 2-(N-morpholino)ethanesulfonic acid, pH 6.5, 50 mM; MgCl₂, 5 mM; and ATP, 0.2 mM in a final volume of 0.5 ml. In the experiment with 0.11 mM 2-deoxyglucose-3H, the specific activity was 1.5 x 10⁶ cpm per amole and 0.2-mg ghosts were added. In the experiment with 1.0 mM 2-deoxyglucose-3H, the specific activity was 4.2 x 10⁶ cpm per amole and 0.5-mg ghosts were added. Tubes were incubated 10 min at 25°.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Product</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1, 0.11 mM 2-deoxyglucose-3H</td>
<td>None</td>
<td>1223</td>
</tr>
<tr>
<td></td>
<td>0.4 mM 3-phosphoglycerate</td>
<td>1232</td>
</tr>
<tr>
<td></td>
<td>0.4 mM citrate</td>
<td>1000</td>
</tr>
<tr>
<td>Experiment 2, 1.0 mM 2-deoxyglucose-3H</td>
<td>None</td>
<td>1839</td>
</tr>
<tr>
<td></td>
<td>0.4 mM 3-phosphoglycerate</td>
<td>3410</td>
</tr>
<tr>
<td></td>
<td>0.4 mM citrate</td>
<td>3248</td>
</tr>
</tbody>
</table>

Fig. 9. Effect of enzyme concentration on the rate of hexokinase in the presence and absence of citrate. Conditions same as Fig. 7. Filled symbols have no citrate and open symbols have 0.5 mM citrate. Buffers used were: 2(N-morpholino)ethanesulfonic acid, pH 5.9 (●, ○); 2(N-morpholino)ethanesulfonic acid, pH 6.8 (■, □); and TEA, pH 8.0 (▲, △).
and hence as an apparent activation of hexokinase. A further
work performed was that not subject to this possibility;
2-deoxyglucose-3H was incubated with yeast ghosts as in Table
V. The extent of phosphorylation by the ghosts was determined
as radioactivity that was retained on an anion exchange column
after removal of cells and ghosts by centrifugation. As was also
seen with purified hexokinase, activations by citrate and 3-phos-
phoglycerate are apparent only at the higher concentrations of
sugar substrate. Since identical results were obtained with both
the isotopic and spectrophotometric assays, it is evident that in
the ghosts, citrate is activating hexokinase directly.

**DISCUSSION**

Two central questions arise from the present studies: what is
the mode of action of the several activators at the molecular level
and what is the importance of these effects in the normal func-
tion of hexokinase in the cell? With respect to both questions,
future work is certainly needed. Attempts to show direct in-
teraction of citrate with yeast hexokinase by equilibrium dialysis
have not been successful. However, the inability to carry out
prolonged dialysis in the presence of both glucose and ATP limits
the value of this experiment. Likewise, the failure to observe
fluorescence changes with citrate and 3-phosphoglycerate is in-
conclusive and the implication from the kinetic studies that the
activators interact with an intermediate of the reaction sequence
limits the possibilities for a number of comparable tests such as
effects of activators on the stability of the enzyme toward ther-
mal or proteolytic denaturation.

However, the argument that these interactions are indirect,
through the chelation of a metal ion inhibitor, does not seem
strong; the activation is slow and the lag in attaining the acti-
ved rate is not shortened by prior incubation of the enzyme
with activators. Previous treatment of all reagents and en-
mymes with Chelex or 8-hydroxyquinoline did not eliminate the
activator effects, although a 2-fold enhancement of the unacti-
ved rate was seen by either of these treatments or by purifying
the commercial ATP on DEAE columns. On the other hand,
several chelating agents such as tris(ethylenediaminetetra-
acetic acid) and 8-phenanthroline do not activate, while some poor chelating agents such as
3-phosphoglycerate and malate are excellent activators at
10^{-5} M. Finally, the activators affect the K_m of both glucose
and MgATP, as well as the observed velocity, so that activation
is not simply a matter of making more enzyme available in ac-
tive form. Possibly the E-glucose·ATP·Mg complex in the
acid form interacts with a tightly bound metal ion already pres-
ent on the enzyme and this is prevented by interaction of the
bound metal, the enzyme, or both with the activator. Although
the specificity studies imply unusual variety among the activa-
tors, ATP, citrate, malate, Pi, and 3-phosphoglycerate, this is not
without previous example, as in the case of phosphofructokinase,
where a similar range in the nature of effectors is known.

Scheme 1 represents the observations of the effects of pH and
activators on the enzyme-substrates ternary complex. A rep-
resents activators; [Φ], active enzyme with both substrates bound;
[Γ], inactive enzyme with both substrates bound. The equilib-
rium for the allosteric conversion (Step 1) is far toward the
inactive form as judged from the fact that in the unactivated
low pH condition, the enzyme has only a small fraction of the
activity that it has at pH 5.0 (Fig. 2A). Activators react only, or
much more strongly, with the minor, active component of the
equilibrium of acid forms. This interaction is not competitive
with protons, as would appear if A reacted only with Φ since
K_A of citrate is independent of pH. Glycerol inhibits the en-
zyme at all pH values, but part of the inhibition seen at acid pH
seems to be reversed by activators. Thus, the effect of glycerol
appears to be to shift the allosteric equilibrium toward inactive
form. Such an effect may be interpreted to indicate that this
form has more of its polar groups buried (20). The loss of some
functional groups on the enzyme in the inactive form would be
consistent with its failure to interact as well with the polar ac-

The variety in the nature of the activators suggests that dif-
ferent activator sites may be involved for each class of com-
pound, exemplified perhaps by P_i, 3-phosphoglycerate, malate,
citrate, and ATP. This possibility requires further study. pH
is important in determining the range of effectiveness of the
various activators. P_i shows a pH-dependent K_A, whereas
citrate does not. However, in the range where citrate becomes
protonated (pK_a = 6.4), the extent of activation (v - v_o) falls to low values, suggesting that the dianion form binds but
does not activate.

The kinetic behavior of the activators has been interpreted to
mean that they interact favorably only with the active form of
the enzyme. The allosteric conversion to inactive form does
not occur at the free enzyme stage, as shown by the fact that
when either one or both substrates and activator are added
together to enzyme that has been at pH 6.5, the initial rate is
the same as that of the fully activated enzyme, whereas if both
substrates are added prior to activator this rate is achieved only
after a considerable lag. Thus only the ternary complex can
undergo conversion to the inactive form.

The question of physiological role is one that is appropriate in
view of the recent report that phosphofructokinase of yeast does
not show the typical inhibition by ATP at acid pH values (21)
which correspond to those for the yeast cytoplasm. As the
present study shows, the unactivated rate of hexokinase falls
rapidly with [H^+]^{-2} in the neutral region (Fig. 2). Thus it may
be considered that in yeast, hexokinase as well as phos-
phofructokinase is a target for multiple control. It may be of fur-
ther interest to recall that Rothman and Cabib (22) have dem-
strated that various allosteric inhibitors of yeast glycogen
synthetase are much more effective at pH 0 than at pH 7.5.

A role of P_i concentration in the control of yeast glycolysis was
postulated long ago on the basis of its elevation during the aerobic
to anaerobic transition (23-25) when glucose utilization is in-
creased. However, more recent concepts of the control of fer-
mentation in yeast favor a limitation in glucose transport rather
than at the phosphorylation step, based largely on the
very low concentration of intracellular glucose found in cells
under steady state condition (26, 27). Both the very high K_A
of P_i under acid conditions (Fig. 3) and the insensitivity to


\[
\begin{align*}
S &\xrightarrow{2H^+} 1 \\
\text{S}^{-2H^+} &\xrightarrow{1} \text{S}-2H^+ \\
\text{S}^{-2H^+} &\xrightleftharpoons{2H^+} \text{A}
\end{align*}
\]

Scheme 1
activators at low glucose concentration (Fig. 6) tend to discourage the elaboration of simple control schemes at this time. However, the use of the semipermeable yeast cell preparation of Schlenk and co-workers (11, 19) provides an ideal means of determining the kinetic properties of enzymes in concentrations and milieu approaching those of the intact cell, and may contribute further insights into the behavior of hexokinase in the cell.

REFERENCES

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