Problem Set 6 (PLP, TPP and glycolysis and regulation)

Problem 1. Pyruvate ferredoxin oxidoreductase (PFO) and pyruvate oxidase (POX) catalyze the reactions shown in the equations below. In the PFOR case (Eq 1), the oxidant is ultimately ferredoxin oxidized [2Fe22S]$^{2+/1+}$ and the structure is shown in Figure 1.
In POX (Eq 2) the cofactor is FAD.

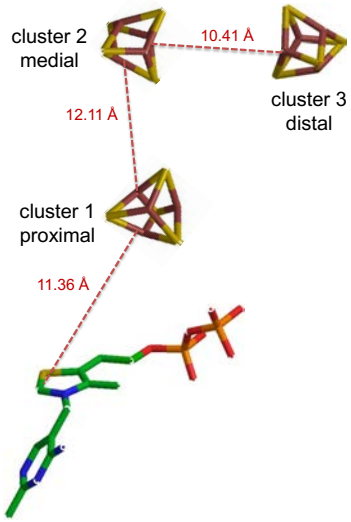

Figure 1 PFOR with TPP and FeS clusters shown. Ferredoxin (Fd) is not shown.

Eq 1 PFOR: $pyruvate + CoA + 2\,Fd_{ox} \rightleftharpoons acetyl\text{-}CoA + CO_2 + 2\,Fd_{red}$

Eq 2 POX: $pyruvate + phosphate + oxygen + H^+ \longrightarrow acetyl\ phosphate + CO_2 + H_2O_2$

Questions:

1) Based on what you now know about the mechanisms of VitB1 and FeS clusters, propose a mechanism (curved arrows with chemical structures) for the transformation of pyruvate to acetlyCoA by PFOR.
2) The mechanism of POX is similar to that for PFOR. Show in the case of POX, how acetyl phosphate could be formed from one of the intermediates in your mechanism in part 1.
3) Propose a role for FAD and O_2. You DO NOT need to draw a detailed chemical mechanism, just suggest in words a proposed function for these substrates.

Problem 2. PGlyM (phosphoglycerate mutase) catalyzes the conversion of 3-PGA to 2-PGA and requires a phosphorylated histidine intermediate for this process (the proposed scheme is shown in Figure 2). Recently PEP (phosphoenol pyruvate), a metabolite near the end of the glycolysis pathway, was shown by mass spectrometric methods to be able to phosphorylate PGlyM:

$$PGlyM - His \;+\; PEP \rightleftharpoons PGlyM - His - {PO_4}^{2-} \;+\; pyruvate$$

Questions:

1) Is this a reasonable chemical reaction to propose? Explain your thinking.
2) What are the consequences of this reaction on the major function of the glycolysis pathway?

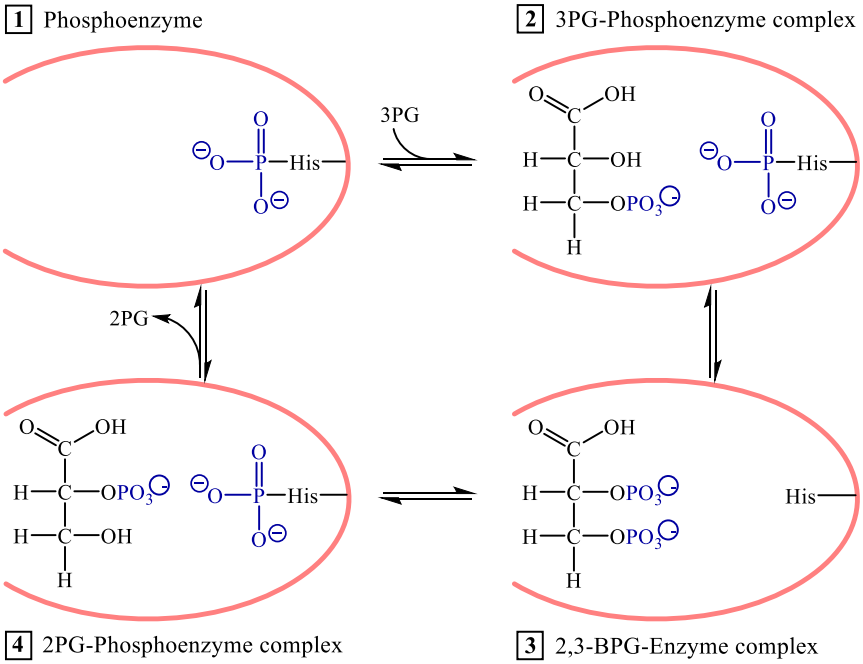

Figure 2. Phosphoglycerate mutase enzyme in the glycolysis pathway and the proposed mechanism of interconversion of 3-PGA to 2-PGA

Problem 3. ATP is a metabolite present in cells at high levels (> mM) and at most, its levels fluctuate 10% under different environmental conditions (resting muscle cell vs muscle cell after sprinting 100 meters). This observation raises the puzzling issue of how altering ATP concentations can control key enzymes in glycolysis such as PFK-1. ATP-Mg^{2+} (ATP is always complexed to Mg^{2+}) concentrations are buffered in two ways in muscle cells: via creatine kinase and by adenylate kinase.

Creatine kinase catalyzes: $Cr - P + ADP\text{-}Mg^{2+} \longrightarrow Cr + ATP\text{-}Mg^{2+}$

Adenylate kinase catalyzes: $ATP\text{-}Mg^{2+} + AMP\text{-}Mg^{2+} \rightleftharpoons 2\ ADP\text{-}Mg^{2+}$ K=0.44

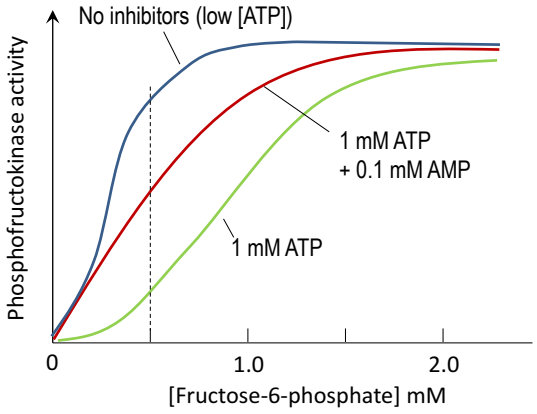

Figure 3. The activity of PFK-1 ($F\text{-}6\text{-}P + ATP\text{-}Mg^{2+} \longrightarrow F\text{-}1,6\text{-}P_2 + ADP\text{-}Mg^{2+}$) and its regulation by ATP-Mg^{2+} and AMP-Mg^{2+}

The concentrations of ATP and AMP as shown in Figure 3, play an important role in controlling the flux through the glycolysis pathway by regulating PFK-1. In general, the concentration of ATP (crude estimate) is 50 x higher than that of AMP and 10 x higher than ADP, and over a short period of time the total adenylate pool (ATP, ADP and AMP) in the cell remains constant.

Questions:

1) Using the data shown in Figure 3, provide an explanation for the low and 1 mM ATP curves, on PFK-1 activity. Does your explanation make sense based on your understanding of the function of the glycolysis pathway?
2) Suppose the concentration of ATP changed from 1 mM to 0.9 mM. What effect would this change have on the concentrations of ADP and AMP? Can this explain the ATP/AMP red curve in Figure 3? Why?

Problem 4. Serine transhydroxymethylase is a PLP requiring enzyme that catalyzes the formation of glycine and formaldehyde from serine (Eq 3). In reality the formaldehyde is transferred directly to another Vitamin (Folate) to form a formylated folate. Both products play an essential role in purine and pyrimidine metabolism. For the purpose of this problem you can ignore the folate participation and assume that Eq 3 describes this reaction. The enzyme requires PLP (pyridoxal phosphate) to effect this transformation. [Hint: Remember in this reaction you are cleaving a C-C bond and this occurs through aldol reactions.]

Eq 3 $\quad serine \rightleftharpoons glycine + formaldehyde\ (H_2CO)$

Question:

1) Given what you have learned about the chemistry of PLP at the α-position of amino acids, propose a mechanism for this transformation (use curved arrows and chemical structures).

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