Pyruvate Kinase Revisited

THE ACTIVATING EFFECT OF K⁺*

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For more than 50 years, it has been known that K⁺ is an essential activator of pyruvate kinase (Kachmar, J. F., and Boyer, P. D. (1953) J. Biol. Chem. 200, 669–683). However, the role of K⁺ in the catalysis by pyruvate kinase has not been totally understood. Previous studies without K⁺ showed that the affinity of ADP-Mg²⁺ depends on the concentration of phosphoenolpyruvate, although the kinetics of the enzyme at saturating K⁺ concentrations show independence of the binding of substrates (Reynard, A. M., Hass, L. F., Jacobsen, D. D. & Boyer, P. D. (1961) J. Biol. Chem. 236, 2277–2283). Here, we explored the kinetics of the enzyme with and without K⁺.

The results show that without K⁺, the kinetic mechanism of pyruvate kinase changes from random to ordered with phosphoenolpyruvate as first substrate. \( V_{\text{max}} \) with K⁺ was about 400 higher than without K⁺. In the presence of K⁺, the affinities for phosphoenolpyruvate, ADP-Mg²⁺, oxalate, and ADP-Cr²⁺ were 2–6-fold higher than in the absence of K⁺. This as well as fluorescence data also indicate that K⁺ is involved in the acquisition of the active conformation of the enzyme, allowing either phosphoenolpyruvate or ADP to bind independently (random mechanism). In the absence of K⁺, ADP cannot bind to the enzyme until phosphoenolpyruvate forms a competent active site (ordered mechanism). We propose that K⁺ induces the closure of the active site and the arrangement of the residues involved in the binding of the nucleotide.

In a now classic paper, Kachmar and Boyer (1) described the activating effect of K⁺ on pyruvate kinase (PK). Since that time, many enzymes that are activated by monovalent cations have been described (2). More recently, high resolution crystallographic structures of Na⁺ and K⁺ in proteins were reviewed (3). In some cases, the structures have been instrumental in the understanding of the mechanism of ion activation and selectivity (4, 5). However, the molecular basis of the selectivity of PK for K⁺ and the mechanism through which K⁺ activates the enzyme are not entirely known.

Nowak and Suelter (6) wrote a historical description of the effect of K⁺ on PK. In 1953, Kachmar and Boyer (1) put forth that the binding of the monovalent cation induces disassociation of adjoining structures and that the expression of catalysis was associated with the magnitude of the displacement. Indeed, several physical studies showed that the conformation of the protein is modified by monovalent cations. Alkali metal ions change the UV, fluorescence polarization, and circular dichroism spectra of the enzyme (7–10). They also alter its immunoelectrophoretic behavior (11) and the sedimentation velocity of the protein (12). Structural modifications of PK have been observed with non-activating monovalent cations; this suggests that conformational changes are involved in catalysis, albeit they do not suffice to support high activities (10, 11).

Later, evidence for the interaction of K⁺ with substrates was reported. This provided important evidence for a direct participation on the catalytic events at the active site. Along this time, kinetic, UV difference spectroscopy, and EPR studies showed a synergistic effect in the binding of Mn²⁺ and K⁺ to the enzyme (13, 14). In NMR proton relaxation rate titration experiments, it was found that K⁺ raised the affinity of the enzyme for PEP and its analogs and that PEP and its analogs raised the affinity of the enzyme for K⁺. It was also found that K⁺ decreased the affinity of analogs of PEP that lack the free carboxyl function (15). Therefore, it was reasoned that monovalent cations facilitate the interaction of PEP to the binary enzyme-Mn²⁺ complex by interacting with the carboxylate group (15). In consonance with this hypothesis, NMR studies showed that the distance between Mn²⁺ and ²⁰²Ti⁺ complexed to PK was shortened by the addition of PEP. Thus, it was rather evident that the monovalent cation binds at the catalytic site and participates directly in the interaction of PEP with the enzyme-Mn²⁺ complex (16, 17). In fact, Suelter (2) proposed that K⁺ assists in the stabilization of the enolate form of pyruvate.

The localization of the active site was suggested by the first crystallographic structure of pyruvate kinase; however, the binding site for the monovalent cation was not ascertained (18). The interaction of K⁺ with the substrates and products was definitely demonstrated by the crystallographic structures of PK complexed with K⁺, Mg²⁺, and phospholactate (19) or ATP (20). The data showed that K⁺ interacts with the phosphoryl oxygen of either PEP or that of γ-phosphate of ATP. All of the crystal structures of PK up to date are in complex with monovalent cations; thus, the effects that K⁺ exerts on the overall structure and function of PK are not well understood. Likewise, it is not clear what residues are involved in the transduction of K⁺ binding to the enhancement of catalytic activity. In this regard, it is relevant that the binding site for monovalent cation is identical in all of the structures reported, regardless of whether they are in an active (closed) or inactive (open) conformation (19) or in complex with K⁺ or the nonactivator cation Na⁺ (20).

Since catalysis is more amenable to kinetic than to structural studies, in this work, we investigated the kinetic features of the enzyme in the absence of K⁺. It should be mentioned that hardly any attention has been given to the characteristics of PK in the absence of monovalent cation. Therefore, we hypothesized that the understanding of the struc-

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3 The abbreviations used are: PK, pyruvate kinase; WT-PK, rabbit muscle pyruvate kinase wild type; E117K-PK, rabbit muscle pyruvate kinase E117K mutant; LDH, lactate dehydrogenase; PEP, phosphoenolpyruvate; Mes, 4-morpholineethanesulfonic acid.
ture and function of the enzyme without K\(^+\) can provide a powerful background for the understanding of the action of K\(^+\) on PK. We also studied PK under conditions in which the enzyme displays a considerable K\(^+\)-independent activity: in water/Me\(_2\)SO mixtures (21) and in the PK mutant E117K (E117K-PK) (22).

**MATERIALS AND METHODS**

Preparation of Chromium-ADP—Chromium-ADP (ADP-Cr\(^2+\)) was prepared according to DePamphilis (23), except that tetramethylammonium bicarbonate was used instead of KHCO\(_3\) in the neutralization step. The purity of ADP-Cr\(^2+\) was ascertained from its visible spectral characteristics and by thin layer chromatography. Tetramethylammonium bicarbonate was prepared by ion exchange chromatography in a Dowex 50WX-4 (15 × 2.5-cm) column.

Subcloning of E117K—Plasmid pET22b containing the E117K-PK gene, plasmid pTrc99A, and Escherichia coli PB25Δ-PKΔ-PKII were kindly provided by Dr. George H. Reed from the University of Wisconsin (Madison, WI) (22) and by Dr. Francisco Bolivar Zapata (National University of Mexico) (24), respectively. E117K-PK originally cloned in pET22b was subcloned in pTrc99A. PCR was performed with the 5'-CATGGAATTCTAATAAGGAGCTGATGGACG-3' primer containing an EcoRI restriction site and the upstream NdeI site of E117K-PK and T7 terminator primer. The PCR-amplified DNA product was cut with EcoRI, inserted to pTrc99A, and transformed to competent E. coli PB25Δ-PKΔ-PKII (PB25-E117K) that contained anti-biotic resistance cassettes to chloramphenicol and kanamycin. Subsequently, sequence analysis was carried out.

Cell Growth and Purification of the E117K Mutant—LB medium containing 100 μg/ml ampicillin, 50 μg/ml kanamycin, and 30 μg/ml chloramphenicol was inoculated with PB25-E117K. Expression was induced with 1 mm isopropyl 1-thio-β-D-galactopyranoside and 20 g/liter of lactose at an A\(_{600}\) of about 0.8. The enzyme was purified as in Ref. 24 with some modifications. Cells were treated with lysozyme and an osmotic shock. DNase and a protease inhibitor mixture were added to the spheroplasts, which were then broken by sonication. The suspension was centrifuged 20 min at 20,000 × g. The supernatant was precipitated with ammonium sulfate at 37%, and the resultant supernatant was collected. A second precipitation with ammonium sulfate at 65% saturation was prepared. The pellet was collected, suspended, and desalted by dialysis. The final steps involved ion exchange chromatography in DEAE and carboxymethyl-Sepharose. The E117K mutant was 95% pure as indicated by SDS-PAGE.

Assays of Pyruvate Kinase Activity—WT-PK and hog muscle lactate dehydrogenase (LDH) were obtained as ammonium sulfate suspensions from Roche Applied Science. Ammonium sulfate-free enzymes were obtained as described (25). Contaminating NH\(_4\)\(^+\), Na\(^+\), and K\(^+\) in reaction mixtures were below the detection limit (10 μM) as indicated (25). The formation of pyruvate was measured at 25 °C either in a coupled reaction mixture was enough to overcome the inhibition of oxalate and K\(^+\) on PK. We also studied PK under conditions in which the enzyme displays a considerable K\(^+\)-independent activity: in water/Me\(_2\)SO mixtures (21) and in the PK mutant E117K (E117K-PK) (22).

**RESULTS AND DISCUSSION**

**Initial Velocity Studies of WT-PK with and without K\(^+\) in Aqueous Medium**—PK catalyzes the transfer of phosphoryl group of PEP to ADP in the presence of two ions of Mg\(^{2+}\) and one of K\(^+\). WT-PK has an absolute requirement for K\(^+\) (1); its activity with and without K\(^+\) is 250 and 0.02 μmol min\(^{-1}\) mg\(^{-1}\), respectively (28, 29). The pioneer work of Boyer’s group (30) showed that at saturating concentration of K\(^+\), WT-PK follows a random order rapid equilibrium kinetic mechanism. This was later confirmed by other authors (31, 32). In our initial kinetic studies carried out in the absence of K\(^+\), we observed that the affinity of ADP-Mg\(^{2+}\) depended on the concentration of PEP, indicating that in the absence of K\(^+\), the binding of PEP affects that of ADP-Mg\(^{2+}\). Thus, we explored the kinetic behavior of the enzyme without K\(^+\).

Initial velocities of the reaction of WT-PK were determined without and with K\(^+\) at variable concentrations of one of the substrates and at fixed variable concentrations of the other (Fig. 1). In the absence of K\(^+\), the double reciprocal plots of initial velocities versus PEP concentrations intersected above the 1/5 axis and to the left of the 1/1 axis, (Fig. 1A). With ADP-Mg\(^{2+}\) concentrations as variable substrate, the lines intersected on the 1/1 axis and above the 1/5 axis (Fig. 1B). Hence, the data indicate a rapid equilibrium sequential ordered mechanism with
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**TABLE ONE**

| Condition                           | Initial velocity intersecting patterns | Kinetic mechanism | $V_{max}$ | $K_m$ WT-PK | $K_m$ ADP-Mg²⁺ | $k_{cat}$ | Log ($k_{cat}$/$K_m$) WT-PK | Log ($k_{cat}$/$K_m$) ADP-Mg²⁺ |
|-------------------------------------|----------------------------------------|------------------|-----------|-------------|----------------|----------|----------------------------|-------------------------------|
| WT-PK 100% H₂O without K⁺          | Intersects to the left of the 1/v axis and above the 1/S axis | Intersects on the 1/v axis and above the 1/S axis | Ordered rapid equilibrium | 0.8 ± 0.04 | 0.61 ± 0.07 | 14.1 ± 1.7 | 3.2 | 3.71 | 2.35 |
| WT-PK 100% H₂O with K⁺             | Intersects to the left of the 1/v axis and on the 1/S axis | Intersects to the left of the 1/v axis and on the 1/S axis | Random rapid equilibrium | 299 ± 11 | 0.13 ± 0.007 | 2.3 ± 0.1 | 1182 | 6.96 | 5.70 |
| WT-PK 40% Me₂SO without K⁺         | Intersects to the left of the 1/v axis and on the 1/S axis | Intersects to the left of the 1/v axis and on the 1/S axis | Random rapid equilibrium | 25 ± 1 | 0.06 ± 0.006 | 1.4 ± 0.1 | 98 | 6.21 | 4.85 |
| E117K-PK 100% H₂O without K⁺       | Intersects to the left of the 1/v axis and on the 1/S axis | Intersects to the left of the 1/v axis and on the 1/S axis | Random rapid equilibrium | 41 ± 2 | 0.24 ± 0.016 | 2.0 ± 0.1 | 161 | 5.83 | 4.90 |

**SCHEME 1.** Ordered (A) and random (B) rapid equilibrium kinetic mechanisms.

Initial velocity experiments were carried out without K⁺ in 40% Me₂SO (w/v) with WT-PK and in 100% water with E117K-PK (experiments not shown). In both cases, the double reciprocal plots were identical to those obtained in the wild type enzyme in 100% water with K⁺. The data were globally fitted to the equation that describes a random order kinetic mechanism (TABLE ONE). In the absence of K⁺, the $V_{max}$ of WT-PK in 40% Me₂SO and of E117K-PK were 30-fold (25 versus 0.8 μmol min⁻¹ mg⁻¹) and 50-fold (41 versus 0.8 μmol min⁻¹ mg⁻¹) higher than the $V_{max}$ of WT-PK in 100% water without K⁺. In the data of TABLE ONE, it is relevant that the $K_m$ values and catalytic efficiencies for the WT-PK with K⁺ in 100% water, 40% Me₂SO without K⁺, and the E117K-PK in 100% water without K⁺ are similar. However, the values are drastically different from those of WT-PK in 100% water without K⁺. For example, the $K_m$ for ADP-Mg²⁺ in WT-PK without K⁺ is 14.1 mM, whereas in the other conditions, it is around 2 mM. Likewise, log ($k_{cat}$/$K_m$) is more than 2 orders of magnitude higher for the enzyme in the first three experimental conditions than for the enzyme without K⁺ in aqueous medium. In summary, these data indicate that the characteristics of the active site in Me₂SO or the mutant resemble those of the enzyme in the presence of K⁺. It has been shown that in the absence of K⁺, 40% Me₂SO induces the acquisition of the active conformation of WT-PK (21).

**Dead End Inhibition Studies**—Dead end inhibitors are powerful tools to probe the kinetic mechanisms of enzymes (39). Here we used oxalate and ADP-Cr²⁺ as dead end analogues of PEP (40) and of ADP-Mg²⁺ (41), respectively (experiments not shown). In the absence of K⁺, the
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patterns of oxalate inhibition versus PEP and ADP-Mg²⁺ were competitive and noncompetitive, respectively. With ADP-Cr²⁺, the inhibition was uncompetitive and competitive with PEP and ADP-Mg²⁺, respectively. With K⁺, the patterns of oxalate inhibition versus PEP and ADP-Mg²⁺ were competitive and noncompetitive, respectively, whereas with ADP-Cr²⁺, the inhibition was noncompetitive and competitive with respect to PEP and ADP-Mg²⁺. In all cases, replots of the slopes or intersects of double reciprocal plots against inhibitor concentration were linear (not shown). The data were globally fitted to the equations that describe linear competitive inhibition, linear noncompetitive inhibition, linear mixed inhibition, or linear uncompetitive inhibition. The inhibition patterns and calculated inhibition constants are shown in TABLE TWO.

Collectively, the data show that with or without K⁺, oxalate is a competitive inhibitor with respect to PEP, whereas ADP-Cr³⁺ is a competitive inhibitor of ADP-Mg²⁺. This indicates that analogues and substrates bind to the same site. Because with or without K⁺, oxalate is a noncompetitive inhibitor of ADP-Mg²⁺, it may be concluded that oxalate forms a nonproductive ternary complex and thereby diminishes V_max without altering the binding of ADP-Mg²⁺. The same argument holds for ADP-Cr³⁺ with respect to PEP in the presence of K⁺. On the other hand, with PEP in the absence of K⁺, the inhibition pattern by ADP-Cr³⁺ was uncompetitive, illustrating that ADP-Cr³⁺ binds exclusively to the enzyme-PEP binary complex. The uncompetitive pattern confirms the data of Fig. 1 and TABLE ONE that show that in the absence of K⁺, WT-PK followed a sequential rapid equilibrium ordered mechanism. In contrast, the overall kinetics in the presence of K⁺ (substrates binding independently) confirm previous reports (30–32) that show that the kinetic mechanism of WT-PK involves a rapid equilibrium random order.

The inhibition patterns in the absence of K⁺ of WT-PK in 40% Me₂SO medium and of E117K-PK in 100% water followed the same patterns as those of WT-PK with K⁺, except that a mixed type pattern with α < 1 for oxalate inhibition versus ADP-Mg²⁺ was observed (TABLE TWO). This difference indicated that the enzyme-oxalate binary complex has a higher affinity for ADP-Mg²⁺ than the free enzyme. Therefore, the data with dead end inhibitors indicate that in the absence of K⁺, WT-PK in medium 40% Me₂SO or E117K-PK in 100% water follow a rapid equilibrium random order mechanism.

The data of TABLES ONE and TWO show that K⁺ increases the affinity of the enzyme for the inhibitors and the substrates. Thus, it is noteworthy that WT-PK in medium with 40% Me₂SO or in the mutation E117K in 100% water, the affinity for the inhibitors is higher than that of PK in water plus K⁺ (TABLE TWO). Along this line, it is relevant that Reed et al. (40) reported that oxalate is an analogue of the transition state. Our data show that the active site of the enzyme in 40% Me₂SO or that of E117K-PK is in a conformation near to the transition state. In fact, it has been described that the inclusion of 40% Me₂SO induces structural arrangements of WT-PK that favor the partition of substrates and cations to the active site (21, 42). Therefore, the low inhibition constants of dead end inhibitors, the high affinity for the substrates, and the high catalytic efficiency of WT-PK in 40% Me₂SO or of PK-E117 indicate that the enzyme is in a conformation prone to bind substrates and carry out catalysis in these conditions.

In regard to the mechanism through which E117K-PK carries out K⁺-independent catalysis, it has been proposed that the positive charge of Lys provides an internal monovalent cation (22). This mechanism cannot explain the K⁺-independent activity observed in Me₂SO. It is noted, however, that in 40% Me₂SO, WT-PK exhibits a conformation similar to that observed in aqueous medium with K⁺ (21); this suggests that the overall conformation of the enzyme contributes to the expression of catalysis.

The Effect of K⁺ and Ligands on the Structure of Pyruvate Kinase—A salient feature of our data is that K⁺ changes the kinetic mechanism from ordered to random. This indicates that the change is related to structural arrangements of the enzyme. Indeed, crystallographic studies of WT-PK show that the enzyme acquires different conformations that depend on the ligands that are at the active site. With PEP analogs, it is open or partially closed, and with PEP analogs and ATP-Mg²⁺, the active site is tightly closed (19, 20) (Fig. 2A).

We determined the spectral center of mass and intrinsic fluorescence anisotropy of free WT-PK and in complex with K⁺ and the ligands shown in TABLE THREE. PK has three Trp residues per monomer, two in the C and one in the B domain (18–20, 43). The C domain remains in the same position in the open and closed conformations, whereas the D domain is highly mobile and differs in 40° in the two conformers (see Fig. 2A) (20). It is likely that Trp¹⁷⁵, which is in the B domain, accounts for the differences in intrinsic anisotropy with and without ligands and reports the movement of this domain.

Oxalate, K⁺, Mg²⁺, and ATP-Mg²⁺ induced a blue shift of the λ_max or spectral center of mass of PK. The transition from the open to the closed conformation was gradually induced by the sequential addition of various active site ligands (TABLE THREE). In consonance with crystallographic data, a first transition was observed as a result of the addition of oxalate and Mg²⁺ to PK; the subsequent addition of K⁺ induced a further shift that corresponds to the partially closed active site (19). A still larger shift was brought about by the addition of ATP-Mg²⁺; this corresponds to the totally closed conformation (20).

The intrinsic fluorescence anisotropies of the same samples were recorded at the λ_max (TABLE THREE). In agreement with the data of the spectral center of mass, the ligands induced stepwise changes in anisotropy values. This indicates that ligands induce a lower mobility of the

**TABLE TWO**

Dead end inhibition patterns and inhibition constants for oxalate and ADP-Cr²⁺ in WT-PK and in E117K-PK

| Inhibition patterns | Dead end analog of PEP:oxalate | Dead end analog of ADP-Mg²⁺:ADP-Cr²⁺ |
|---------------------|--------------------------------|------------------------------------|
|                     | 1/v versus 1/PEP, fixed ADP-Mg²⁺ | 1/v versus 1/PEP, fixed ADP-Mg²⁺ |
|                     |                                | 1/v versus 1/PEP, fixed ADP-Mg²⁺ | 1/v versus 1/PEP, fixed ADP-Mg²⁺ |
| WT-PK, 100% H₂O with K⁺ | C                              | NC                                | C                              |
| WT-PK, 100% H₂O without K⁺ | C                          | NC                                | C                              |
| E117K-PK, 100% H₂O without K⁺ | C                              | MT                                | NC                              |
| WT-PK, 40% Me₂SO without K⁺ | C                              | NC                                | C                              |

| Kᵢ (oxalate) | Kᵢ (ADP-Cr²⁺) |
|--------------|---------------|
| 29 ± 1.8     | 1.3 ± 0.6     |
| 77 ± 3.2     | 2.8 ± 0.6     |
| 4.5 ± 0.2    | 0.9 ± 0.08    |
| 2.4 ± 0.1    | 0.5 ± 0.02    |
occupancy of the active site. In the absence of ligands, the B domain is in the open conformation and highly mobile. The binding of K\(^+\), Mg\(^{2+}\), and PEP analogs to the active site results in either an open or partially closed (19, 43) conformation. The subsequent binding of ATP-Mg\(^{2+}\) brings the enzyme to the totally closed or active conformation (20). These data agree with our fluorescence results that show that the successive addition of ligands gradually closes the active site. These data fit well with the structure of PK in crystals grown with oxalate, Mg\(^{2+}\), K\(^+\), and ATP-Mg\(^{2+}\) (20). The unit cell of this crystal contains two tetramers. Two of the eight subunits contain oxalate, Mg\(^{2+}\), and K\(^+\); the other six also contain ATP-Mg\(^{2+}\). Subunits without nucleotide exhibit an open active site cleft, whereas five of the subunits with ATP-Mg\(^{2+}\) exhibit an angle of rotation of 41\(^\circ\) in reference to the open subunits and are completely closed. When the nucleotide is present, residues Arg\(^{119}\), Lys\(^{206}\), and Asp\(^{177}\) move 6.8, 10.4, and 6.8 \(\AA\), respectively, from their position in the open conformation and establish contacts with ATP-Mg\(^{2+}\) in the closed conformation (Fig. 2, B and C). This indicates that the binding site for the nucleotide is structured only after the cleft has been closed.

Structural studies have not yielded conclusive mechanistic information on the influence of K\(^+\) on the structural arrangements of WT-PK. However, our kinetic data show that K\(^+\) enhances the affinity constants for ADP-Mg\(^{2+}\), oxalate, and ADP-CI\(^{2-}\), although they do not establish direct coordination with K\(^+\) (19, 20). This indicates that K\(^+\) induces the correct geometrical arrangement of the active site residues. This implies that K\(^+\) not only enhances the binding of substrates through the coordination with the phosphoryl oxygen of PEP or ATP but also arranges the active site pocket. Moreover, the effect of K\(^+\) on the conformation of the active site is also illustrated by the influence of the cation on the binding of ADP-Mg\(^{2+}\). With K\(^+\), ADP binds independently of the presence of PEP to the enzyme, whereas without K\(^+\), ADP binds to the enzyme only after PEP is bound. Since the ADP binding site is formed after the B domain has rotated over the A domain (Fig. 2), our data suggest that K\(^+\), PEP, or both contribute to the descent of the B domain. It is relevant to mention that two of the residues that coordinate K\(^+\), Asp\(^{112}\) and Thr\(^{113}\), are positioned at the beginning of the hinge region that links the A and B domains.

In addition to its effect on PK, K\(^+\) is an activator of various other enzymes. In this context, it is particularly relevant that K\(^+\) activates inosine 5’-monophosphate dehydrogenase from Aerobacter aerogenes by changing its kinetic mechanism from random order to ordered with inosine monophosphate and NAD\(^+\) as the first and second substrate, respectively (44, 45). The authors suggest that K\(^+\) induces a conformational change that permits the binding of NAD\(^+\) (44, 45). Crystallographic and kinetic studies of this enzyme suggest that K\(^+\) controls the conformation of the active site loop by stabilizing the conformation that binds NAD\(^+\) (46–48).

In summary, our data show that the role of K\(^+\) as an essential activator of WT-PK is not limited to coordination of the phosphate group of PEP or ATP, as shown quite clearly by crystallographic studies (19, 20). Our data show that in the absence of K\(^+\), ADP cannot bind to the active site until PEP arranges the ADP binding site (ordered mechanism with PEP as the first substrate). On the other hand, K\(^+\) induces the active (closed) conformation of the enzyme, allowing either PEP or ADP to bind independently (random order mechanism). In all likelihood, K\(^+\) is directly involved in the movement of the B domain and the acquisition of the active conformation of PK.

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**TABLE THREE**

| \(\lambda_{\text{max}}\) | SCM | \(r\) |
|------------------|-----|-----|
| PK               | 334.4 | 348.1 ± 23.6 | 0.1087 ± 0.0023 |
| PK-Mg\(^{2+}\)-oxalate | 331.6 | 346.5 ± 25.5 | 0.1468 ± 0.0024 |
| PK-Mg\(^{2+}\)-oxalate-K | 330.8 | 346.5 ± 25.6 | 0.1489 ± 0.0023 |
| PK-Mg\(^{2+}\)-oxalate-ATP | 330.9 | 345.8 ± 24.6 | 0.1591 ± 0.0025 |
| PK-Mg\(^{2+}\)-oxalate-ATP-K | 330.5 | 345.0 ± 23.2 | 0.1682 ± 0.0028 |

Trp residues of WT-PK until the compact closed conformation of the active site (with ATP-Mg\(^{2+}\)) is attained. The overall fluorescence data indicate that the occupancy of the active site and the movement of domain B are intimately related.

**The Role of K\(^+\) in the Structure and Kinetics of Pyruvate Kinase**—The results outlined show that the inclusion of K\(^+\) induces a change in the kinetic mechanism of WT-PK from ordered to random. In order to define the dependence of the kinetics on K\(^+\) on the structure of PK, the crystallographic structure of the enzyme was examined. Each subunit of tetrameric WT-PK is formed by four structural domains: the N-terminal, A, B, and C domains. The active site is in a cleft formed by A and B domains. Rotation of the B domain over the A domain closes the active site cleft (19, 20) (Fig. 2A). The interdomain movement depends on the
