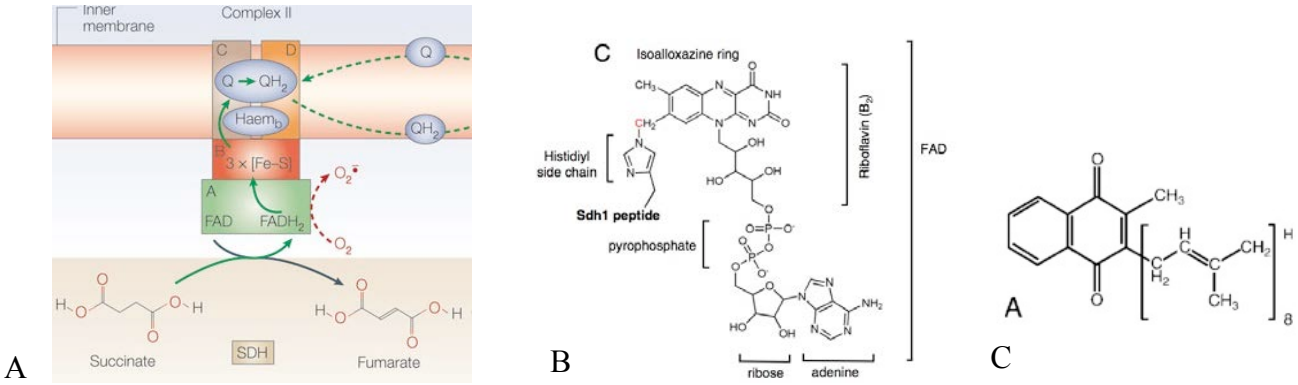
Chemistry 5.07 2013
Problem Set 5 Answers

Problem 1

Succinate dehydrogenase (SDH) is a heterotetramer enzyme complex that catalyzes the oxidation of succinate to fumarate with concomitant reduction of ubiquinone (Q) to ubiquinol (QH2). Subunit 1 contains the deeply buried flavin (Fig 1B) and subunit 2 contains additional cofactors that include three distinct iron sulfur cofactors. Ultimately the electrons reduce the quinone that is bound at the interface of subunit 2 and the membrane-spanning subunits (subunit 3 and subunit 4)[see Figure 2].

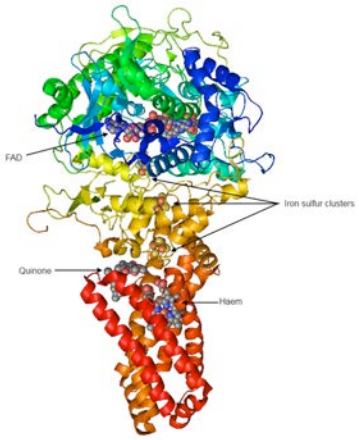


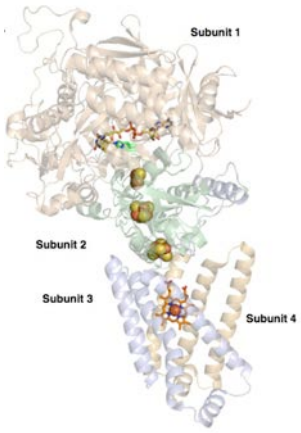
A

Courtesy of Nature Publishing Group. Used with permission.

Courtesy of Elsevier. Used with permission.

Figure 1. A. *A cartoon of the SDH with its cofactors, its location in the inner mitochondrial membrane and the domains of the protein for the conversion of succinate to fumarate and Q to QH2.* ***B.*** *The covalent linkage of the flavin to subunit 1.****C.*** *The structure of Q.*





© Steve Cook. Licensed under a Creative Commons License CC-BY-SA-3.0.
All rights reserved. This content is excluded from our Creative Commons license.
For more information, see https://ocw.mit.edu/help/faq-fair-use.

Courtesy of Elsevier.
Used with permission.

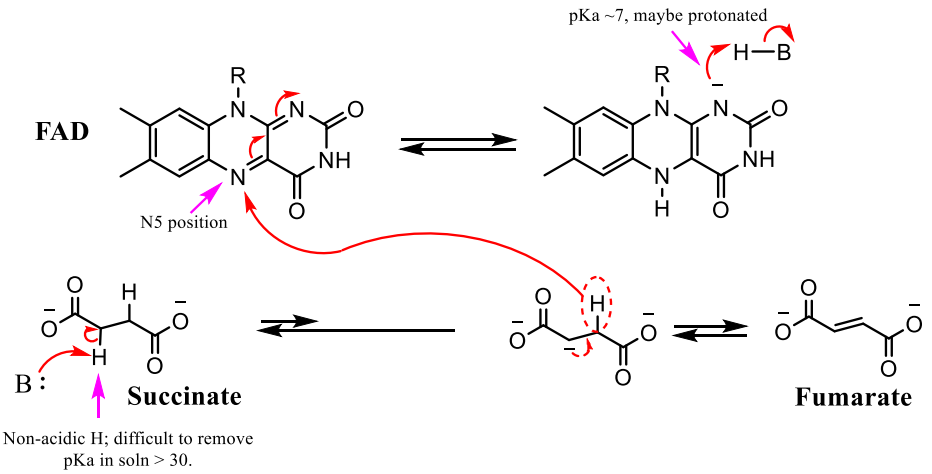
Figure 2. *Two different views of SDH (Upside down compared to Figure 1). Note the position of the FAD and the quinone in the structure on the left. The structure on the right has the different subunits more clearly delineated and the FeSs are easier to see. Also there is a heme present that is not directly involved in the actual oxidation process. As discussed in class FADH2 can rapidly react with O2 to form reductive metabolites of oxygen such as O2 • (Fig 1A). You will discuss this uncoupling reaction soon, but it should remind you of Hb where its heme cofactor gets oxidized to Fe3+ and loses its ability to reversible bind O2 or the α-ketogluratate dioxygenase involved in hydroxylating proline in collagen biosynthesis.*

Questions:

1. Propose a mechanism (chemical structures and curved arrows showing how the electrons are transferred) for the oxidation of succinate to fumarate by FAD. Given our discussions of pKas, what is likely to be the most challenging step in this process?
2. The FADH2 formed in the above reaction is covalently bound to the enzyme (see Figure 1B). Thus it must be reoxidized on the protein. Given the proximity of the cofactors shown in Figure 2, what is the oxidant and propose a mechanism for how this oxidation might proceed.
3. The spacing between the FAD----2Fe2S cluster----4Fe4S cluster-----3Fe4S cluster----Q are 12.2. 9.8, 8.9. 7.1 Å. Do these long distances make sense? Why? Provide an explanation for your answer.
4. Propose a chemical mechanism (curved arrows to show electron flow) for how Q is reduced to QH2.

Answers:

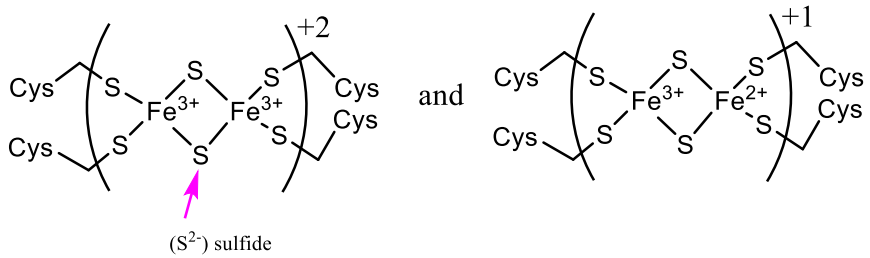
1.

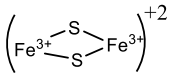


There are fumarate reductases that work under "anaerobic" conditions and SDH in complex II (aerobic conditions). The former has been better characterized crystallographically. They have very similar active sites. The most challenging

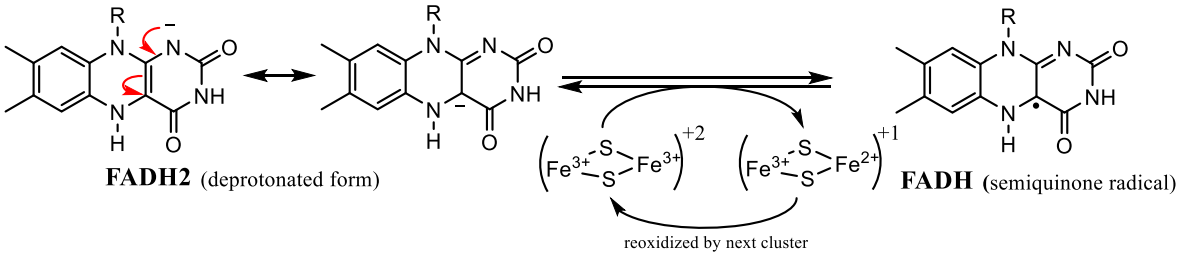
step is generation of the carbanion adjacent to the carboxylate. The proposal is that "R" is involved.

2. The nearest oxidant is the [2Fe2S]$^{2+/1+}$ cluster. The only two oxidation states available are +2 and +1.

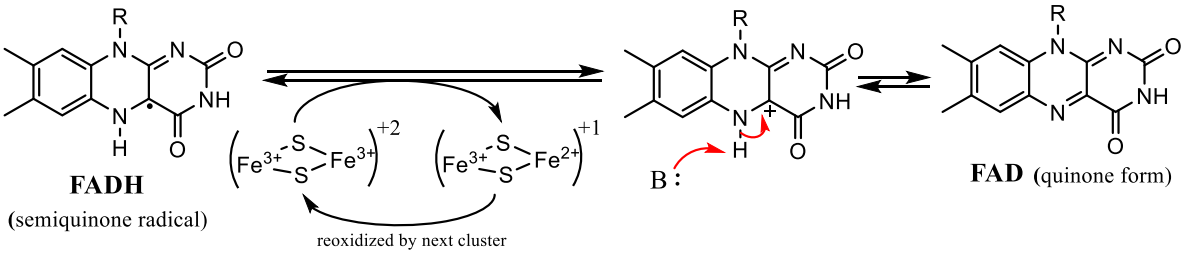


Thus, the electrons removed from the reduced flavin ($FADH_2$ (if protonated)), must occur one at a time. To drive the reaction to the right, once the [2Fe2S]$^{+2}$ is reduced to [2Fe2S]$^{+1}$, it can be reoxidized by the next FeS cluster. Thus, it is reset in the  **state to receive a second electron from the one electron oxidized flavin.**

Reaction:

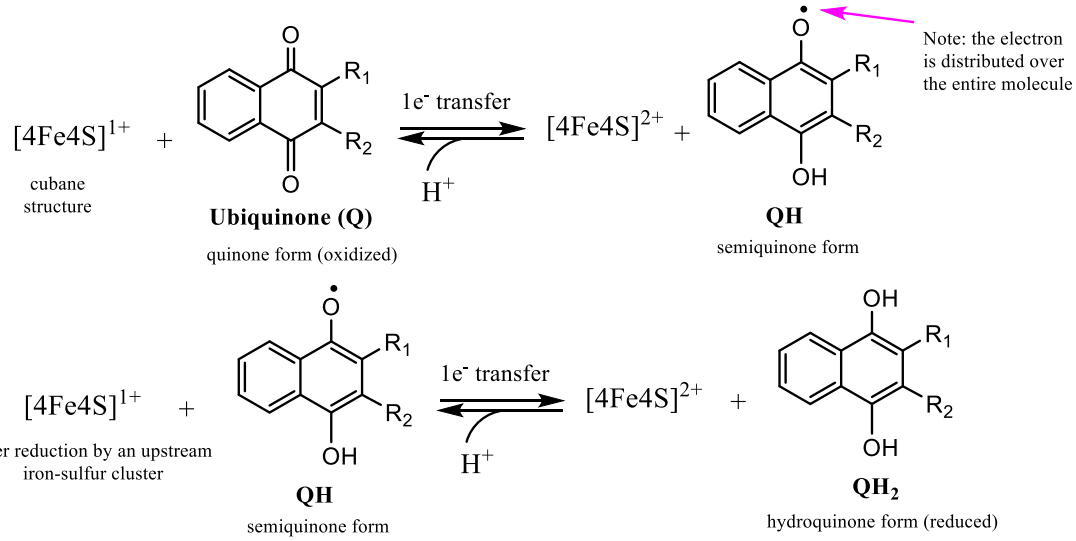


The [2Fe2S] cluster gets reoxidized by the next cluster and a second electron can be transferred, along with the loss of a H$^+$ to generate FAD.



3. Nature has a design principle that has emerged for electron transfer (ET) processes. The centers are spaced 10 – 15 Å from the flavin. Thus, the ET occurs via a "tunneling" process associated with the wave behavior of electron associated with its small mass. ET reactions occur at <u>very fast</u> rates even at 10 Å distances and are associated with the orbital overlap of the donor and acceptor; the $\Delta G^{o'}$ – driving force for the oxidation process and λ(the reorganization energy – change in geometry or solvation) are also associated with the redox change. The rate constant for ET is described by Marcus equation given in class.

4. The quinone in Fig 2 is very far removed from the FAD. Quinones also can mediate either 1-electron or 2-electron reactions. The only electron donor in the structure is the $[4Fe4S]^{2+/1+}$ cluster. Thus, the proposal is that the quinone is reduced to the hydroquinone by two sequential 1-electron transfers and two H^+ transfers.



Problem 2

The oxidation of $FADH_2$ in succinate dehydrogenase (via the electron transport chain you will be discussing soon) yields sufficient energy to drive the synthesis of two moles of ATP per mole of $FADH_2$.

Overall reaction: $FADH_2 + ½\ O_2 + 2ADP + 2\ P_i \rightarrow FAD + 3H_2O + 2ATP$

Think about the two reactions that could lead to the overall reaction:
i. $FADH_2 + ½O_2 \rightarrow FAD + H_2O$
ii. $2ADP + 2P_i \rightarrow 2ATP + 2H_2O$

Calculate the standard reduction potential of the enzyme bound $FADH_2$ in succinate dehydrogenase assuming 37% efficiency of the energy conservation reactions in vivo. That is, calculate $E^{o'}$ for $FAD + 2H^+ + 2e^- \rightarrow FADH_2$.

You are given the following half reaction to help you make this calculation:
$½O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $E°' = +0.816$ V.

Answer:

Both NADH and $FADH_2$ feed into the respiratory chain and the energy released on their oxidation can be used to generate a proton gradient across the inner mitochondrial membrane that can then be used to make ATP. You will discuss this process in detail in the coming week.

The overall reaction above can be considered as the sum of the two reactions i and ii.

i. $FADH_2 + ½O_2 \rightarrow FAD + H_2O$ $\Delta G_2°' = ??$ kJ/mol
ii. $2ADP + 2P_i \rightarrow 2ATP + 2H_2O$ $\Delta G_3°' = 2(+30.5$ kJ/mol$) = 61$ kJ/mol
(or $2 \times 7.3 = 14.6$ kcal/mol)

$FADH_2 + ½\ O_2 + 2ADP + 2\ P_i \rightarrow FAD + 3H_2O + 2ATP$ $\Delta G_1°' = \Delta G_2°' + \Delta G_3°'$

If the energy required to make 2ATPs (reaction ii) represents 37% of the energy released in reaction i, (in other words, only 37% of the energy released gets converted into ATP), then $\Delta G_2°' = 61/0.37 = 165$ kJ/mol (39.4 kcal/mol) is released. Since oxidation is favorable, the ΔG is negative. $\Delta G_2°' = -165$ kJ/mol.

Therefore, $\Delta G_1°' = \Delta G_2°' + \Delta G_3°' = -165$ kJ/mol + 61 kJ/mol = -104 kJ/mol.

Now, let's return to the actual redox chemistry, where you have the two half reactions:

$FAD + 2H^+ + 2e^- \rightarrow FADH_2$ $E_1°' = ??$ V
$½O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $E_2°' = +0.816$ V

Sum: $FADH_2 + ½O_2 \rightarrow FAD + H_2O$ $\Delta E°' = E_2°' - E_1°'$

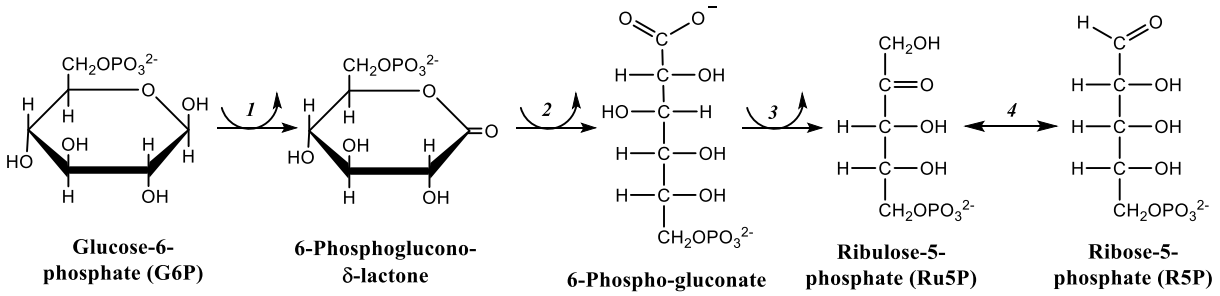
But this is also reaction i above, for which $\Delta G_2°' = -165$ kJ/mol = -39.4 kcal/mol.

$\Delta G_2°' = -nF\Delta E°'$. Therefore $\Delta E°' = -\Delta G_2°'/nF$
$\Delta E°' = -(-39.4$ kcal/mol$)/(2(23.063$ kcalV^{-1}mol^{-1}) $= 0.854$ V.

Therefore, $E_1°' = E_2°' - \Delta E°' = 0.816 - 0.854 = -0.038$ V.

Problem 3

Glucose-6-P can be converted to ribose-5-P by using four enzymes in the pathway shown below. Two of these reactions involve organic redox cofactors and neither of the proteins as isolated are yellow or have any detectable absorption spectrum. Recall that proteins absorb at 280 nm due to their Y and W residues.

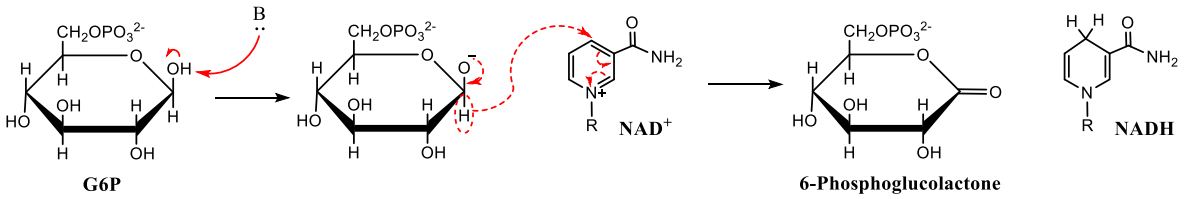


Questions: 1. Which two of the reactions require a redox cofactor that acts as a co-substrate? Draw a chemical mechanism for each of these reactions using structures and curved arrows. 2. The other two reactions should also be familiar. Draw a chemical mechanism for each of these reactions.

Answers:

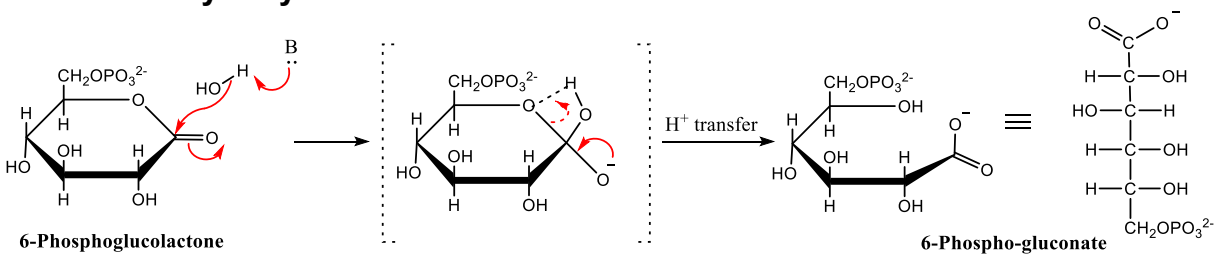
1.

Reaction1: you are doing an oxidation. Given that neither protein in this pathway is "yellow", this indicates the redox cofactor is NAD^+ and not FAD. All FAD-requiring proteins are bright yellow.



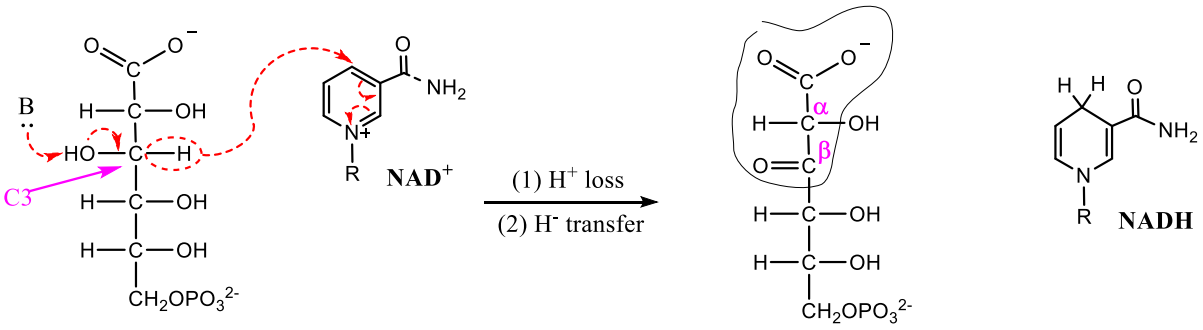
This reaction involves H^+ (proton) and H^- (hydride) transfer, almost always in separate steps.

Reaction 2: hydrolysis of lactone.



Hydrolysis occurs through a tetrahedral or high energy intermediate.

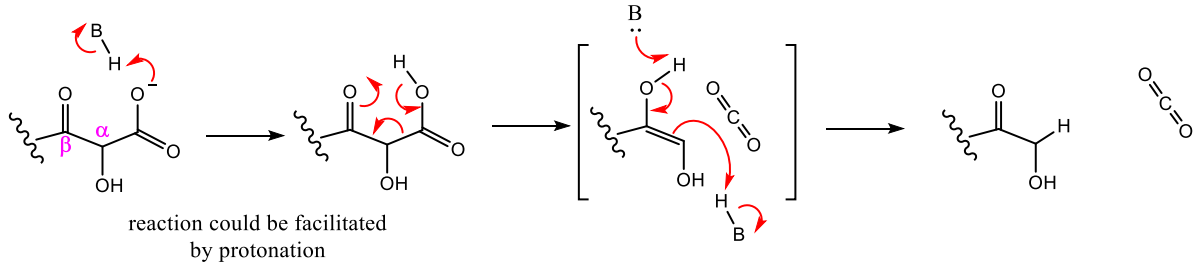
In step 3, you lose "CO_2" and go from C_6 to C_5. From the structures of the starting material and product, it appears as if C_3 is oxidized. NAD^+ is again the oxidant.



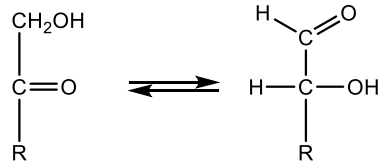
6-Phospho-gluconate

6-Phospho-3-keto-gluconate

You now have a β-keto acid, which readily undergoes loss of CO_2 (a decarboxylation) to give the product.

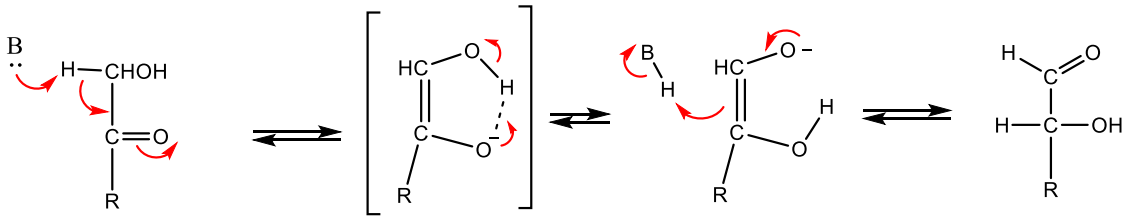


In the last step, the ketone is isomerized to the aldehyde. This reaction is analogous to triose phosphate isomerase in the glycolysis pathway.



Ketone Aldehyde

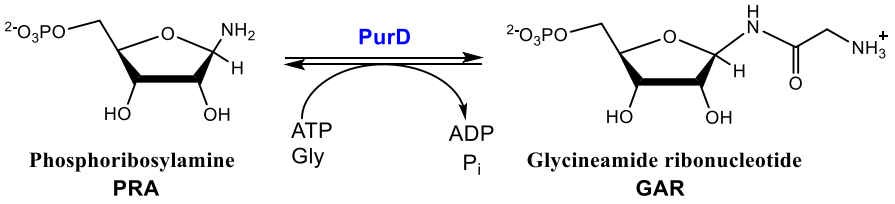
Mechanism:



Ketone cis-ene-diolate Aldehyde

Problem 4

In the purine biosynthetic pathway (the pathway that makes the adenosine of ATP) there are four members of the ATP grasp superfamily of enzymes. One of them, glycineamide ribonucleotide (GAR) synthetase (called PurD) catalyses the reaction shown below of phosphoribosylamine (PRA), ATP, and glycine to give glycineamide ribonucleotide (GAR) and ADP and Pi:

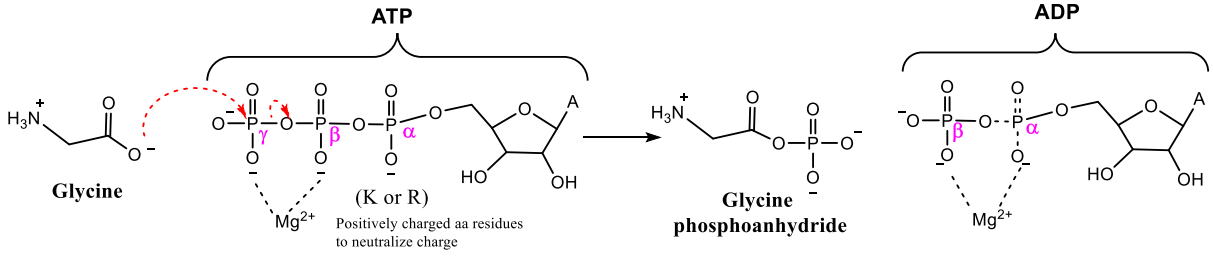


Questions:

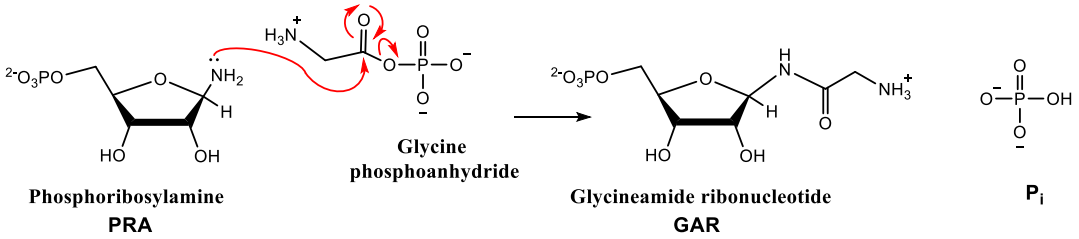
1. Draw an arrow pushing mechanism for this interconversion clearly showing the role of ATP. What is the additional cofactor that is missing?
2. Phosphoribosyl amine (PRA) produced by the first step in the purine biosynthetic pathway has a half life of only 10 sec under physiological conditions (pH 7). What would be the breakdown products of this molecule under these conditions (pH 7)? (Think about the carbohydrate chemistry discussed in Lecture 13).

Answers:

1. The carboxylate of glycine needs to be converted into a better leaving group. Since the product is ADP and P_i, ATP is used to phosphorylate glycine to a phosphoanhydride. The missing cofactor is Mg^{2+}, essential for all reactions involving ATP.

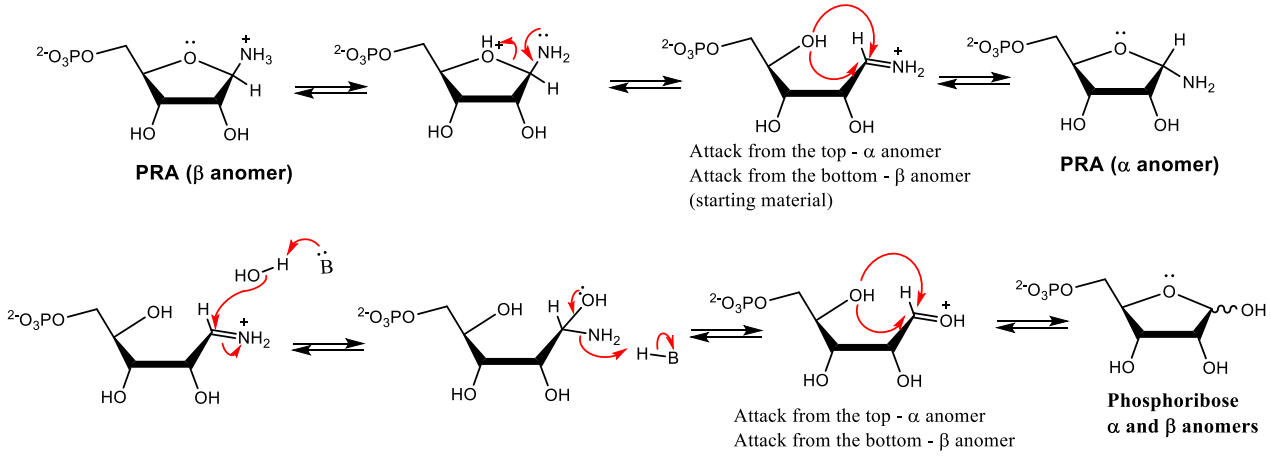


Note that Mg^{2+} moves to first activate the γ-phosphate for nucleophilic attack and then moves between α and β phosphates to facilitate ADP to "leave".



Note that PRA must be deprotonated to attack the activated glycine.

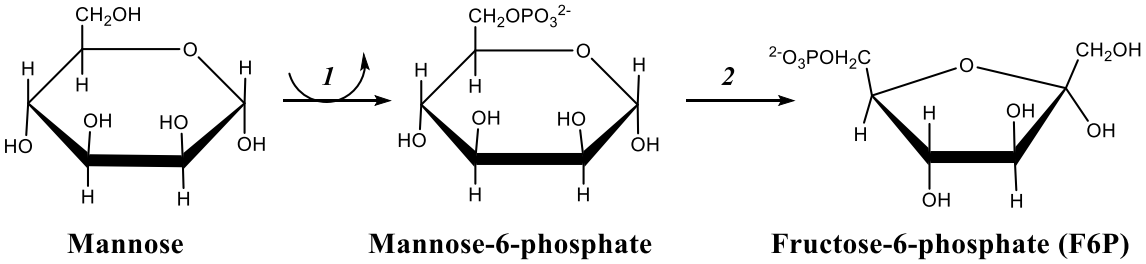
2. Upon protonation, PRA can ring open. The resulting iminium species can ring close to form the α anomer or it can hydrolyze to give phosphoribose anomers.



Thus, PRA rapidly anomerizes and hydrolyzes.

Problem 5

Mannose is the product of digestion of polysaccharides and of glycoproteins.

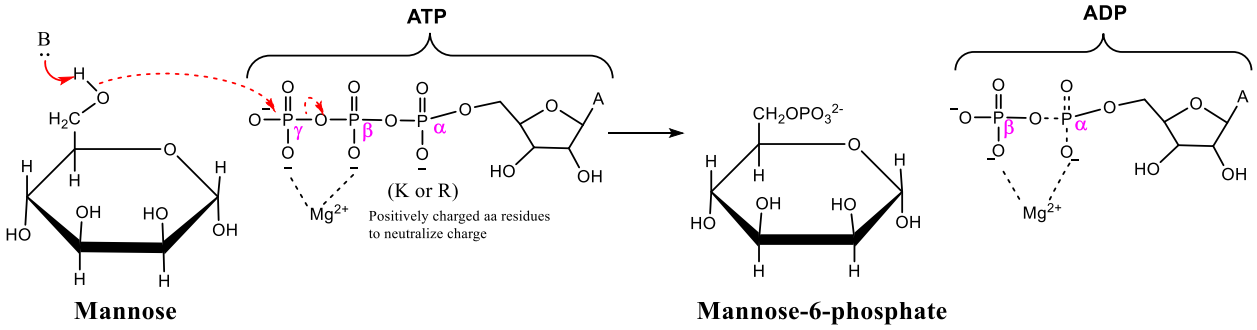


It can be used in the glycolysis pathway through reaction 1 and 2 in the above equation.

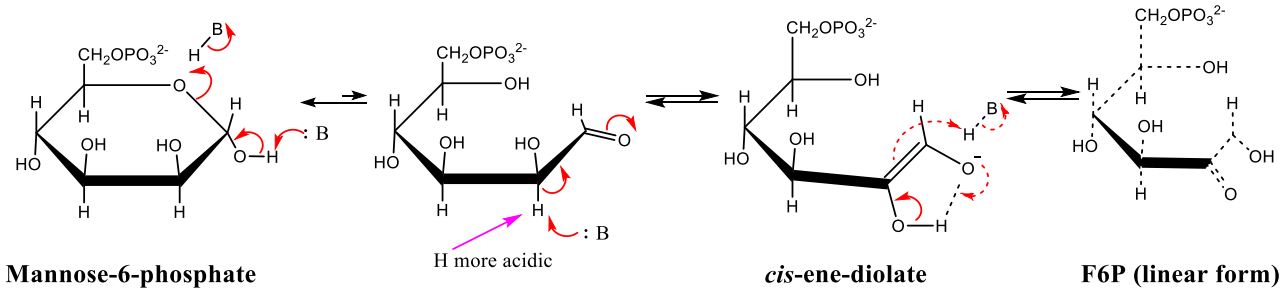
Questions:
1. What is the likely second substrate required for step 1 and what is the name of the enzyme that catalyzes this type of reaction? Show the chemical transformation using structures and curved arrows.
2. Propose a chemical mechanism (structures and curved arrows) for the conversion of mannose-6-P to F6P.
3. If the substrate for the second enzyme was mannose and not mannose-6-P, would you obtain the same product? Explain your answer which should explain why the phosphorylation step is important.

Answers:

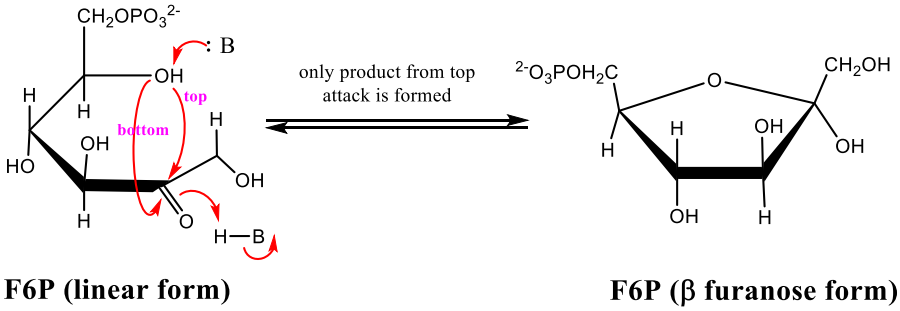
1. The second substrate is Mg^{2+}ATP. The phosphorylation of mannose is catalyzed by a *kinase*, also yielding ADP.



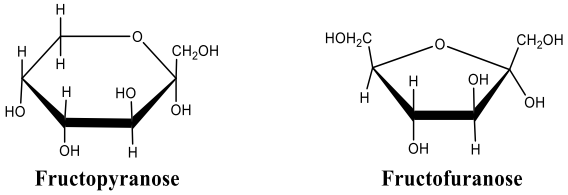
2. There are <u>NO</u> reactive positions in mannose-6-P. Therefore, the sugar must be ring-opened to the linear form to allow chemistry to occur. Then the isomerization of aldehyde to ketone happens as above (Problem 3).



Finally, the ring closure for F6P happens stereospecifically inside the enzyme. The attack is from the top face.



3. 6-membered ring sugars (pyranoses) are, in general, more stable than 5-membered ring sugars (furanoses). Without the phosphate, the reaction would produce fructopyranose, not fructofuranose.



MIT OpenCourseWare
https://ocw.mit.edu

5.07SC Biological Chemistry I
Fall 2013

For information about citing these materials or our Terms of Use, visit: https://ocw.mit.edu/terms.