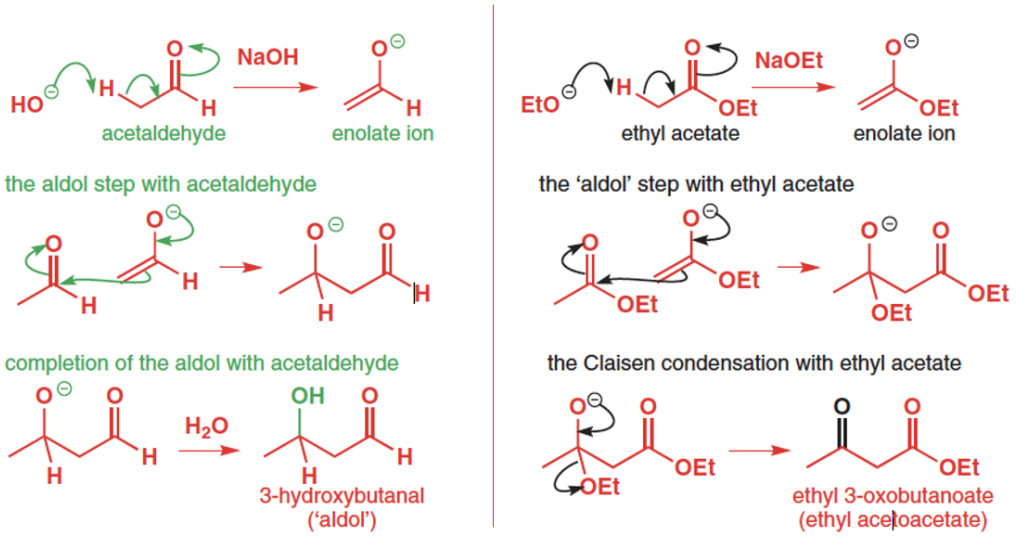
12.1 Claisen Reaction

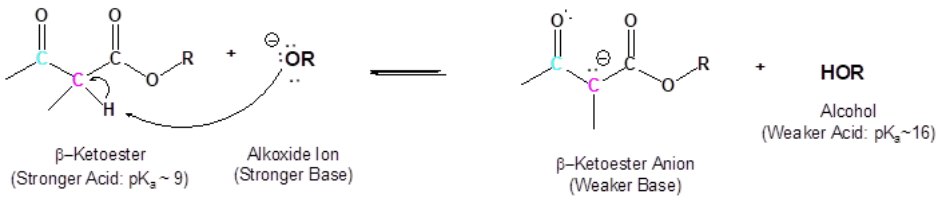
a. Although they may appear similar, there are a number of fundamental differences between an aldol and Claisen condensation. Draw the mechanisms of aldol addition and Claisen condensation and indicate their differences.

During the mechanism of Claisen condensation, the formed tetrahedral alkoxide intermediate is not protonated to from an "aldol" type product. Rather, the alkoxide intermediate will reform the C=O carbonyl bond and eliminate a (-OR) leaving group to produce a nucleophilic acyl substitution product.



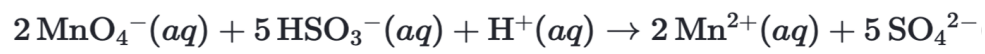
b. Claisen product will not form unless it contains an alpha hydrogen acidic enough to react completely with the reaction base. Draw the mechanism of the irreversible deprotonation and explain its role in the reaction progression.

Deprotonation of b-ketoester results in a stable enolate product (due to the formation of conjugated pi electron system). Thus, the *β*-keto ester condensation products are removed from the equilibrium by this deprotonation, which causes the reaction to be driven forward by Le Chatelier's principle.



12.2 Recognizing when a molecule is being oxidized or reduced

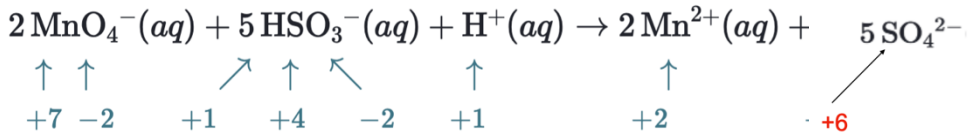
I. Which element is oxidized in the reaction represented above, and how does its oxidation number change?



$$2\,MnO_4^-(aq) + 5\,HSO_3^-(aq) + H^+(aq) \rightarrow 2\,Mn^{2+}(aq) + 5\,SO_4^{2-}$$

Answer:

To identify the element that is oxidized in the reaction, we first need to assign oxidation numbers to the atoms of each element in the equation. Then, we can determine which element's oxidation number increases over the course of the reaction (indicating oxidation). So, the element that is oxidized in the reaction is S (oxidation number changes from +4 to +6).



II. Each of the biochemical transformations shown below is a step in amino acid metabolism. For each reaction determine the state of the final product: is being oxidized, reduced, or neither oxidized nor reduced.

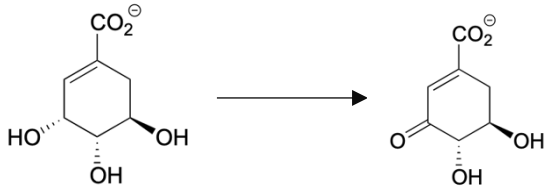
Answer :

Oxidation of an organic compound results an *increase* in the number of carbon-heteroatom bonds, and/or a ***decrease*** in the number of carbon-hydrogen bonds.

Reduction of an organic compound results in a *decrease* in the number of carbon-heteroatom bonds, and/or an ***increase*** in the number of carbon-hydrogen bonds.

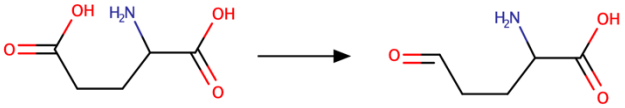
Heteroatoms such as oxygen and nitrogen are more electronegative than carbon, so when a carbon atom gains a bond to a heteroatom, it loses electron density and is thus being **oxidized**. Conversely, hydrogen is less electronegative than carbon, so when a carbon gains a bond to a hydrogen, it is gaining electron density, and thus being **reduced**.

A) From aromatic amino acid biosynthesis. Oxidation (Alcohol to ketone)



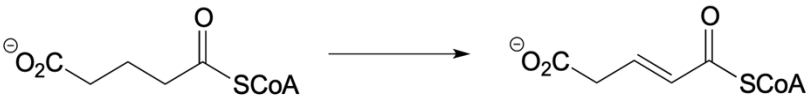
shikimate 5-dehydroskikimate

B) From biosynthesis of proline. Reduction (Carboxylic acid to ketone)

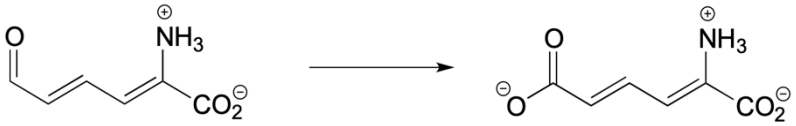


Glutamate GSA

C) From the catabolism of lysine. Oxidation (alkane to alkene)



D) From the catabolism of tryptophan. Oxidation (aldehyde to carboxylate)

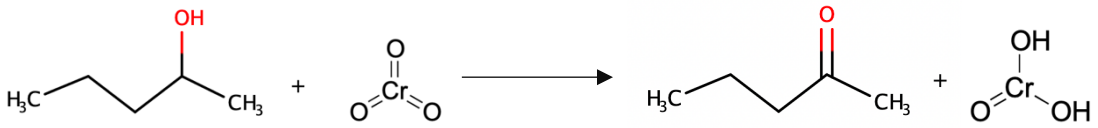


E) From the catabolism of threonine. Not redox (one carbon is reduced – alcohol to alkane and one is oxidized – amine to ketone)

12.2 Chemical reagents to mediate redox reactions

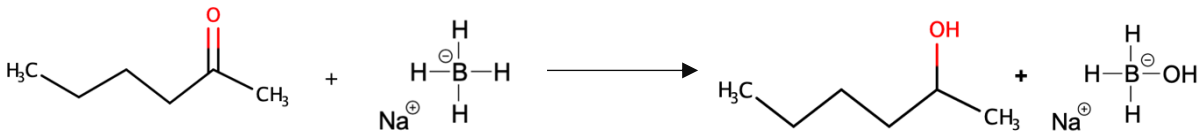
Alcohol (ketone for the reaction in B) gets oxidized in the reaction, **oxidizing agent (CrO_3)** gets reduced in the reaction, Cr lots a bond to oxygen.

A)



B)

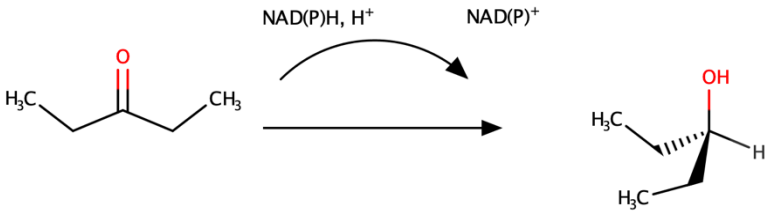
C)



Ketone gets reduced in the reaction, **reducing agent ($NaBH_4$)** gets oxidized in the reaction, boron lost a bond to hydrogen, gained a bond to oxygen.

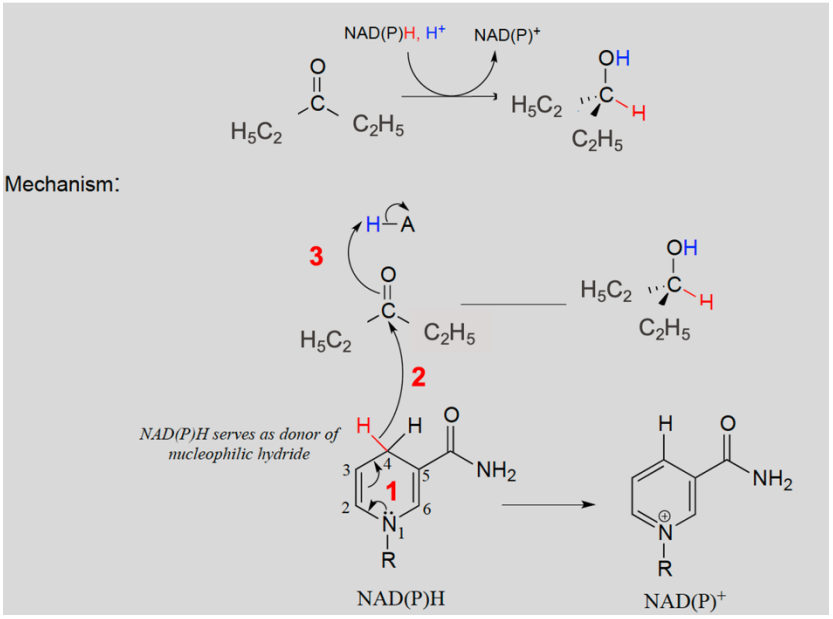
12.3 Proficient with hydrogen transfer reactions

Draw the mechanism of NADPH-dependent hydrogenation for the following reaction:



Answer:

Nicotinamide adenine dinucleotide - a hydride transfer coenzyme. 1 – shuffling of electron to activate hydride, **2** – Hydride from NADH attacks the reactive carbon without forming a free hydride ion intermediate, **3** – Attack of the oxygen to acidic proton.

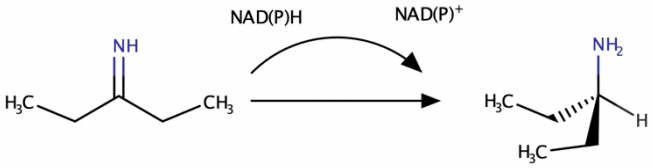


As an enzymatic group transfers a proton to the ketone oxygen, the carbonyl carbon loses electron density and becomes more electrophilic, and is attacked by a hydride from NADH. Because carbon of NADH is bound in such close proximity to the electrophile, this step can occur without generating a free hydride ion intermediate – the two hydride electrons can be pictured as shifting from one carbon to another. Note the products of this reaction: the ketone (which accepted a hydride and a proton) has been reduced to an alcohol, and the NADH cofactor (which donated a hydride) has been oxidized to NAD^+.

12.4 Stereochemistry effect of reactions

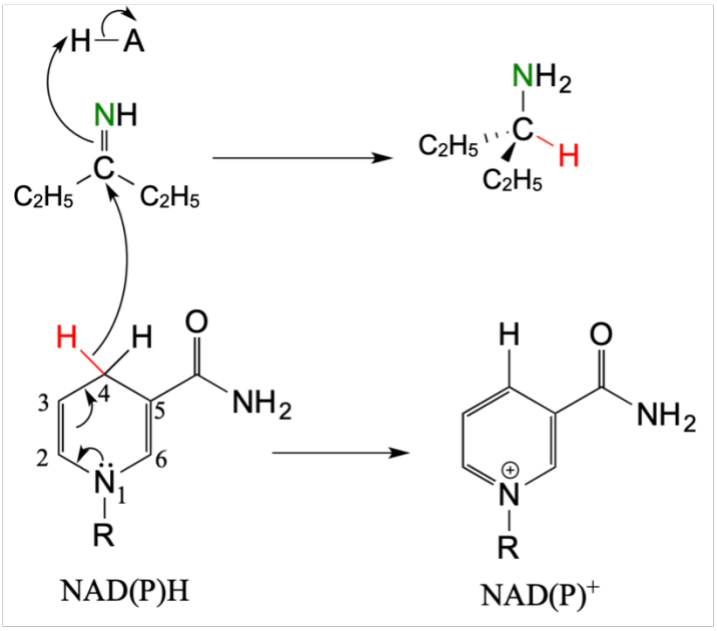
I. Draw the mechanism for hydrogenation/dehydrogenation of imine and amine in the following reactions:

A)

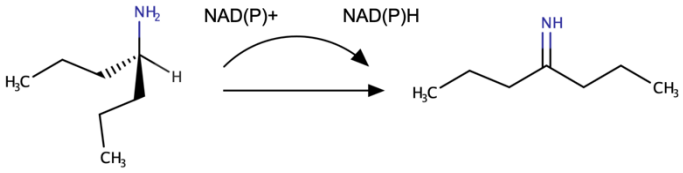


Answer:

It is a **<u>hydrogenation reaction</u>** of imine, where NAD(P)H transfer the hydrogen. Products of the reaction: the amine (which accepted a hydride and a proton) has been reduced to an **<u>amine</u>**, and the NADH cofactor (which donated a hydride) has been oxidized to **<u>NAD^+</u>**. The explanation of the mechanism is the same like in the ex 3, just it is a hydrogenation of imine: **1**- shuffling of electron to activate hydride, **2**- Hydride from NADH attacks the reactive carbon without forming a free hydride ion intermediate, **3** – Attack of the nitrogen to acidic proton.

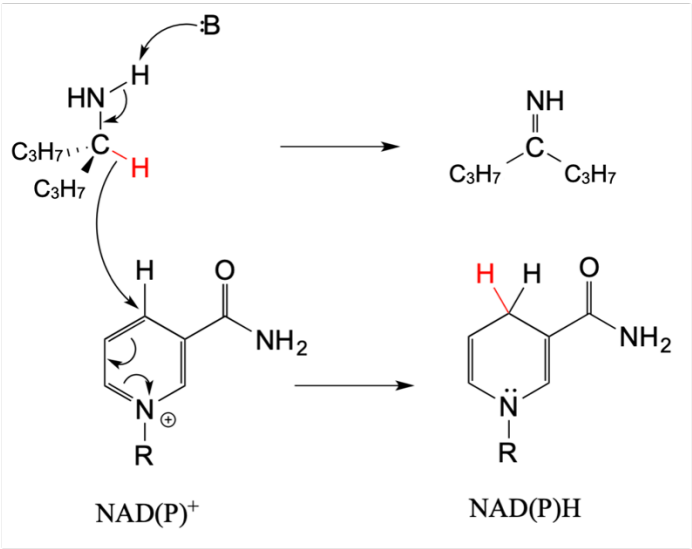


B)



Answer:

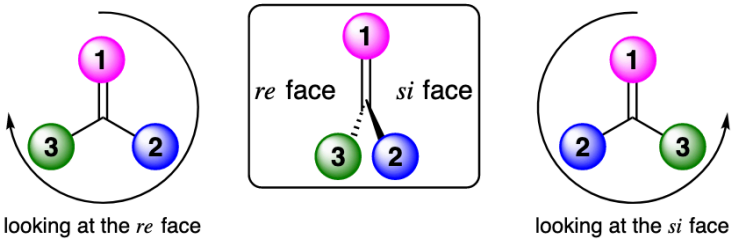
It is a **dehydrogenation reaction** of amine. The dehydrogenation of an amine by NAD^+ is simply the reverse of a imine hydrogenation: NAD(P)+ serves as acceptor of hydride leaving group. An enzymatic base positioned above the carbonyl removes a proton, and the electrons in the O-H bond shift down and push out the hydride, which shifts over to carbon of NAD^+.



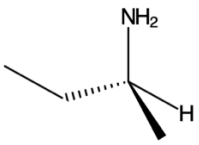
II. For the following compounds, indicate which face of the substrate is the coenzyme positioned next to in the active site of the enzyme?

The stereochemical configuration of the product depends on which side of the ketone/imine substrate the NAD(P)H coenzyme is bound in the active site. Any given enzyme will catalyze its reaction with one of these two stereochemical outcomes, not both.

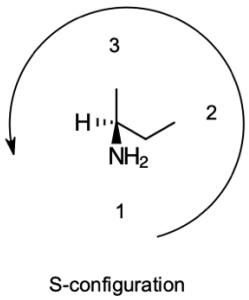
Trigonal planar, sp^2-hybridized carbons can be prochiral centers if they are bonded to three different substituents. There are two faces of a prochiral sp^2 carbon, called the re and si faces. To determine which is the re face and which is the si face of a planar organic group, we use the same priority rankings as in the R/S system. Trace a circle from the highest to the lowest priority substituent: the re face corresponds to a clockwise (right-handed) direction, and the si face corresponds to a counterclockwise (left-handed) direction. If a nucleophile attacks from the re face and becomes the highest priority substituent, the resulting product will have the R configuration.



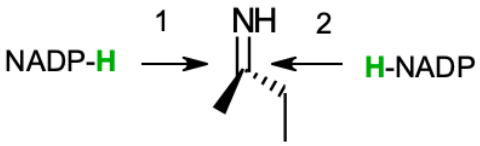
A)



Well, the product is in an S-configuration:

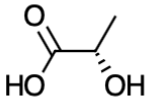


Coenzyme can be positioned in the enzyme's pocket on one of the two sides relative to the substrate:

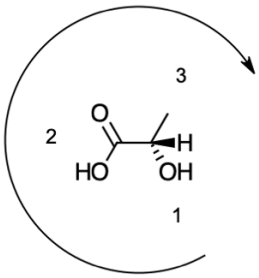


As a result of the positioning to the left (1), we would obtain an R-configuration product. To achieve an S-configuration, the coenzyme should be positioned to the right (2) of the specified molecule.

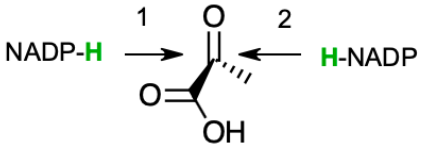
C)



We have an S-configuration of lactic acid here (even though the ranking is in the clockwise direction, the hydrogen is pointing towards us):

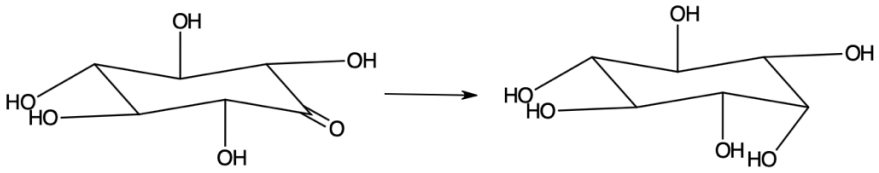


Again, two possible ways to attach the ketone:



Positioning to the left (1) will yield an S-configuration, while positioning to the right (2) will yield the desired R-configuration.

C)



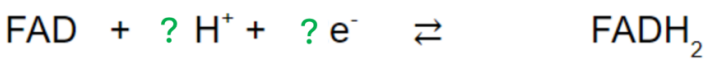
Simply put, for the OH group to point down, the hydride nucleophile needs to attack from the top.

12.5 Nicotinamide- vs flavin-catalyzed reactions

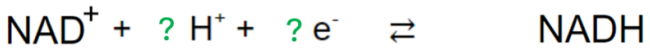
Both FADH2 and NADH undergo redox reactions and assist cellular metabolism. There are certain differences in their mechanisms of action. Let's find them out!

I. Finish the following equations:

A)

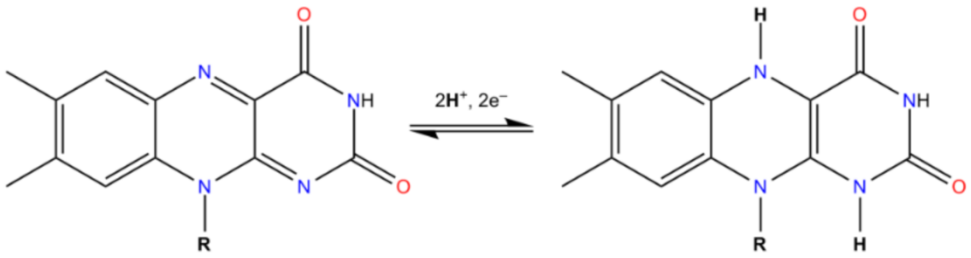


B)

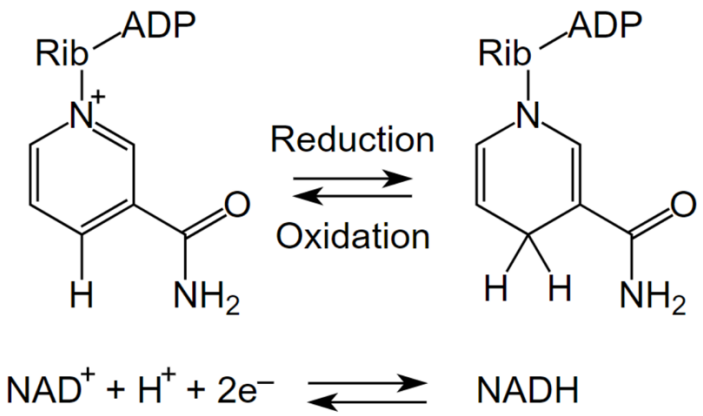


Answer:

FAD accepts two electrons and two hydrogen ions (H+) to be reduced to FADH2. This involves the addition of two hydrogen atoms (each consisting of one electron and one proton) to the flavin ring of FAD.

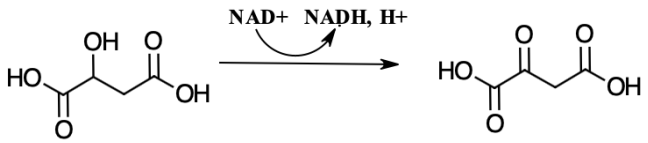


Both NAD+ and NADP+ can undergo two electron redox steps, in which a hydride is transferred from an organic molecule to the NAD+ or NADP+, with the electrons flowing to the positively charged nitrogen of NAD+ which serves as an electron sink. All NAD+/NADH reactions in the body involve 2 electron transfers. This process involves the transfer of a hydride ion (H-, which has two electrons and one proton).

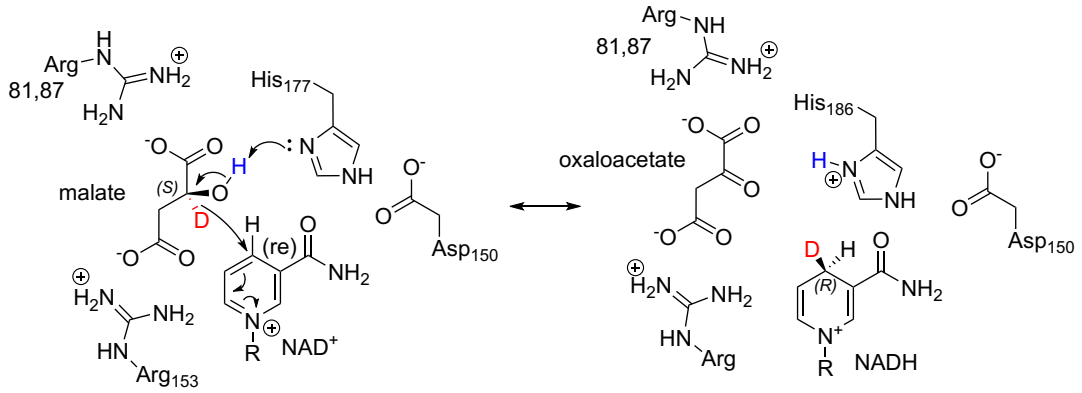


II. Predict starting substrate structures, draw mechanisms, specify hydride donor and acceptor, and identify which compound undergoes oxidation and which undergoes reduction.

A)

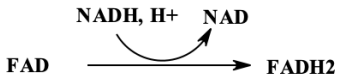


Mechanism (there is no need to draw the enzyme pocket; simply denoting the base involved is sufficient):

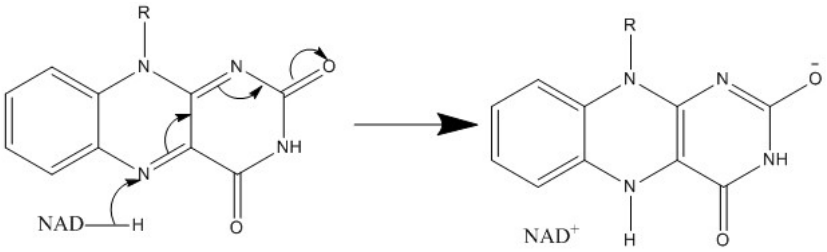


In this reaction, malate is a hydride donor, and NAD+ is a hydride acceptor. This means that NAD+ is getting reduced (gains electrons) and malate is being oxidized (loses electrons).

B)

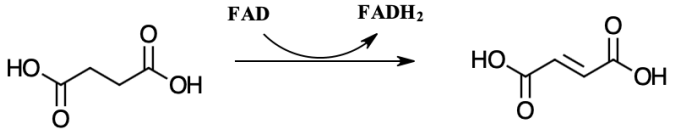


Mechanism:

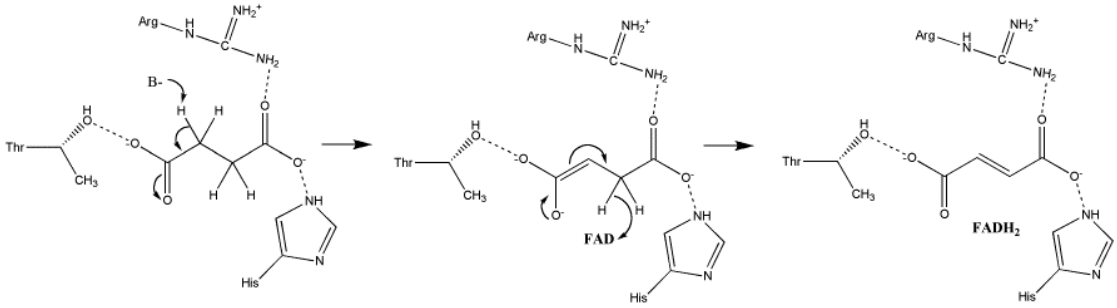


In this reaction, NADH is a hydride donor, and FAD is a hydride acceptor. This means that FAD is getting reduced (gains electrons) and NADH is being oxidized (loses electrons).

C)

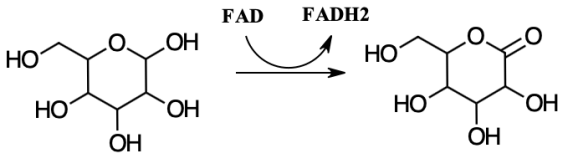


Mechanism:

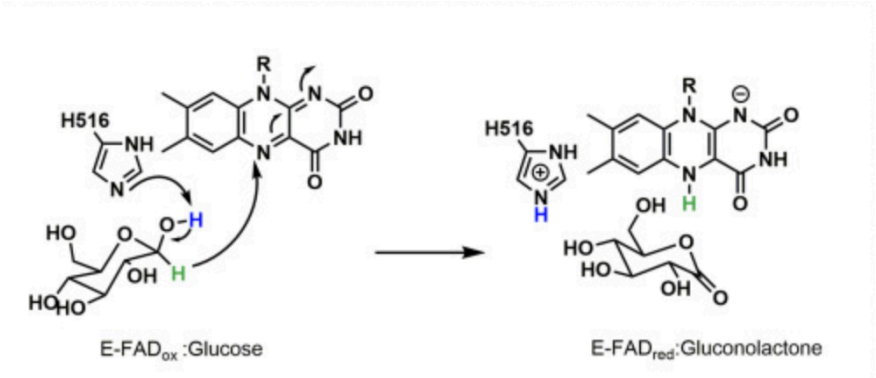


In this reaction, succinate is a hydride donor, and FAD is a hydride acceptor. This means that FAD is getting reduced (gains electrons) and succinate is being oxidized (loses electrons).

D)



Mechanism:



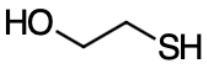
In this reaction, glucose is a hydride donor, and FAD is a hydride acceptor. This means that FAD is getting reduced (gains electrons) and glucose is being oxidized (loses electrons).

12.6 Cysteine reactions

Draw the mechanism for the disulfide exchange reaction in the presense of:

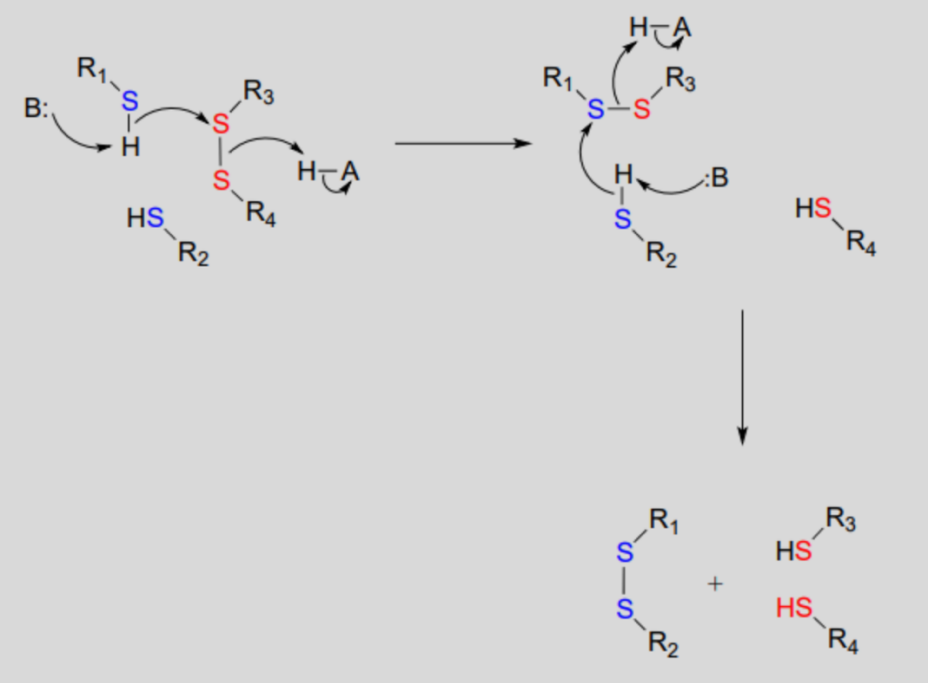
A) Glutatione

B) β-mercaptoethanol (BME). BME is used in the biochemical lab for reducing protein disulfide bonds prior to polyacrylamide gel electrophoresis.

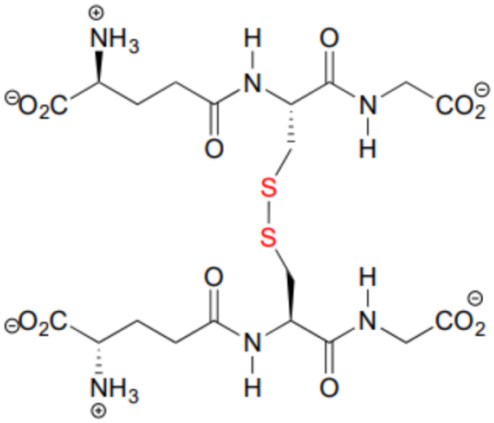


β-mercaptoethanol

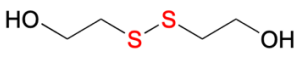
The general mechanism is the following:



Substitute R3 and R4 with Glutathione or β-mercaptoethanol.



glutathione dimer (GSSG)
oxidized state



oxidized BME