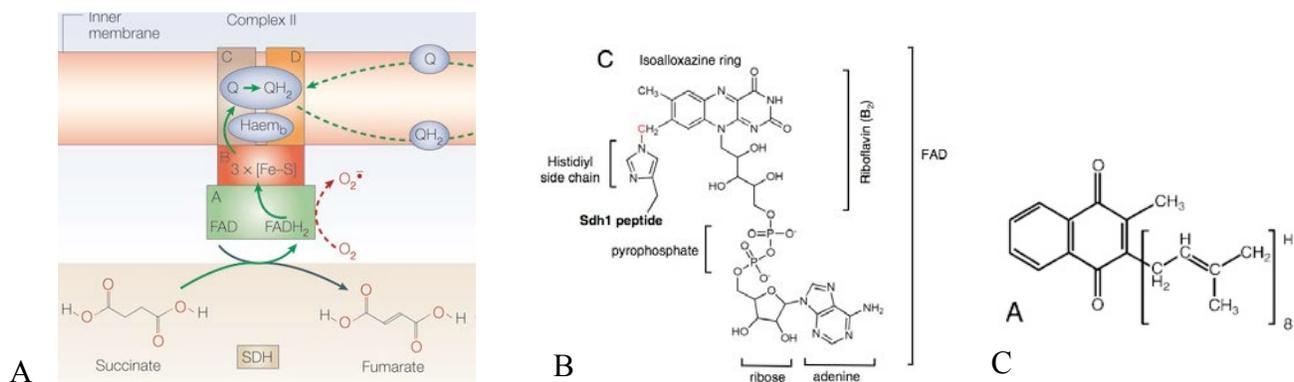


Chemistry 5.07 Problem Set 5

(redox cofactors and oxidation reactions; carbohydrate chemistry, and introduction to metabolism)

Problem 1

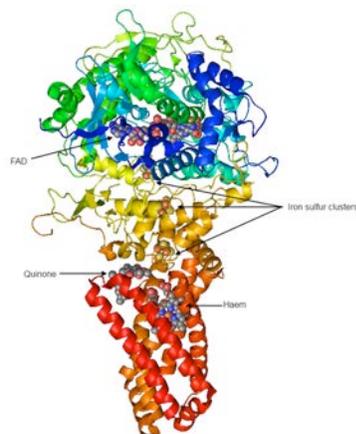
Succinate dehydrogenase (SDH) is a heterotetramer enzyme complex that catalyzes the oxidation of succinate to fumarate with concomitant reduction of ubiquinone (Q) to ubiquinol (QH₂). Subunit 1 contains the deeply buried flavin (Fig 1B) and subunit 2 contains additional cofactors that include three distinct 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].



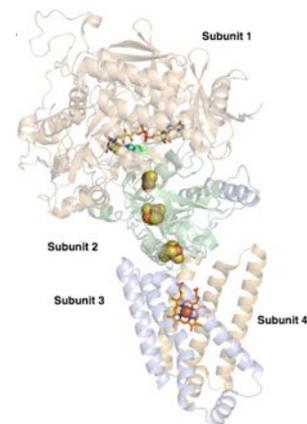
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Figure 1. A. A cartoon of the SDH with its cofactors, its location in the inner mitochondrial membrane and the domains of the protein for the conversion of succinate to fumarate and Q to QH₂. **B.** The covalent linkage of the flavin to subunit 1.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



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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-ketoglutarate 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 pK_as, 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.

Problem 2

The oxidation of FADH₂ 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 FADH₂.



Think about the two reactions that could lead to the overall reaction:

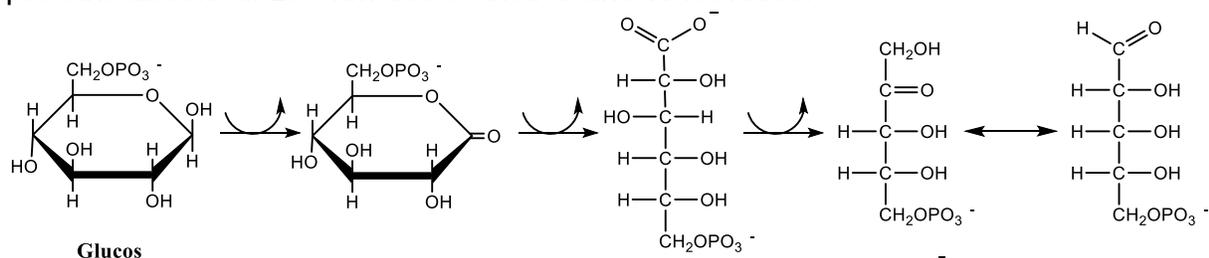
- i. $\text{FADH}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O}$
- ii. $2\text{ADP} + 2\text{P}_i \rightarrow 2\text{ATP} + 2\text{H}_2\text{O}$

Calculate the standard reduction potential of the enzyme bound FADH₂ in succinate dehydrogenase assuming 37% efficiency of the energy conservation reactions in vivo. That is, calculate E^{o'} for $\text{FAD} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{FADH}_2$.

You are given the following half reaction to help you make this calculation:
 $\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}$ E^{o'} = +0.816 V.

Problem 3

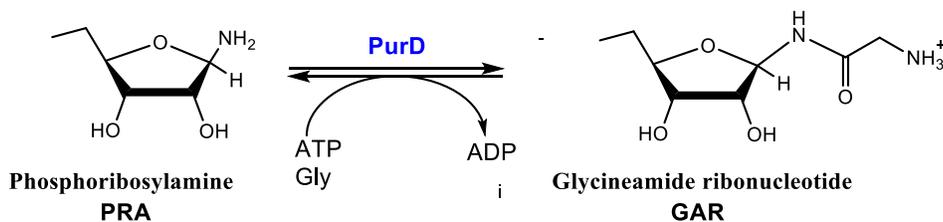
Glucose-6-P can be converted to ribose-5-P by using four enzymes in the pathway shown below. Two of these reactions involve organic redox cofactors and neither of the proteins as isolated are yellow or have any detectable absorption spectrum. Recall that proteins absorb at 280 nm due to their Y and W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

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:

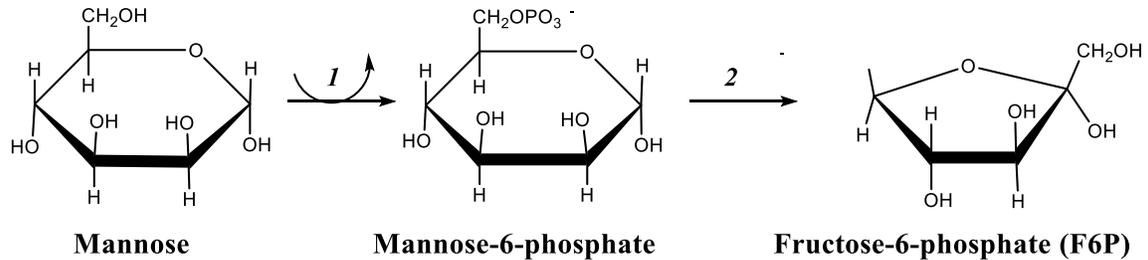


Questions:

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).

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.

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