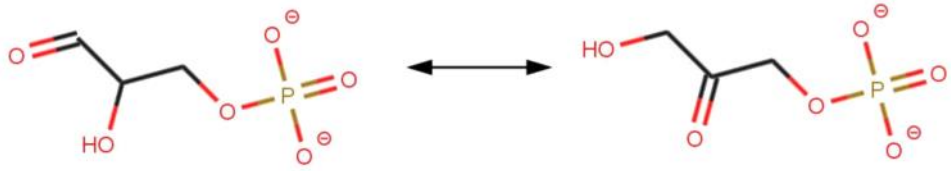
Enzymes and Cofactors

Tuesday, October 11, 2016 7:32 AM

Enzymes and Cofactor Chemistry

TIM

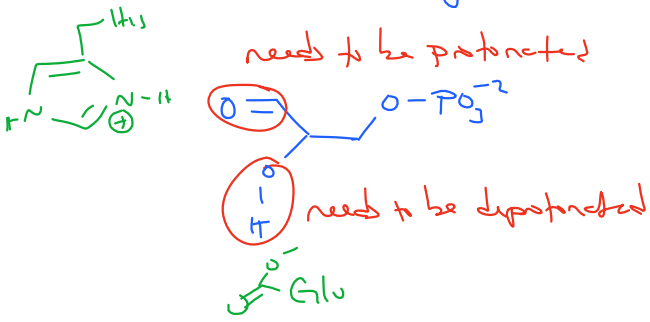
TIM catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). This reaction is shown below.



1. What class of enzyme catalyzes this reaction? Isomerase

2. Studies have identified Glu165 as a general base and His95 as a general acid that is important in this reaction. Based on that information, propose a mechanism by which this reaction proceeds.

As discussed in class, we only want to focus on understanding the active site - don't worry about the mechanism



3. Replacing Glu165 with an Asp could affect the catalytic rate. Give one explanation for why it would influence the rate and one reason why it may not.

would: asp is one carbon shorter - it may not be able to get close enough to deprotonate the substrate

would not: Asp has the same functionality as Glu so it should be able to do the same thing

4. This side chain of histidine has a pKa of 6.07. What protonation state would this residue be in at a physiological of pH 7.2? What protonation state does it need to be in to act as a general acid? Can you explain why these factor contradict?

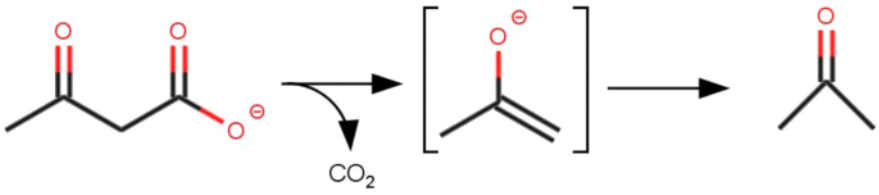
pH > pKa → this means His is deprotonated

However, His need to be the general acid, so it must be in the acidic form.

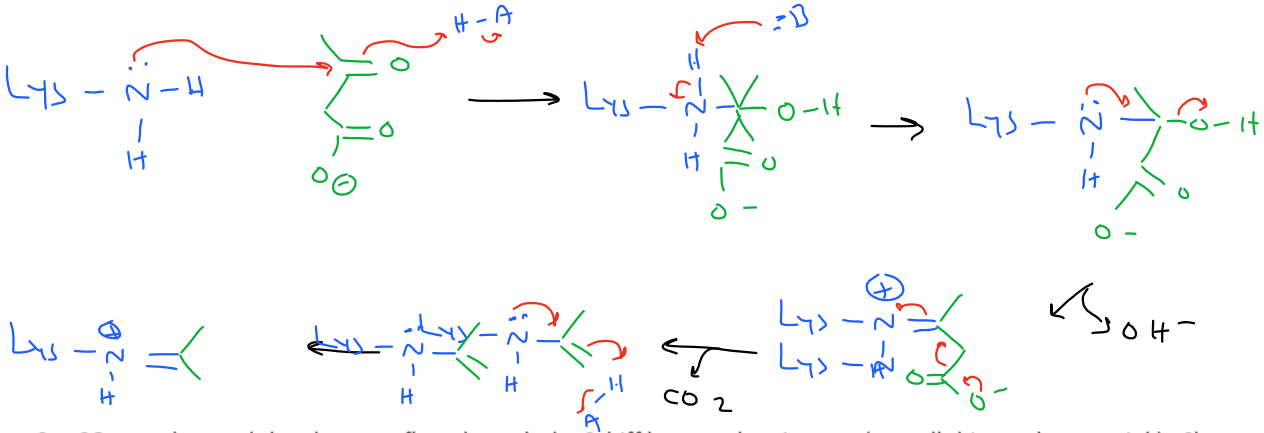
The pKa must be shifted by the chemical environment

Acetoacetate Decarboxylase – Schiff bases and electron sinks.

Acetoacetate decarboxylase catalyzes the removal of CO_2 from acetoacetate as shown in the image below. Your textbook describes this reaction as a very difficult one using standard chemical strategies because of the unstable enolate intermediate that forms (shown as the transition state below). The enzyme bypasses this by forming a Schiff base. Let's explore this mechanism.



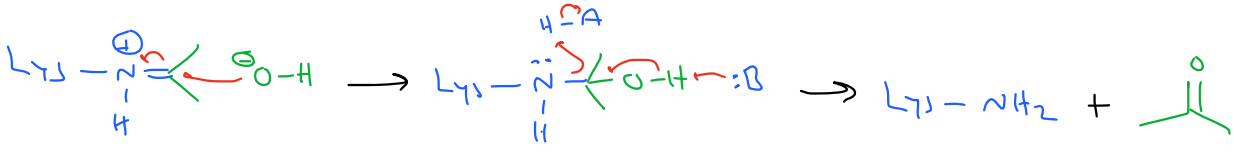
5. In the catalyzed reaction, the first step is the formation of a Schiff base between C3 (the carbonyl) of acetoacetate and a lysine residue from the enzyme. The steps of this formation are: 1. A deprotonated lysine attacks C3. 2. The nitrogen from the lysine gets deprotonated by a general base. 3. The lone pair on nitrogen forms a double bond with the carbon and hydroxide is lost. Sketch the mechanism for this process.



6. CO_2 now lost and the electrons flow through the Schiff base to the nitrogen (we call this an electron sink). Show this mechanism and compare the product to the transition state shown above. Why is it much lower energy?

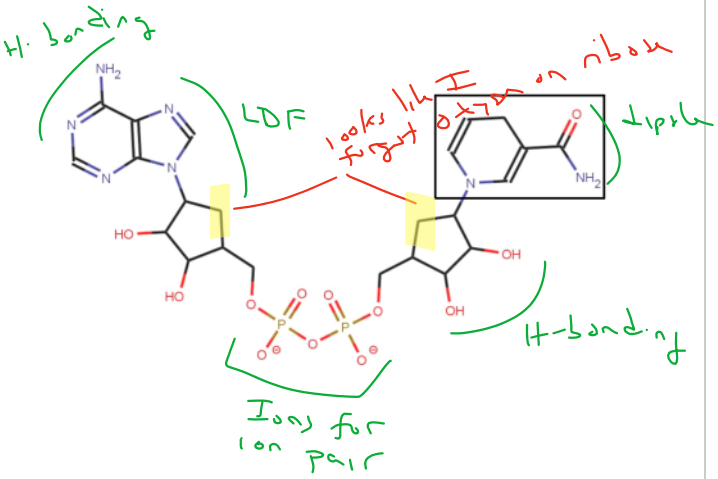
See mech above. The N cation serves as an electron sink that is pulling e⁻ to it. Once they "arrive", you have a stable intermediate that has formed

7. The final step is to reverse the Schiff base formation to release acetone. Show the mechanism for this process.



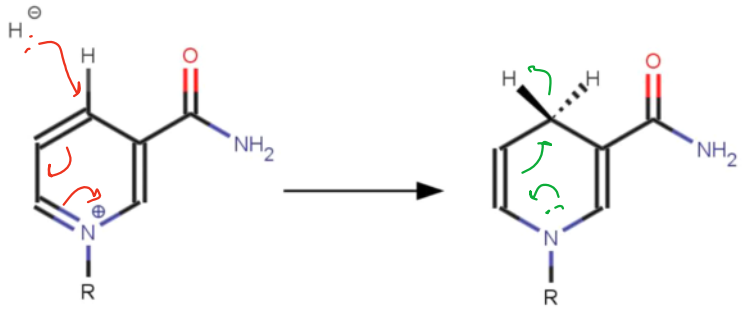
NADH and Lactate Dehydrogenase

Nicotinamide adenine dinucleotide (NADH) is shown to the right. The business end of this molecule is shown with a box around it.

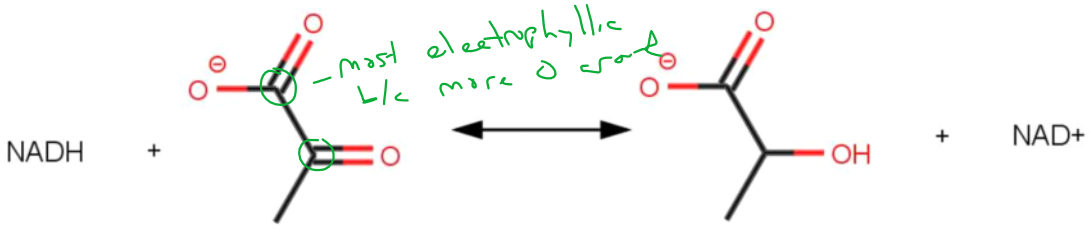


9. Examine this image and comment on what features NADH presents that could be recognized by enzymes that use it.

10. NADH is an important cofactor for oxidoreductases – enzymes that catalyze electron transfer reactions. This cofactor contributes to these reactions by facilitating the transfer or electrons in the form of a hydride anion (H^-[1]). The reaction below shows the oxidized (NAD^+, left) and reduced (NADH, right) form of the cofactor. On this diagram, push electrons to show how hydride interacts with the cofactor.



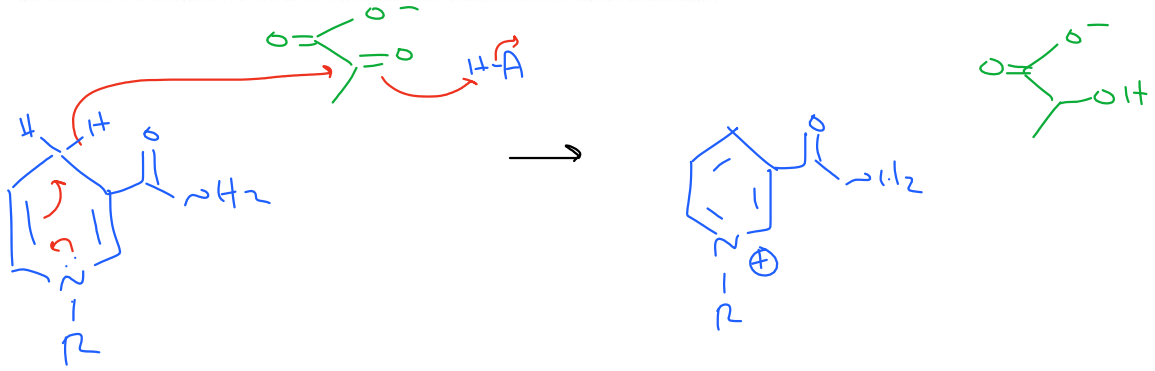
11. NADH is a required cofactor for the interconversion of pyruvate (left) and lactate (right). On the reaction scheme below, identify all electropyllic carbons. Which is the most electrophyllic? Explain your choices.



12. Sketch a mechanism for the reduction of pyruvate to lactate. Hydride ($H:^-$) initiates the reaction.

Note here that careful inspection of the react/prod shows that H:⁻ should attach @ C_2, not C_1. As discussed later, the enzyme can ensure this!

13. As it is indicated above, NADH is the source of hydride for the reduction. Redraw your mechanism showing the production of hydride from the business end of NADH (see above).



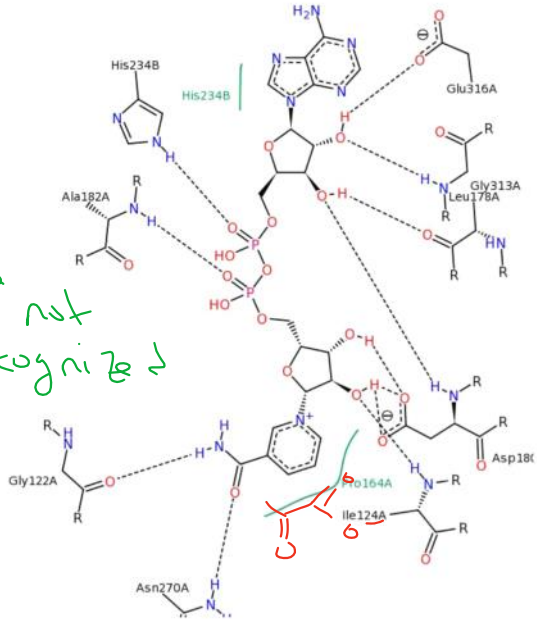
14. In problem 7, you may have indicated that C2 is not the most electrophyllic carbon, yet it is the target of the hydride anion in this reaction. Considering what you know about where the hydride is coming from, how do you think that the enzyme overcomes this complication?

It positions the NADH in a way that it can only attack C_2

15. The image below shows the binding pocket for NADH (actually NAD+, but the position of the cofactor does not change).
    a. How did you do in problem 5? Did you accurately predict how NADH is recognized by the enzyme?

Perhaps the one surprise is that the Watson/Crick face is not recognized

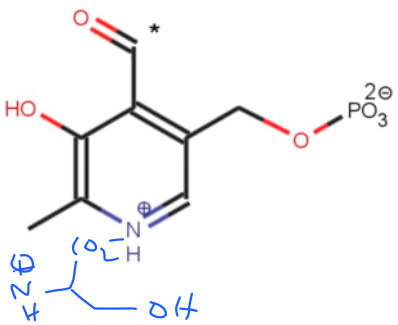
    b. On the image, indicate where pyruvate would bind. Make sure to consider which carbon should be in closest proximity to the hydride.

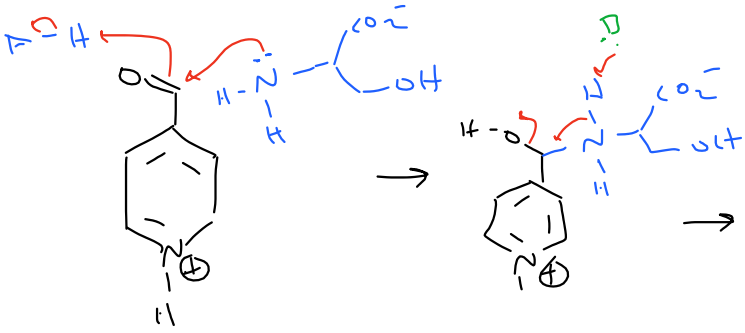


PLP and Serine Decarboxylase.

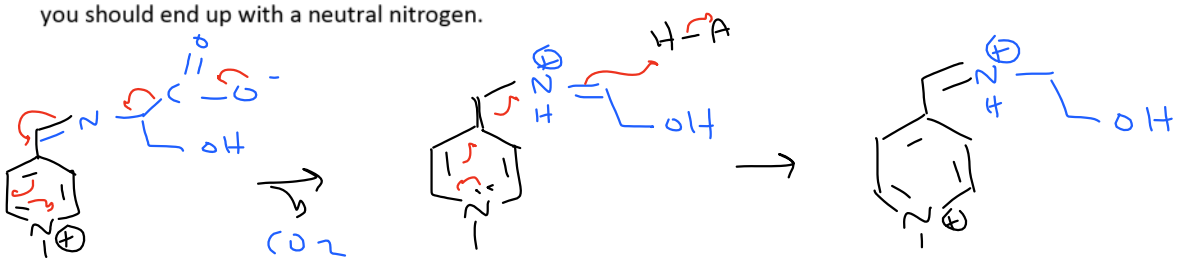
Pyridoxal phosphate (PLP) is a hugely important cofactor in biochemistry. It is the cofactor that is responsible for most transformations of amino acids (this includes, but is not limited to the formation of neurotransmitters and the biosynthesis of amino acids from common TCA cycle intermediates). It serves this role by making a covalent linkage (Schiff base) with the amino group of the amino acid backbone and subsequently becomes a great electron sink because electrons can freely flow through the pi system to the nitrogen cation in the ring. Let's explore this cofactor a little bit.

16. On the right, the business end of PLP is shown. The location of the Schiff base is indicated with a star. Using what you learned earlier in this activity, show the formation of the Schiff base between serine and PLP.





17. This intermediate is now able to facilitate the decarboxylation of serine. Show the mechanism for this step – you should end up with a neutral nitrogen.



18. The next step involves electrons flowing back through the PLP and allowing the beta carbon (or I guess it's not the beta carbon anymore) to pick up a proton from a general acid. Show this step.

See last step above

19. Lastly, the decarboxylated serine is released by reversing the Schiff base.