II. EFFECT OF pH ON ITS ALLOSTERIC BEHAVIOR*

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SUMMARY

Type L pyruvate kinase from rat liver has been further studied. The allosteric properties of the enzyme are strongly affected by changes in the hydrogen ion concentration of the assay medium. At pH values lower than 7, the enzyme obeys Michaelis-Menten kinetics with respect to one of the substrates (phosphoenolpyruvate (PEP)) and cannot be activated by fructose 1,6-diphosphate (FDP). In addition, a cooperative effect of ATP is observed at a low concentration of this substrate.

At pH values higher than 7.2 the enzyme has a sigmoidal response to PEP. This is transformed into a normal hyperbolic relationship in the presence of FDP and the apparent affinity of the enzyme for this substrate increases.

On the other hand, ATP shows cooperative effects at low pH values, at 7.5 in the presence of 0.002 mM FDP, or at high PEP concentration (4 mM).

When the enzyme kinetics was studied within a narrow physiological range of pH (6.8 to 7.25), the homotropic effect of PEP was altered, becoming more sigmoidal as the pH was increased.

It is concluded that small variations in the intracellular pH could be important in the regulation of pyruvate kinase activity by metabolites in vivo.

RESULTS

Stability of Enzyme—As previously reported (5), crude as well as partially purified pyruvate kinase (type L) from mouse liver is highly susceptible to inactivation under a variety of experimental conditions. The loss of activity of the enzyme is partially prevented by addition of 1 mM dithiothreitol under the ammonium sulfate step. The same result was observed with the enzyme from rat liver. Recently, Tanaka et al. (3) reported that the best protection of the pyruvate kinase (type L) could be achieved by the addition of 0.5 mM ADP, 0.5 mM PEP, and 1 mM dithiothreitol.

It was found that the undiluted DEAE-cellulose fraction type L on the concentration of one of its substrates (phosphoenolpyruvate) indicates a multimolecular kinetics, as has been reported in previous papers (4–7). Moreover, in the presence of fructose 1,6-diphosphate the kinetics are in agreement with the Michaelis-Menten equation and the apparent affinity of the enzyme for PEP increases. Recently it has been proposed that the activity of pyruvate kinase type L is modulated through activation by PEP or FDP and inhibition by ATP (7), but relatively little attention has been given to the physiological importance of other types of changes in the environment.

In the preceding paper it was shown that the FDP stimulation is pH dependent (5). In order to gain further insight into the nature of the regulatory mechanism involved, a more detailed study of the influence of pH on the kinetic parameters of hepatic pyruvate kinase (type L) was undertaken.

EXPERIMENTAL PROCEDURE

Materials and methods were as described in the preceding paper (5) unless otherwise indicated. Dithiothreitol, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, and N-tris(hydroxymethyl)methyl 2 aminoethanesulfonic acid were purchased from Calbiochem.

Pyruvate kinase (type L) was purified from rat liver by means of the procedure described for the mouse enzyme (5) with slight modifications. The specific activity of the DEAE-cellulose fractions was essentially similar to that of the mouse enzyme preparations. Pyruvate kinase activity was measured spectrophotometrically by coupling the system with lactate dehydrogenase as already described (8).

Recent reports (1, 2) have shown that there are at least two forms of pyruvate kinase (EC 2.7.1.40) in liver, type M and type L. Tanaka et al. (3) found that the level of the latter is under hormonal and dietary regulation. They also showed that the ratio of type L to type M under normal conditions is approximately 3.

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and then readjusted to 8.15 and 5.9, respectively. The activity of a very narrow pH range variation, in the absence or presence of FDP (Fig. 1A), and this metabolite is necessary for maximal activity even at relatively high PEP concentrations (up to 2.5 mM).

Fig. 1, B and D shows the kinetics at pH 5.9. The substrate saturation curve has a hyperbolic shape, and FDP does not produce any stimulatory effect. Inverse plots of the rate as a function of PEP concentration gave straight lines both in the presence and absence of the effector (FDP).

The same preparation was also tested at a pH value near the optimum of the enzyme (results not shown in the figure). At pH 6.8 there is a very slightly cooperative homotropic effect for PEP in the absence of FDP (n = 1.3).

The data were replotted in terms of the Hill equations (9, 10) and the concentration of PEP at half-maximal velocity (Kₐ) was calculated. The Kₐ for this substrate was 0.3 mm at pH 5.9 and increased to 1.9 mm at pH 8.15. Correspondingly, n, the slope of the Hill plot, was greater at higher pH values; the presence of FDP at pH 8.15 increases the apparent affinity of the enzyme for PEP to values of Kₐ similar to those obtained at pH 5.9.

Effect of Different Buffers—In the experiments depicted in Fig. 1, the pyruvate kinase activity was determined with Tris-maleate as buffer. In order to verify that the pH effect found was independent of the buffer employed in the assay medium, the FDP stimulation on enzyme activity was studied with different buffers.

A low PEP concentration (0.2 mM) was chosen for these experiments, because at this substrate level the FDP effect is magnified, as can be seen in Fig. 1. The data of Table IA show that FDP activation is approximately the same in all of the buffers used, although the activity of the enzyme is somewhat different. Table IB illustrates the effect on pyruvate kinase activity of a very narrow pH range variation, in the absence or presence of FDP. These results also indicate that the FDP stimulation on enzyme activity is a function of pH and it is independent of the nature of the buffers used.

Reversibility of pH Effect—To test the possibility that the lack of sensitivity of the enzyme to FDP at low pH values is not due to destruction of the FDP-sensitive site, two different experiments were carried out. In the first experiment, two aliquots of the enzyme (1 ml) were exposed to pH 5.9 and pH 8.15 and the activity was immediately tested (Table IIA, Lines 1 and 4). Both preparations were kept at 4° for 20 min at each pH value and then readjusted to 8.15 and 5.9, respectively. The activity was again immediately tested (Table IIA, Lines 2 and 5). These results show that the treatment of the enzyme at a high pH value does not alter its property of being insensitive to FDP when measured at the lower pH. In addition, they also show that prior incubation at acidic pH does not result in the loss of FDP stimulation when the enzyme is tested at alkaline pH values. The preparation which was previously incubated at pH 5.9 and then adjusted to pH 8.15 was then kept for 20 min at 4°, the pH was again lowered to 5.9, and the activity was tested in the absence or presence of FDP (Table IIA, Line 3). Again, at the low pH value there is no activation by FDP, although the

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Variation of the activity of pyruvate kinase (type L) with PEP concentration in the presence or absence of 0.1 mM FDP at different pH values. A and B show direct plot; C and D show Lineweaver-Burk transformation. Assay conditions were as described under "Experimental Procedure" with some modifications. The incubation mixture was as follows: 100 mM Tris-maleate buffer (pH 5.9 or 8.15 as indicated), 100 mM KCl, 10 mM MgCl₂, 2 mM ATP, 0.2 mM NADH, excess of commercial lactate dehydrogenase, and different concentrations of PEP. The incubation was carried out at 30°.

<table>
<thead>
<tr>
<th>Buffer 0.1 M</th>
<th>pH</th>
<th>Enzyme activity* FDP</th>
<th>FDP</th>
<th>Activation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-maleate</td>
<td>5.9</td>
<td>23</td>
<td>23</td>
<td>1.00</td>
</tr>
<tr>
<td>Tris-maleate</td>
<td>6.1</td>
<td>67</td>
<td>71</td>
<td>1.00</td>
</tr>
<tr>
<td>Tris-maleate</td>
<td>8.1</td>
<td>0.89</td>
<td>78</td>
<td>88</td>
</tr>
<tr>
<td>Tris-maleate</td>
<td>8.1</td>
<td>1.78</td>
<td>96</td>
<td>54</td>
</tr>
<tr>
<td>Tris-HCl</td>
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<td>0.71</td>
<td>78</td>
<td>110</td>
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<tr>
<td>HEPES</td>
<td>6.8</td>
<td>97</td>
<td>36</td>
<td>1.33</td>
</tr>
<tr>
<td>HEPES</td>
<td>7.2</td>
<td>53</td>
<td>20</td>
<td>4.94</td>
</tr>
<tr>
<td>TES*</td>
<td>54</td>
<td>38</td>
<td>70</td>
<td>1.8</td>
</tr>
<tr>
<td>TES*</td>
<td>54</td>
<td>59</td>
<td>37</td>
<td>1.55</td>
</tr>
<tr>
<td>TES*</td>
<td>54</td>
<td>61</td>
<td>37</td>
<td>1.55</td>
</tr>
<tr>
<td>HEPES</td>
<td>7.2</td>
<td>13</td>
<td>50</td>
<td>3.84</td>
</tr>
</tbody>
</table>

* The pyruvate kinase activity was measured by the standard assay (Fig. 1) except that the concentration of PEP was 0.2 mM and different buffers were used as indicated.

* N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

* N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

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(about 1 mg of protein per ml of solution) in the presence of 25 mM Tris-HCl buffer, pH 7.5, 1 mM dithiothreitol, 0.25 mM sucrose, and 0.15 mM KCl, is stable at 4° for at least 4 days. Its regulatory properties, as far as the ATP inhibition and FDP activation, are preserved.

Dependence of Activity on pH—It was demonstrated in the preceding paper that the FDP stimulation is dependent on the pH of the medium (5); therefore the kinetic properties of the enzyme at different pH values have been studied in more detail. Plots of initial velocities against PEP concentration at a saturating ADP level (2 mM), in the presence or absence of FDP, are shown in Fig. 1. It can be observed that the change in pH produces a marked effect on the substrate cooperative interactions. This effect is maximal at pH 8.15 (Fig. 1A). The same data, when plotted in double reciprocal form, give a non-linear relationship (Fig. 1C). The sigmoidal curve is transformed to a hyperbola of the classical Michaelian type in the presence of FDP (Fig. 1A), and this metabolite is necessary for maximal activity even at relatively high PEP concentrations (up to 2.5 mM).

TABLE I

<table>
<thead>
<tr>
<th>Buffer 0.1 M</th>
<th>pH</th>
<th>Enzyme activity* FDP</th>
<th>+ FDP</th>
<th>Activation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Tris-maleate</td>
<td>5.9</td>
<td>23</td>
<td>23</td>
<td>1.00</td>
</tr>
<tr>
<td>Cacodylate</td>
<td>5.9</td>
<td>67</td>
<td>71</td>
<td>1.00</td>
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<tr>
<td>Tris-maleate</td>
<td>8.1</td>
<td>0.89</td>
<td>78</td>
<td>88</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>8.1</td>
<td>1.78</td>
<td>96</td>
<td>54</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>8.1</td>
<td>0.71</td>
<td>78</td>
<td>110</td>
</tr>
<tr>
<td>B. Tris-maleate</td>
<td>6.8</td>
<td>97</td>
<td>36</td>
<td>1.33</td>
</tr>
<tr>
<td>Tris-maleate</td>
<td>7.2</td>
<td>53</td>
<td>20</td>
<td>4.94</td>
</tr>
<tr>
<td>TES*</td>
<td>54</td>
<td>38</td>
<td>70</td>
<td>1.8</td>
</tr>
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<td>54</td>
<td>59</td>
<td>37</td>
<td>1.55</td>
</tr>
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E. Rozengurt, L. Jiménez de A&á, and H. Carminatti 3143
TABLE II

Reversibility of pH effect on enzyme activity

Experiments A-1 and A-2: The DEAE-cellulose enzyme (pH 7.5) was quickly adjusted to the desired pH (5.9 and 8.15) by adding small volumes of appropriate maleic or Tris solution. Pyruvate kinase activity was measured at each pH as described in Table I; then the enzyme was kept at 4°C for 20 min before subsequent pH changes. The activity of the enzyme was measured at the pH value. For other conditions of the experiment see text. Experiments B-1 and B-2: Same as A-1 and A-2 except that the change of the pH of the enzyme solution was achieved by means of 1.5 h dialysis against the appropriate buffer.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pH treatment of enzyme</th>
<th>Enzyme activity*</th>
<th>Activation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>− FDP</td>
<td>+ FDP</td>
</tr>
<tr>
<td>A-1</td>
<td>7.5 → 5.9</td>
<td>10.5</td>
<td>10.25</td>
</tr>
<tr>
<td></td>
<td>7.5 → 5.9 → 8.15</td>
<td>0.46</td>
<td>15.25</td>
</tr>
<tr>
<td></td>
<td>7.5 → 5.9 → 8.15 → 5.9</td>
<td>5.5</td>
<td>5.0</td>
</tr>
<tr>
<td>A-2</td>
<td>7.5 → 8.15</td>
<td>0.7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>7.5 → 8.15 → 5.9</td>
<td>12</td>
<td>12.8</td>
</tr>
<tr>
<td>B-1</td>
<td>7.5 → 5.9</td>
<td>12.6</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>7.5 → 5.9 → 8.15</td>
<td>0.21</td>
<td>3.8</td>
</tr>
<tr>
<td>B-2</td>
<td>7.5 → 8.15</td>
<td>0.2</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>7.5 → 8.15 → 5.9</td>
<td>12.1</td>
<td>14.7</td>
</tr>
</tbody>
</table>

* Activity was measured at the pH indicated last in first column and is expressed in millimicromoles per min.

activity of the enzyme was markedly decreased. These results would indicate that the effect of pH on the enzyme activity is essentially reversible.

In a second set of experiments the pH was changed by means of dialysis. The results confirmed the previous conclusion (Table II).

Fig. 3. A, effect of ATP concentration on reaction rate of pyruvate kinase (type L) in the absence or presence of FDP. Enzymatic assays were conducted as described in Fig. 1 except that the pH was 7.5 and the concentrations of ADP and PEP were 0.3 mm and 0.5 mm, respectively. The concentration of ATP was varied as indicated. R, the Hill plot of the same data was obtained from the equation given by Jensen and Nester (13). Triangles represent points obtained by interpolation in the corresponding curves of A.

Variation with pH of Apparent K0.5 and Hill's Coefficient for PEP—The effect of pH on the apparent K0.5 and the slope of the Hill plot (n) for PEP is shown in Fig. 2. Although no functional significance can be ascribed to the value of n at this time, a relationship appears to exist between its value and the hydrogen ion concentration in the assay medium. The slope of the Hill plot increases from 1.0 at pH 5.9 to 3.0 at pH 8.35, and this increase is associated with an augmentation in the apparent K0.5 for PEP from 0.3 mm at pH 5.9 to 2.3 mm at pH 8.35. A saturating level of the other substrate (ADP) was present in all of the experiments.

The variation of the apparent K0.5 for PEP with pH may be analyzed as described by Dixon and Webb (12). When tangents having slopes of 0 and 1 are drawn on the curve of −log K0.5 for this substrate as a function of pH (Fig. 2) inset, they intersect near pH 6.9, indicating the involvement of an ionizing group having this pK value. The close correlation between the variation of n and the K0.5 with pH between 5.9 and 8.35 suggests that the ionization of a group having a pK of about 6.9 may be associated with the cooperative interaction for this substrate (PEP). It should be mentioned that different enzymatic preparations were used in the experiments depicted in that figure. However, the above interpretation of Fig. 2 is highly speculative because at present the Dixon and Webb (12) treatment has been shown to apply to hyperbolic systems. As far as we know a similar analytical treatment has not been developed for allosteric systems.

Kinetics of Inhibition by ATP at Different pH Values—Recently Tanaka et al. (7) reported that liver pyruvate kinase (type L) is inhibited by ATP and that the inhibition is reversed by FDP. In the present paper the nature of this inhibition was further studied. Curves of the percentage of activity as a function of ATP concentration in the absence or presence of FDP are given in Fig. 3. In the absence of the activator, the value of the slope of the Hill plot for ATP is 1.0, a typical result for an inhibitor which follows Michaelis-Menten kinetics and does not show cooperative interactions. The presence of 0.002 mm FDP increases the value of n to 2.9. The data suggest that FDP at
low concentrations allows the homotropic cooperativity of the inhibitor to be expressed at a given substrate concentration which otherwise would not occur. Furthermore, the effect of FDP overcoming ATP inhibition is clearly observed at a concentration of 0.1 mM. The results of Fig. 3 also show that in the presence of 0.002 mM FDP, ATP at high concentrations gives an inhibition of only 50% of the control values (in the absence of inhibitor). In addition, the ATP concentration which gave half-maximal inhibition was calculated from the Hill graph. These values were 0.15 mM and 0.9 mM in the absence or presence of FDP, respectively.

In Fig. 4A, the percentage of enzyme activity has been plotted against inhibitor concentration at three different levels of PEP. At low concentration of PEP (0.5 mM) no cooperative effect of ATP is detectable, as was already shown in Fig. 3. On the other hand, at higher substrate levels such as 2 mM and 4 mM, the homotropic interaction of the inhibitor is clearly observed. The slope of the curves in the Hill graph increases from 1.0 at low substrate level to 2.6 at high concentration.

The heterotropic interaction of ATP on the PEP saturation curve is depicted in Fig. 4B. It can be observed that the sigmoid nature of the rate-substrate relationship becomes more pronounced in the presence of ATP. Conversely, when 0.1 mM FDP is simultaneously added with the inhibitor, the response to PEP concentration is transformed into a hyperbolic curve.

The experiments described in Figs. 3 and 4 were performed at pH 7.5. To investigate the dependence of the hydrogen ion concentration of the assay medium on the ATP inhibition kinetics, two different pH values were used to study the effect of increasing concentration of inhibitor on the enzyme activity (Fig. 5). While ATP inhibits the enzyme in both cases, several differences can be noted; the most striking is that the enzyme at the acidic pH value is much less inhibited within a certain range of ATP concentration. Furthermore, cooperative effects are clearly observed at this pH value. In contrast, at pH 8.15 the ATP inhibition curve is a classical hyperbola with 0.15 mM ATP, giving half-maximal inhibition, as was observed at pH 7.5.

Effect of Variations at Physiological pH Range on Enzyme Kinetics—The experiments already described were carried out at two extreme pH values (8.15 and 5.9). In order to get a good knowledge of the importance of the pH effect in vivo, the enzyme kinetics was studied within a narrow physiological range of hydrogen ion concentration (14). The results of such an experiment at three pH values, with varying amounts of PEP and ADP at a fixed level, are shown in Fig. 6. As the pH was raised, the curves became increasingly sigmoidal. At the low pH values (6.8) the plots were more hyperbolic, with a corresponding decrease in susceptibility to FDP activation. In other words, the cooperat...
Fig. 7. Effect of ATP concentration on the reaction rate of type L pyruvate kinase at physiological pH values. The assay conditions were similar to those described in Fig. 5.

Fig. 8. Plot of data from Figs. 1 and 4 as \( \frac{Y}{F} \) with respect to \( \alpha \) (PEP concentration/\( K_{eq} \)) with theoretical lines calculated from the equation of Monod et al. (15) as written in the figure, considering \( n = 3 \) and different values of \( L' \) as indicated. A, data from Fig. 4B. \( \circ \), control without additions; \( \Delta \), in the presence of 2 mM ATP; \( \bullet \), in the presence of 0.1 mM FDP and 2 mM ATP. B, experimental values were obtained at pH 8.35 in the presence (\( \square \)) or absence of 0.1 mM FDP (\( \circ \)). Data from Fig. 1B (at pH 5.9) (\( \Delta \)) is also shown.

The allosteric properties of type L pyruvate kinase are strongly affected by changes in the hydrogen ion concentration of the medium. At pH values lower than 7, the enzyme obeys the Michaelis-Menten kinetics with respect to the substrate (PEP) and cannot be activated by FDP. In addition, a cooperative effect of ATP is observed even at a low concentration of PEP.

Various models have been proposed to account for the kinetic behavior of regulatory enzymes (11, 15-17). Although kinetic data alone are generally insufficient for distinguishing between alternative models of subunit interaction (17), the results presented here indicate that predictions from the model of Monod et al. (16) with respect to the behavior of the enzyme exposed to different combinations of substrates and effectors are usually fulfilled in a qualitative fashion. Because of the limited amount of kinetic data the choice of this model is an arbitrary one, but it provides a working hypothesis on which to predict experiments for the present work.

One of the most important features of this kinetic model is the assumption that the enzyme can exist in two interconvertible forms, R and T, and that the effectors modify the equilibrium between the two states by binding preferentially to one of them. In other words, the nature of the saturation curve for the substrate would be an indication of the statistical distribution of the forms in a particular ionic environment and in the presence or absence of different modifiers.

In the case of pyruvate kinase (type L) it is possible to assume that PEP and FDP cooperate in shifting the equilibrium toward the R state, while ATP would favor the T state. The present paper shows that pH drastically changes the allosteric properties of the enzyme. It is reasonable to suggest that at the acid pH the enzyme exists in the R state, while at alkaline pH it is in the T state. The plausibility of this suggestion is supported by the experiments depicted in Figs. 1 and 4B, in which the influence of pH variation closely resembles the effect of the modifiers (ATP and FDP) on the homotropic interaction of the substrate. According to this model, at the acid pH, the saturation curve for PEP is hyperbolic, FDP has no stimulatory effect, and the ATP inhibition curve shows cooperative interactions (Fig. 5).

Conversely, at high pH where the equilibrium is supposed to be displaced toward the T configuration, the homotropic cooperativity of the substrate is more pronounced and the FDP stimulation at low PEP is maximal. At these pH values, FDP also
alters the shape of the substrate saturation curve from a sigmoid to a hyperbolic pattern, increasing the affinity of the enzyme for PEP, and the ATP inhibition curve is now a classical hyperbola. Moreover, the results reported in Figs. 1B and 4B almost fit the theoretical curves calculated from the equation given by Monod et al. (16) when it is assumed that there are three binding sites for PEP and the apparent allosteric constant L' is varied in each case as indicated in Fig. 6.

In conclusion, it appears that the transition between the two different conformational states of the type L pyruvate kinase can be represented schematically as follows:

\[
\text{Pyruvate kinase}_\text{I} + \text{OH}^- \rightleftharpoons \text{Pyruvate kinase}_\text{II} + \text{H}^+
\]

Such pH influence may provide a great flexibility in the regulation of enzyme activity.

Although the kinetic data indicate that PEP, ATP, and FDP interact in an allosteric manner with the enzyme, further work such as desensitization of the enzyme is needed to prove this model.

The most important implications of the data presented in this paper refer to the physiological function of pyruvate kinase within the cell. In previous communications from this laboratory (5, 18) it has been reported that type L pyruvate kinase from mouse liver is strongly inhibited by Cu\textsuperscript{2+} and that this inhibition is reversed by FDP. In addition it was suggested that the Cu\textsuperscript{2+}-FDP interaction might exert a physiological control over the activity of this enzyme in vivo. Further work on this problem showed that the Cu\textsuperscript{2+} effect is only partially counteracted by FDP. Therefore it appears that this cation has two simultaneous inhibition effects on the enzyme, one that is reversed by FDP and the other which is time dependent and seems irreversible.\(^3\) These results, which are essentially similar to those recently reported by Bailey, Stirpe, and Taylor (19), cast some doubts on the possible physiological role for Cu\textsuperscript{2+} in the regulation of pyruvate kinase. In addition, it is not known whether free Cu\textsuperscript{2+} is available to interact with the enzyme in the hepatic cell.

From the report of Tanaka et al. (7) and the data presented in this paper, it is suggested that the enzyme activity is regulated in vivo by the fluctuations in the level of one of the substrates (PEP), the allosteric inhibitor (ATP), and the positive effector (FDP). Small changes in the concentration of these compounds will produce marked variations in pyruvate kinase activity at a physiological hydrogen ion concentration\(^3\) (14). In addition, small variations in pH within the physiological range can either magnify or lessen the ATP or FDP interactions with the enzyme (Figs. 6 and 7).

It should be mentioned that in all of the experiments performed in the present paper, the Mg\textsuperscript{2+} level was held constant at 10 mm, which was found to be optimum for enzyme activity (5). The presence of this cation in considerable excess over ADP and ATP had the advantage that variations in the adenine nucleotides concentration essentially represented variation in the Mg\textsuperscript{2+}-nucleotide concentration. Nevertheless, in view of the fact that most of the Mg\textsuperscript{2+} within the hepatic cell is in the bound form, such levels of Mg\textsuperscript{2+} can be considered not physiological.

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