Pyruvate Kinase

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Abstract. Pyruvate kinase is a vital glycolytic enzyme that catalyzes the formation of pyruvate in the final step of glycolysis. In this reaction, pyruvate kinase facilitates the transfer of the phosphate group on phosphoenolpyruvate to adenosine diphosphate. This reaction produces pyruvate and adenosine triphosphate. Pyruvate can further be converted to ethanol and carbon dioxide, lactate, and acetyl-CoA, all of which play key roles in energy production. Adenosine triphosphate is used within cells to power multiple significant chemical reactions. The structure of pyruvate kinase in the majority of organisms is a homotetramer. Each subunit of pyruvate kinase is divided into three principle domains, the A, B, and C domain. In all cells except for prokaryotes, there is a small N domain analogous to the N-terminus. The secondary structure of pyruvate kinase consists of several alpha helices and beta sheets. Pyruvate kinase consists of four isozymes. The allosterically regulated isozymes are the L, R, and M_2 isozymes. These isozymes follows sigmoidal kinetics and are regulated via a process of feed-back inhibition by adenosine triphosphate and feed-forward activation of fructose 1,6-bisphosphate. The only nonregulated isozyme is the M_1 isozyme. This isozyme displays non-sigmoidal Michaelis-Menten kinetics. Pyruvate kinase is regulated by phosphoenolpyruvate, fructose 1, 6-bisphosphate, adenosine triphosphate and pyruvate. A deficiency in pyruvate kinase can cause a disruption in the anaerobic production of erythrocyte adenosine triphosphate which leads to the shortening of cell life. This disease is aptly named Pyruvate Kinase Deficiency. Studies have shown that individuals who are heterozygous for Pyruvate Kinase Deficiency possess protective factors against malaria.

Introduction

Pyruvate has three important fates in organisms. Pyruvate is a key player in two anaerobic energy-harvesting mechanisms. The first of these mechanisms is the creation of ethanol and carbon dioxide normally conducted by yeast and some bacteria. The second mechanism is the production of lactate which the muscles in the human body require to function properly. Pyruvate can also react to form acetyl-CoA. The importance of acetyl-CoA in metabolism cannot be understated. Its main role is in the Krebs cycle where it takes part in energy production. The physiological significant of pyruvate justifies why its formation is undeniably important. The enzyme that catalyzes the reaction to form pyruvate is pyruvate kinase.

Pyruvate kinase is an important glycolytic enzyme that plays a critical role in cellular metabolism. In the final step of the glycolytic pathway, pyruvate kinase acts as a catalyst by assisting the transfer of phosphate from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP).[1] This reaction yields pyruvate and adenosine triphosphate (ATP). For each mole of glucose that is metabolized, a net gain of 2 moles of ATP are produced from ADP.[2] Under physiological conditions, this reaction is fundamentally irreversible because of the

strong exergonic quality of the transformation of the substrate PEP to the product pyruvate.[2] PEP and pyruvate have an influence in many energetic and biosynthetic pathways.[1] Pyruvate kinase's ability to allosterically respond to multiple effectors is of great significance for its role in cellular metabolism.[1] Considering this information, pyruvate kinase has an undeniable effect on cellular metabolism.[1, 2]

The structure of pyruvate kinase is extremely homologous across species. In the majority of organisms, pyruvate kinase is a homotetramer.[1] The tetrameric structure of pyruvate kinase is shown in Figure 1. Pyruvate kinase can exists in several forms ranging from monomer to decamer.[1] Figures 2A, 2B, and 2C illustrate one subunit of pyruvate kinase. Each subunit of pyruvate kinase is organized into three principle domains, an A, B, and C domain. A small domain that corresponds to the N-terminus is present in all cells with the exception of prokaryotes.[1] These multiple domains allow pyruvate kinase to have an impact on metabolic regulation in the cell.

Pyruvate kinase deficiency (PKD) has been demonstrated to have a significant effect on the body. PKD is caused by a mutation in the PKLR gene, which main function is to encode red blood cell pyruvate kinase.[3] PKD can result in chronic hemolytic anemia.[3] PKD has been shown to have some benefits for individuals that are heterozygous for the alleles. Several in vivo and in vitro studies have been conducted on the relationship of PKD and resistance to malaria.[4] These studies have demonstrated that PKD provides not only protection against infection, but also, protection against the replication of *Plasmodium falciparum*, the cause of malaria, in human erythrocytes.[5] This information is particularly important in areas where malaria is endemic because it indicates that a protective advantage against malaria may be obtained from the mutant pyruvate kinase alleles.[5]

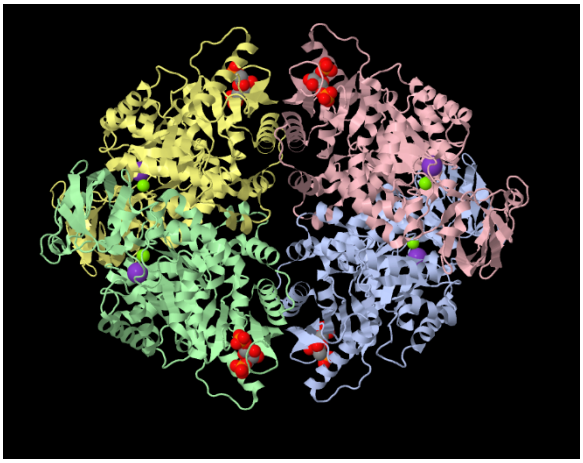


Figure 1: Four Subunits of Human Pyruvate Kinase M2. This figure is the ribbon representation of the human pyruvate kinase M2 tetramer. The pyruvate kinase tetramer has individual subunits colored light blue, pale green, pink, and yellow. FBP, shown as a combination of gray, red, and orange bonded spheres, is bound to the allosteric site. Potassium ion (purple) and magnesium ion (lime green) are shown as spheres. They are bound to the active site. Each chain is a different pastel color. Protein alpha helices and beta strands are shown as ribbons, with arrowheads pointing towards the carboxy termini. Random coil is shown as smoothed backbone trace ropes.[6]

Figure 2: One Subunit Pyruvate Kinase. This figure shows the ribbon (A), secondary structure (B), and rainbow sequence (C) of pyruvate kinase. (A) Ribbon structure of pyruvate kinase. (B) Secondary structure of pyruvate kinase. Alpha helices are shown as pink rockets. Beta strands are shown as yellow planks. Purple arrowheads point towards the carboxy termini. This structure is 47.7% alpha helices, 20.3% beta strands, and 32% neither. (C) Rainbow structure of pyruvate kinase. The N termini is blue and the C termini is red. Between termini, colors follow a spectral rainbow sequence. In all figures, fructose-1,6-bisphosphate (FBP) (gray/red/orange), potassium ion (purple), and magnesium ion (lime green) are shown as spheres.[6]

Structural Details

As mentioned earlier, pyruvate kinase is normally a homotetramer consisting of 50-60 kDa depending on the species.[7] The secondary structure of pyruvate kinase is composed of alpha helices and beta sheets. Pyruvate kinase's quaternary structure is organized in four domains consisting of the A, B, and C domains along with the N-terminal. These domains are illustrated in Figure 3. The active site is located in between the A and B domains.[7] Approximately 39 Å from the active site is the effector site. The effector site is located in the C domain.[7] The B domain controls access to the active site by forming a mobile lid at one end of the A domain.[7]

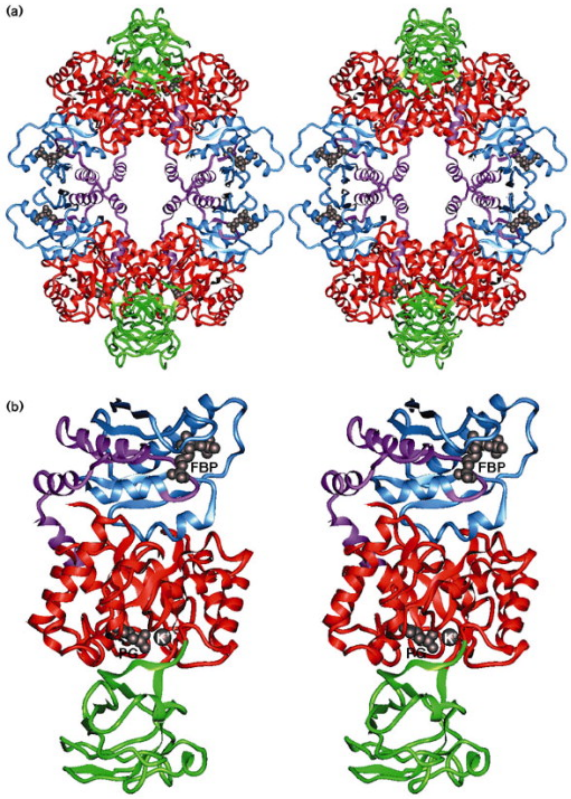


Figure 3: Pyruvate Kinase Structural Model. The figure to the right shows the A domain (red), the B domain (green), and the C domain (blue). The purple section is the residue of the alternatively spliced exon in humans. Bound ligands in the structure are gray van der Waals models. The distance between the allosteric and actives sites is depicted in the second set of images. The active site is located between the A and B domains and PG, Mn^{2+}, and K^{+} are bound to it (black, bottom). The allosteric site is located in the C domain and FBP is bound to it (black, top).[8]

A Domain

The A domain consists of (β/α)8 barrel topology.[1] It is formed by two separate stretches of amino acids. These stretches are illustrated in Figure 4 and consist of residues 19-88 and 189-360.[8] These two amino acid stretches form the two additional α helices and the (β/α)8 barrel.[8] Adjacent A domains form the large interface or A-A.[7]

Figure 4: A Domain Structural Composition. This figure illustrates (β/α)8 barrel topology of the A domain of pyruvate kinase. The α helixes are shown in pink and the β planks are shown in yellow. The purple arrows point towards the carboxy termini.[6]

B Domain

The B domain, shown in Figure 5, is β-stranded and located between the α3 helix and β3 strand of the A domain.[1] The B domain consists of residues 89-188 and forms a cap over the active site.[8]

Figure 5: β-stranded Structure of Domain B. In this figure, the β-stranded planks are shown in yellow. A small section of the A domain is shown as a pink α helix to illustrate the transition between the A and B domains. The B domain forms a cap over the active site.[6]

C Domain

The third domain, the C domain, has a combination of α + β topology.[1] The C domain, illustrated in Figure 6, is known as the regulatory domain and consists of the residues 361-567. It is located at the C terminus.[8] The effector site is located in the C domain approximately 39Å away from the active site.[7] Adjacent C domains in the tetramer form the small interface or C-C.[7] Lys^{382} has been shown to play a part in activator binding and the allosteric transition mechanism.[8]

Figure 6: Regulatory C Domain. This figure illustrates the α (pink) and β (yellow) structure of the C domain. The allosteric site of pyruvate kinase where FBP binds is located in this domain.[6]

N Domain

A final small domain, the N domain, corresponds to the N-terminus and is present in all cells with the exception of prokaryotes.[1] Shown in Figure 7, the N domain consists of the residues 1-18 and is a short α-helical stretch.[8]

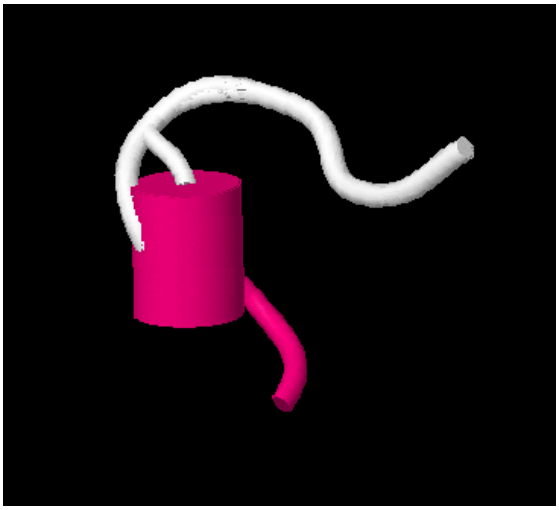


Figure 7: Eukaryotic N Domain. This figure illustrates the very short α-helical stretch (pink) of the N domain.[6]

Tetrameric Structure

The tetrameric structure of pyruvate kinase is extremely homologous across species. In mammals, trypanosomatids, yeast, and bacteria there are three distinct ligand-binding sites.[7] These sites have affinities for numerous small molecules. The three sites are the active site, the effector site, and the amino acid binding site.[7] PEP binds to the active site allosterically in most pyruvate kinases.[7] Pyruvate kinase activators and inhibitors bind the effector site.[7] The activators are the L, R, and M_2 isozymes. The lone inhibitor is the M_1 isozyme. The amino acid binding site can have amino acids such as proline and phenylalanine bind to it in order to reduce the activity of the M_1 isozyme.[7]

Conformational States

Pyruvate kinase has two main conformational states. These states are the tight (T) state and the relaxed (R) state. The T-state is the inactive state and is very low in substrate affinity. The R-state is the active state and is very high in substrate affinity. The switching from the inactive to active state is a result of domain and subunit rotations along with changes in the conformation of the active site.[1]

Enzyme Kinetics: Inhibitors and Allosteric Regulation

Pyruvate kinase consists of the isozymes L, R, M_1, and M_2.[8] All of these isozymes have different kinetic properties determined by the metabolic requirements of the tissue expressed. The L and the M genes in humans encode for all four of the isozymes shown in Figure 8.[8] The L isozyme which is located in the liver and the R isozyme which is located in the red blood cells are encoded by the L gene.[8] The M_1 isozyme which is

located in the skeletal muscles and brain and M_2 isozyme which is located mainly in fetal tissues are encoded by the M gene.[8] The structural differences between the M_1 and M_2 isozyme are illustrated in Figure 9. Since the M_2 isozyme is found on the rapidly proliferating fetal tissues, it is replaced overtime with the three other isozymes.[8]

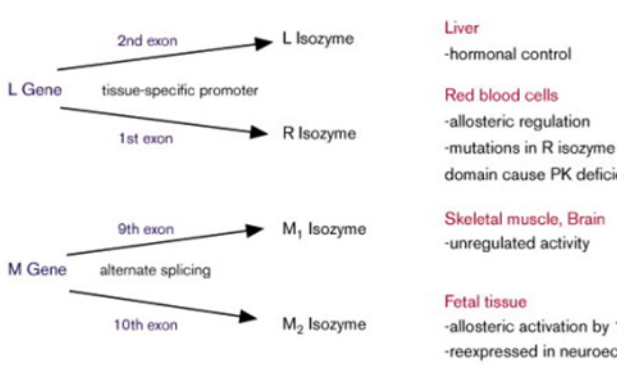


Figure 8: Function of the L, R, M_1, and M_2 Isozymes. In this figure, the location and function of each isozyme is described.[8]

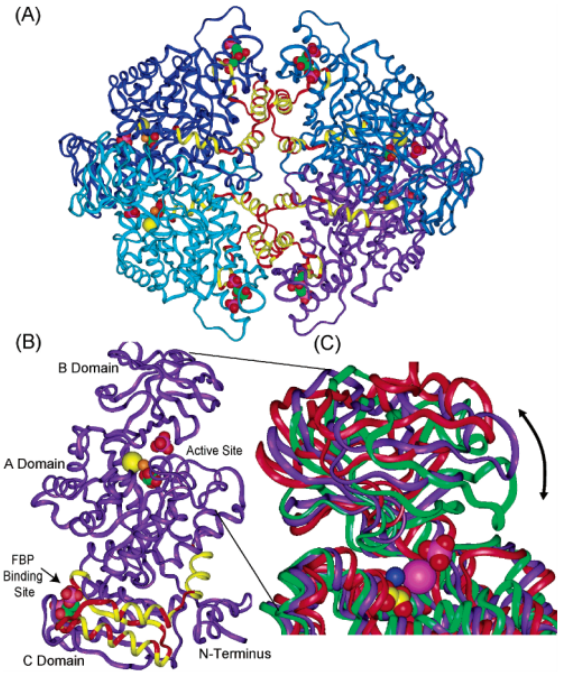


Figure 9: Structural Differences between Human Pyruvate M_1 and M_2. (A) The tetrameric structure of human pyruvate kinase M_2. (B) The A, B, C, and N domains along with the active site and FBP binding site labeled on one of the monomers of the human M_2 pyruvate kinase. Colored yellow is the 56 amino acid stretch that makes up the exons 9 and 10 which are alternately spliced in the M_1 and M_2 isoforms. The red section is the 24 amino acids that differ in these exons. (C) Enlargement of the active site (purple) including the superimposed active sites of the closed subunit (green) and open subunit (red) of the M_2 rabbit muscle.[6]

Pyruvate kinase is regulated by PEP, ATP, pyruvate, and alanine. The more substrate present, PEP, the faster the pyruvate kinase activity. ATP and pyruvate are both negative allosteric inhibitors. This means that when either ATP or pyruvate binds to pyruvate kinase, the affinity for PEP to bind at the active site decreases. Alanine is a negative allosteric modulator.[9] The L, R, M_1, and M_2 isozymes can be sorted into two groups, the nonregulated and regulated isozymes.

Nonregulated Isozymes

The only nonregulated isozyme is the type M_1. This is because under most metabolic conditions, the isozyme displays mainly non-sigmoidal Michaelis-Menten kinetics, hyperbolic kinetics, during the binding of its substrates and activators .[9] This is illustrated in Figure 10. The binding of PEP to the active site is the rate limiting step in this reaction.[9] This means that PEP plays a role in enhancing the activity of the reaction. It has been shown that this reaction does not exhibit rapid dissociation of the products indicating that the products of the reaction, ATP and pyruvate, inhibit the reaction of pyruvate kinase by reversing the reaction.[9] In summary, ATP and pyruvate are non-competitive inhibitors of pyruvate kinase as shown in Figure 11.[9]

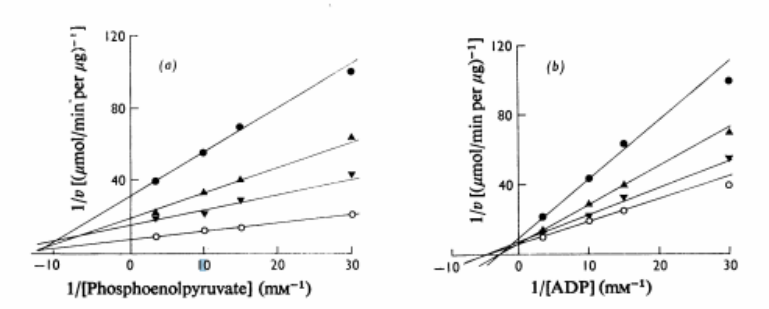


Figure 10: Michaelis-Menten Kinetics of Pyruvate Kinase. In this figure, the reciprocal plots of velocity versus PEP concentration (A) and velocity versus ADP concentration (B) are represented. (A) The concentrations of PEP were from top line to bottom: 33 µM, 66 µM, 100 µM, and 300 µM. (B) The concentrations of ADP were from top line to bottom: 33 µM, 66 µM, 100 µM, and 300 µM.[9]

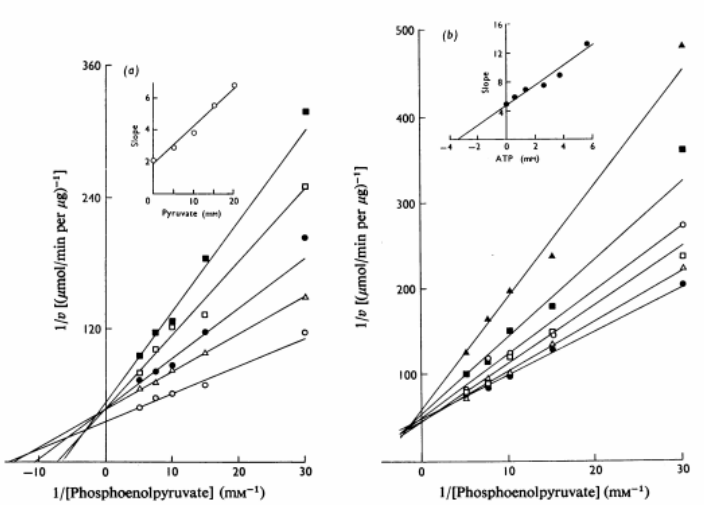


Figure 11: Noncompetitive Inhibition of Pyruvate Kinase by ATP and Pyruvate. This figure shows the reciprocal plot of velocity versus PEP concentration in comparison to ATP and pyruvate concentration. (A) The pyruvate concentrations were from bottom to top: 0 mM. 5 mM, 10 mM, 15 mM, and 20 mM. (B) The ATP concentrations were from bottom to top: 0 mM, 0.55 mM, 1.30 mM, 2.60 mM, 3.7 mM, and 5.55 mM.[9]

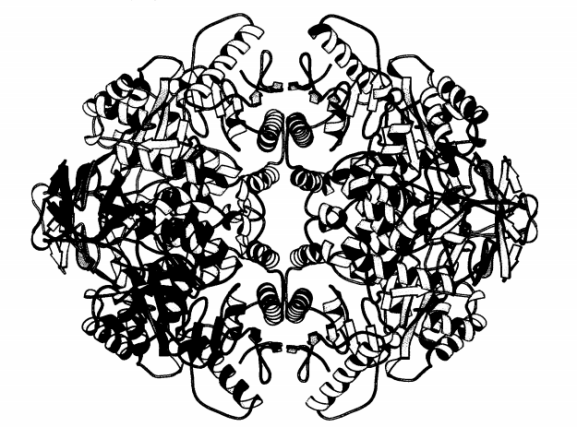
Regulated Isozymes

The allosterically regulated isozymes are the L, R, and M_2 isozymes. These isozymes have sigmoidal kinetic responses to PEP, metal ions, and fructose 1,6-bisphosphate (FBP). These three isozymes are allosterically regulated via a process of feed-back inhibition by ATP and feed-forward activation of FBP.[7]

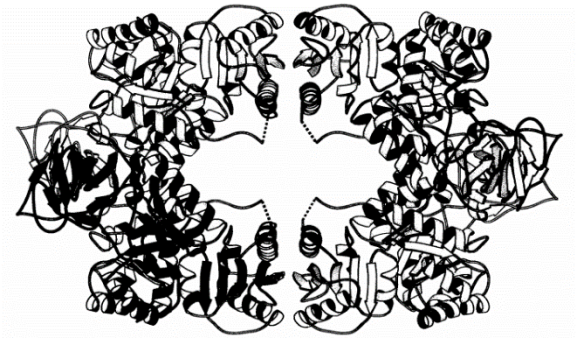
Conformational Shape Change Effects

Allosteric regulation is the primary mode of control for pyruvate kinase. As pyruvate kinase transitions from the R-state to the T-state, the PEP binding site is altered by 29 degrees (Figure 12).[10] This is a result of the (β/α)8 barrel in domain A being shifted by only 1 Å. The alteration in binding site justifies the low substrate affinity of the T-state.[10] A structural transition is also experienced by the effector binding pocket. Research shows that the enzyme activator binds in between the A and C domains in a cleft.[10] A cluster of positively charged residues is located here. This cleft shrinks which prevents effector binding when the C domain rotates to form the T-state.[10] The R-state is the active state and has a very high substrate affinity.

(A)



(B)



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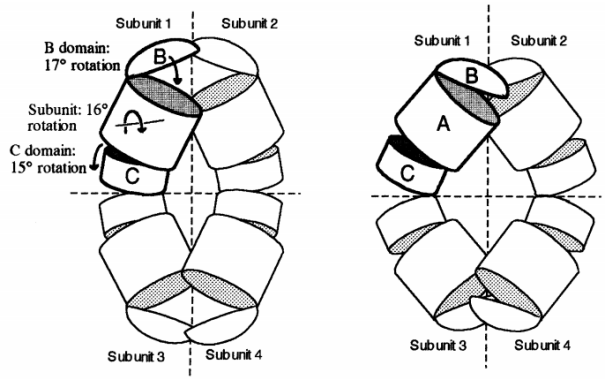

Figure 12: Conformational Shape Change of Pyruvate Kinase. This figure shows the tight T-state (A) and the relaxed R-state (B). (C) The domain and subunit rotations causing the T-state to R-state transition. The B domain rotates by 17 degrees, the C domain rotates by 15 degrees, and the subunit rotates by 16 degrees.[10]

R Isozyme

Pyruvate kinase in red blood cells, the R isozyme, is allosterically activated by FBP.[1] ATP takes on the role as an inhibitor to red blood cell pyruvate kinase.[1] The low affinity T-state and high affinity R-state have the same V_{max} value of 350 U/mg.[1] Pyruvate kinase has the same turnover number when effectors are present and when they are absent. This k_{cat} value is 355/s.[1] On the contrary, the presence of effectors affects the apparent affinity ($S_{0.5}$ or K_m) and Hill coefficient (nH). In the absence of effectors, the K_m and nH values towards PEP are 1.1 mmol/l and 1.6 respectively.[1] In the presence of the FBP effector, the K_m and nH values towards PEP are 0.18 mmol/l and 1.05 respectively.[1] Red blood cell pyruvate kinase is inhibited by ATP (IC_{50} at 0.1 mmol/l PEP, 0.53 mmol/l) .[1] Amazingly, under physiological conditions, the FBP activation almost completely cancels out the ATP inhibition.[1] Red blood cell pyruvate kinase also exhibits hyperbolic behavior. This behavior is directed at ADP with a K_m value of 0.17 mmol/l.

Role of K^+ Concentration

The kinetic mechanism of pyruvate kinase can be either ordered rapid or ordered random depending on the concentration of K^+ (Figure 13 and 14).[11] When the K^+ concentration is low, the mechanism is ordered with PEP being the first substrate. The binding of ADP is dependent of the initial binding of PEP to form a competent active site when low K concentrations are present.[11] Without K^+, the V_{max} for the ordered rapid equilibrium kinetic mechanism is 0.80 ± 0.04 umol/min mg and the k_{cat} was 3.2 s^{-1}.[11] When the K^+ concentration is high, the mechanism is random allowing either PEP or ADP to bind independently. With K^+, the V_{max} for the random rapid equilibrium kinetic mechanism is 299 ± 11 umol/min mg and

the k_{cat} was 1182 s^{-1}.[11] This V_{max} is approximately 400 times greater than when no K^+ was present. Also, the affinities for PEP and ADP were between two and six times greater when K^+ was present. This data demonstrates that the presence of K^+ is involved in the formation of the active confirmation of pyruvate kinase.[11]

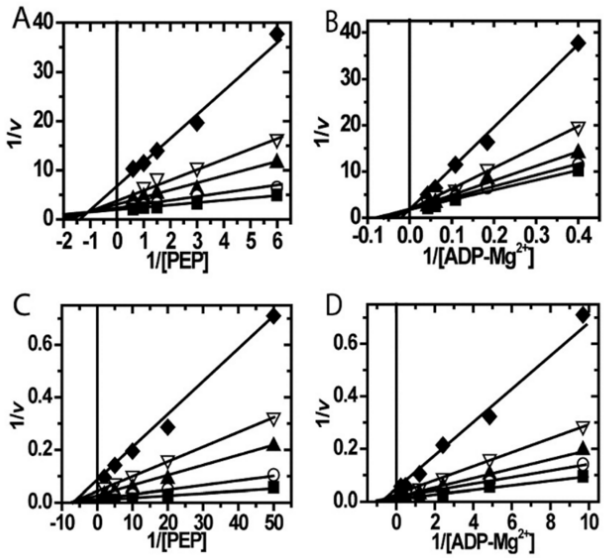

Figure 13: Reciprocal Plots in the Presence and Absence of K^+. In this figure, four reciprocal plots were created of velocity versus PEP concentration in the absence (A and B) and the presence of 90 mM K^+ (C and D). (A) The variable fixed concentrations of PEP from top to bottom are: 5.43 mM, 9.22 mM, 16.96 mM, and 23.46 mM. (C) The variable fixed concentrations of PEP from top to bottom are: 0.1 mM, 0.2 mM, 0.41 mM, 0.83 mM, and 2.1 mM. (B) The variable fixed concentrations of ADP-Mg^{2+} from top to bottom are: 0.17 mM, 0.33 mM, 0.67 mM, 1.0 mM and 1.67 mM. (D) The variable fixed concentrations of ADP-Mg^{2+} from top to bottom are: 0.02 mM, 0.05 mM, 0.1 mM, 0.2 mM, and 0.5 mM.[11]

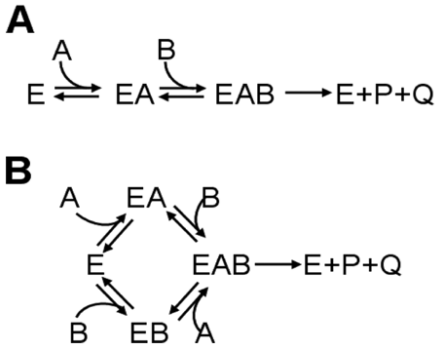

Figure 14: Rapid Equilibrium Kinetic Mechanisms. This figure illustrates an ordered (A) and random (B) rapid equilibrium kinetic mechanism.[11]

Role of Oxalate

Independent of the K^+ concentration, oxalate has been shown to be a competitive inhibitor of PEP and a noncompetitive inhibitor of ADP-Mg^{2+}.[11] It has been suggested that oxalate forms a nonproductive ternary complex which reduces V_{max}. This does not alter the binding of ADP-Mg^{2+}.[11]

Reaction Mechanism

Pyruvate kinase plays a key role in glycolysis. In the final step of the glycolytic pathway, pyruvate kinase acts as a catalyst by facilitating the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP).[1] This reaction yields pyruvate and adenosine triphosphate (ATP). This reaction is demonstrated in Figure 15 and Figure 16. This reaction requires one potassium ion and two magnesium ions to proceed.[8] These positively charged ions along with the positively charged sidechains of Arg-49 and Lys-269 are located in the pocket.[8] The negative charges of the phosphate group before the formation of the transition state are dissipated as a result of this positively charged electrostatic pocket. This causes the phosphorous atom to be more vulnerable to

nucleophilic attack as a result of the phosphorous atom being more electrophilic in this positive pocket.[8]

The first step is the nucleophilic attack of the phosphorous atom in PEP by the negatively charged oxygen not stabilized by the magnesium ion in ADP.[12] This is the phosphorylation reaction pyruvate kinase catalyzes. This step forms one molecule of ATP and enolpyruvate. ATP leaves the reaction and can be used for energy in the body. The second step of this reaction is the tautomerization of enolypyruvate.[12] After the phosphate group is removed, the enol group rearranges itself into a keto group. A proton is needed for this step to bind to the third carbon in the structure. The final product, pyruvate, is acquired after this reorganization.[12] For each mole of glucose that is metabolized in glycolysis, a net gain of 2 moles of ATP are produced from ADP.[2] Under physiological conditions, this reaction is fundamentally irreversible because of the strong exergonic quality of the transformation of the substrate PEP to the product pyruvate.[2]

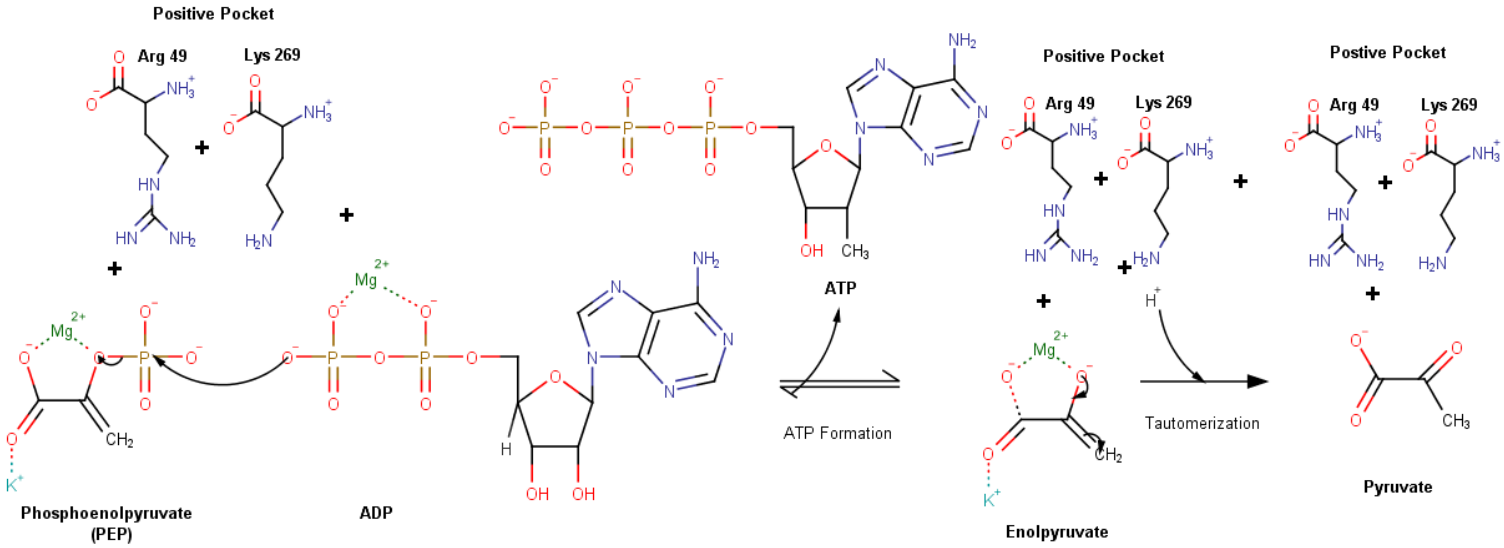

Figure 15: Proposed Step-Wise Mechanism of Pyruvate Kinase. The figure shows the positive pocket containing the positive Arg-49 and Lys-269 sidechains along with the potassium and magnesium ions. PEP is phosphorylated by ADP to from enolpyruvate and ATP. Enolpyruvate is tautomerized to pyruvate with a proton from the solution. [8, 12]

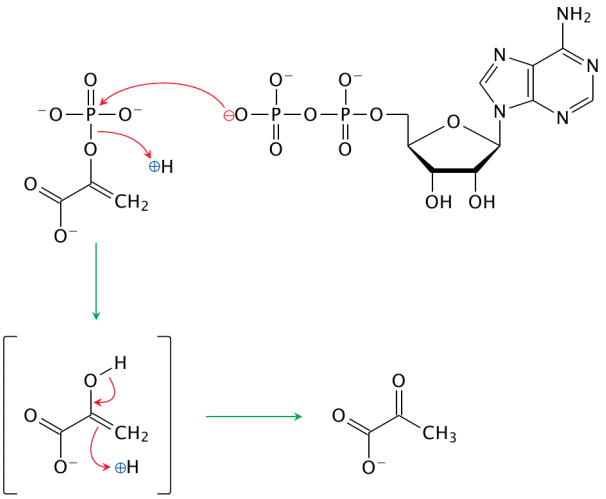

Figure 16: Simplified Mechanism of Pyruvate Kinase: In the above mechanism, the first step is the transfer of the phosphate group on phosphoenolpyruvate to ADP. This forms an intermediate product called enolpyruvate. The removal of the phosphate group enables the enol group to rearrange itself into a keto group which is the second step of the reaction. This step is very exergonic and is required to counterbalance the energy required to convert the phosphoester to the anhydride. This pushes the reaction toward the side forming ATP and the reaction is practically irreversible under physiological conditions.[12]

Disease Implications

Pyruvate Kinase Deficiency (PKD)

As mentioned earlier, a deficiency in pyruvate kinase can result in chronic hemolytic anemia.[3] The anaerobic production of erythrocyte ATP is disrupted leading to the shortening of cell survival. Cell death is caused by the impairment of iron homeostasis, reduction of membrane deformability, and an increase in 2,3-diphosphoglycerate(2,3-DPG) .[3] This increase in 2,3-DPG catalyzes a chain of inhibitions starting with hexokinase and then glycolic function.[3] PKD is caused by loss-of-function mutations in PKLR and follows an autosomal recessive inheritance pattern.[5] PKD is characterized by over 180 mutations in the PKLR gene.[9] For every 20,000 people, there is an estimated 1 case of homozygous PKD.[5]

The severity of PKD varies dramatically among individuals with homozygous recessive individuals being impacted the most, followed by heterozygous individuals, and then homozygous dominant individuals who do not have the disease. The homozygous recessive individuals normally have very poor health overall consisting of severe anemia and are forced to rely on blood transfusions for survival.[13] These individuals might also require a splenectomy. Regardless, homozygous recessive individuals normally require life-long care because of the severity of their disorder. Considering the phenotypic spectrum of PKD is extremely variable, individuals that are mildly affected do not normally require a neonatal transfusion or splenectomy.[3] Heterozygous individuals have been shown to have an advantage over their homozygous recessive and dominant counterparts.[4]

PKD and Malaria Resistance

The second most common erythrocyte enzyme disorder, PKD has been discovered in all regions of the world previously endemic of malaria.[13] The impact malaria has had on the population of the world cannot be understated. Malaria causes one million deaths and 500 million clinical cases annually.[5] One of the causes of malaria is *Plasmodium falciparum*. Recent research has indicated that PKD provides protection against infection and replication of *P. falciparum* in human erythrocytes.[5] A dual mechanism was used to explain this conclusion, shown in Figure 17. First, erythrocytes showed a decreases in invasion

by *P. falciparum* parasites in PKD patients when compared to a control group without PKD.[5] Second, erythrocytes of individuals with PKD that also were infected with *P. falciparum* experienced more extensive phagocytosis than did individuals from the control group that were also infected with *P. falciparum*.[5] Based on this mechanism, individuals with PKD may be protected against malaria as a result of a decrease in the number of erythrocytes infected with parasites or as a result of a decrease in the parasite affliction in general.[5]

Heterozygous individuals benefit from having this protective effect against malaria, while not having all the negative effects associated with recessive homozygous mutations in PKLR causing severe, life-threatening cases of PKD. Unfortunately for homozygous dominant individuals, they do not possess the protective factor against malaria, however, they do not have PKD.

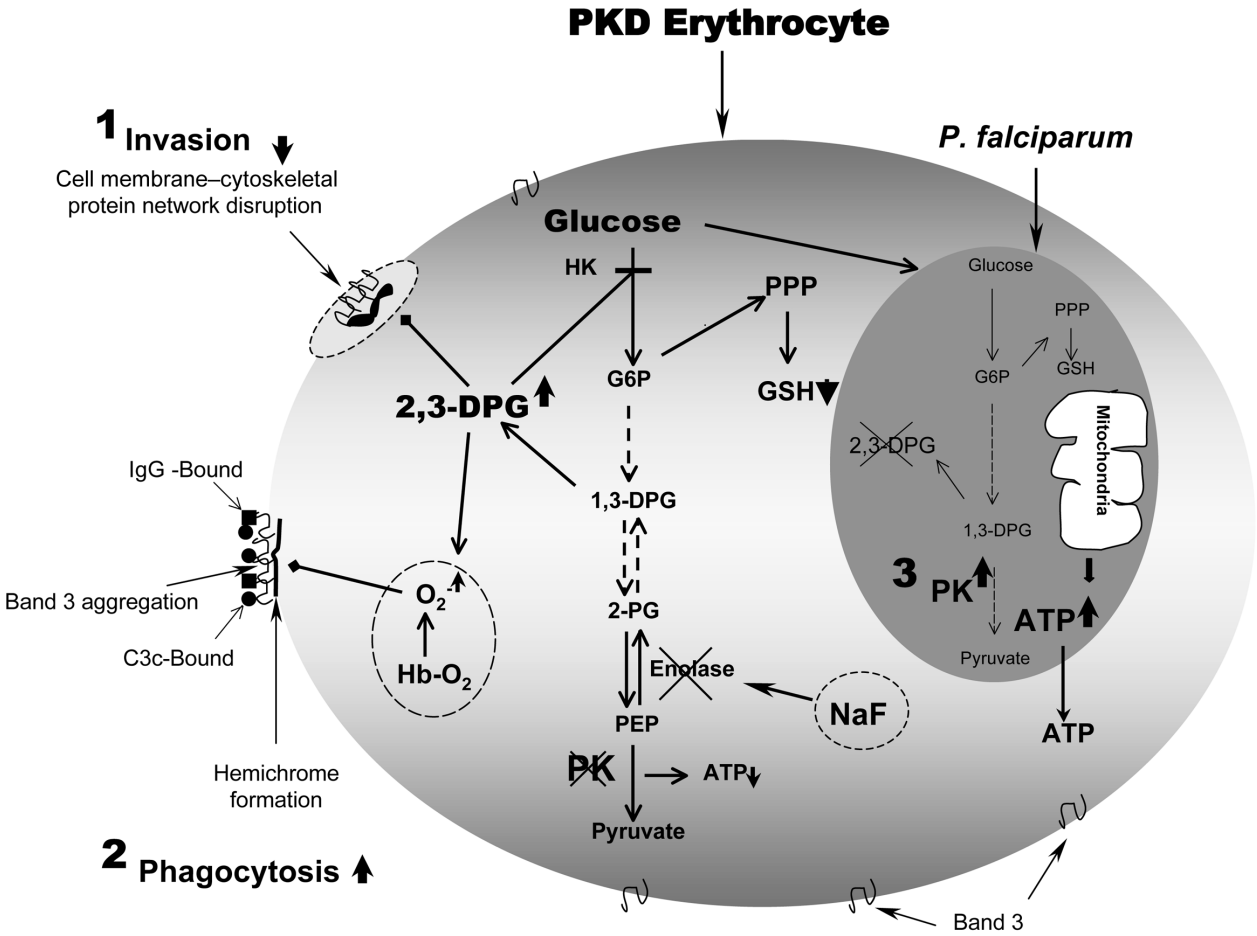

Figure 17: Mechanism of PKD against Malaria. An overview of the mechanism is shown in the above figure. Decreased levels of erythrocyte ATP and increased levels of 2,3-DPG are characteristics of PKD. When combined, these may interfere with the cell membrane and cytoskeleton protein network, which decreases invasion of the erythrocyte increased clearance of ring-stage erythrocytes with parasites. Increased radical production, hemichrome formation, and band 3 aggregation may further damage the PKD erythrocyte as a result of the parasite invasion. The limited number of parasites that actually invade the PKD erythrocyte survive by increasing parasite ATP production. Key: 1,3-DPG, 1,3-

diphosphoglycerate; 2-PG, 2-phosphoglycerate; G6P, glucose-6-phosphate; GSH, glutathione; Hb-O_2, oxyhemoglobin; HK, hexokinase; O_2^-, superoxide; PEP, phosphoenolpyruvate; PPP, pentose phosphate pathway.[13]

Resources

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