Triose Phosphate Isomerase – At the Core of Glycolysis

**Glycolysis**

Glycolysis is one of the most conserved pathways in all of evolution because of its centrality to the generation of cellular energy. Glucose is of course the starting material for the pathway, and through a series of chemical conversions, it generates ATP for use in cellular processes. Glucose is an aldose, meaning that it has a terminal aldehyde. After glucose intake, it is phosphorylated by expending a molecule of ATP becoming glucose-6-phosphate. This is then converted to the ketose form, called fructose. The conversion involves an enediol intermediate. Draw out the mechanism for practice (it is an important mechanism that you should know!) Fructose is then further phosphorylated by expending another molecule of ATP to become fructose 1,6-bisphosphate shown to the left. All sugars can take both a cyclic form and an acyclic form. The acyclic form of fructose 1,6-bisphosphate is shown to the left, and the cyclic form is shown below, along with the mechanism for interconversion. Know this simple acid-catalyzed mechanism. Both forms are observed in the cell, although it is the linearized form which undergoes further steps of the glycolysis pathway.

After linearization of the fructose 1,6-bisphosphate, it undergoes a reverse aldol reaction to give two isomeric interconvertible triose sugar molecules, called Glyceraldehyde 3'-Phosphate (GAP) and Dihydroxyacetone Phosphate (DHAP). It is the role of Triose Phosphate Isomerase (TIM) to interconvert these two, and this process is discussed in detail below. After this, GAP is further metabolized.

**Fructose Diphosphate Aldolase**

Fructose Diphosphate Aldolase (FDA) is the enzyme which performs the reverse aldol reaction yielding two triose molecules from fructose 1,6-bisphosphate as shown here. The arrow-pushing mechanism is very simple and shown above. The enediol above is protonated, yielding DHAP. One interesting question is whether the enediol above is indeed cis (E) as shown above. We can do a Newman Projection to determine what will be the most stable conformation. Shown to the right is the most stable conformation of the transition state. Remember that the breaking bond must be oriented to have good overlap with the carbonyl antibond (C=O π*), and this is achieved with a ~109° angle (the Dunitz-Burgi Trajectory). Hydrogen bonding positions the
carbonyl in a syn orientation with the two alcohols as shown and indeed this yields the E-enediol that is observed. Why is this important, you may ask. Consider that the transition state in TIM for the conversion of DHAP to GAP is an E-enediol and think about enzyme-to-enzyme transfer of intermediate (called enzymatic intermediate transfer). Here is a beautiful example of how direct chemical analysis such as that shown above can lead to a testable biological hypothesis. Indeed, if you search the literature, you will find information on intermediate transfer between FDA and TIM!

**Triose Phosphate Isomerase (TIM) – A Most Efficient Enzyme**

The equilibrium shown to the left is favorable to DHAP, which is present at a concentration 20 times greater than that of GAP in the cytoplasm of a cell. However, GAP is required for the final production of pyruvate in glycolysis and is the active substrate. Thus, TIM is a necessary equilibrase which quickly reequilibrates the system so as to ensure a constant supply of GAP for the progress of glycolysis. TIM is one of the most efficient enzymes known – it is only limited by the rate of diffusion of its substrates!

TIM gives us full appreciation for the chirality of enzymes and the consequent asymmetry of enzyme catalyzed processes. The first appreciation is that the enzyme active site only allows for a salt-bridge with positively charged Lysine-13.

Further Suggested Reading: Bugg Chapter 7 (pages 158 – 162), Chapter 4 (pages 60 – 67).

DHQ Synthase – A Sheep in Wolves’ Clothing
The Industrious Use of Substrate Analogs

DHQ Synthase – The Overview of the Pathway

DHQ synthase is part of the shikimic acid pathway, a pathway used in the biosynthesis of the aromatic amino acids Phenylalanine, Tyrosine, and Tryptophan. DHQ synthase catalyses a 5 step process that converts 3-deoxyarabinohexulosonate 7-phosphate (DAHP) into 3-dehydroquinate (DHQ). Shown to the left is the progress of the enzyme-catalyzed reaction. The enzyme is NAD$^+$ dependent and involves the oxidation and re-reduction of the 5'-hydroxyl. Step 1 is the oxidation of the alcohol to the corresponding ketone, which allows for enolization in step 2a. It is important to note that the phosphate acts as the base for deprotonation in this step, and this was proven through the use of substrate analogs. The enol then undergoes E1cb type elimination of the phosphate in step 2b. Since the only purpose of the ketone was the catalysis for the deprotonation through conjugation, it is at this stage rereduced using the now reduced form NADH which acts as the hydride donor. At this point, a basic residue in the enzyme catalyzes ring opening at the hemiacetal, leading to the acyclic enolate seen in step 4a. This enolate then rotates as seen in step 4b, now positioned for direct attack on the ketone. The enolate attacks, closing the ring, giving us DHQ. The step-by-step analysis of this process was done through the use of transition state analogs, which are discussed in detail here.

Step 1 – Does the Oxidation Actually Occur?

This analog of the natural substrate replaces the phosphate with a phosphonate, which is not a leaving group (the breaking of a carbon-carbon bond is required!). Thus, once step 1 is complete, Step 2a is feasible, but the substrate undergoes no further reaction. Thus an equilibrium is established between the keto and hydroxy forms of the analog, and both can be observed in experimentation. This demonstrates that oxidation does indeed occur.

Step 2 – How do we know the Phosphate Acts as the Base?

Well, the first step is showing that it is even feasible. We realize that there are two prochiral hydrogens on the methylene group attached to the

...
phosphate. Substituting one for deuterium allows us to determine the stereochemistry of the final product. We realize that in order to deprotonate the ring hydrogen, the phosphate must be in the syn-conformation as shown. If the phosphate is twisted away, it cannot access the hydrogen. If we analyze the reaction, we see that it is indeed a syn-elimination which occurs from the geometry of the product alkene. Notice the positioning of the D and H are identical to the positioning of the D and H in the syn-conformation. This shows that the phosphate is a possible base, but we require further evidence that it is indeed the base!

**Step 2 – Proving the Phosphate is the Base**

Using these conformationally rigid analogs, we lock the phosphate into either a syn or anti conformation with respect to the ring proton. Now putting this substrate analog along with the enzyme in a D2O solution allows us to observe proton exchange. As we know, once again, this substrate is stuck at step 2a. It can get oxidized and deprotonated, but its only course of action is to equilibrate between steps 1 and 2a. Thus, equilibration of step 2a in D2O produces a deuterated substrate if the substrate is deprotonated under the reaction conditions. Since we see deuteration only in the syn-conformer, we realize that deprotonation is highly dependent on the phosphate conformation. This gives us reasonably conclusive evidence that it is indeed the phosphate which deprotonates the proton!

**Further Suggested Reading**

Chorismate Mutase – An Introduction to Biological Pericyclic Reactions

Pericyclic Reactions
A Pericyclic Reaction is characterized as a change in bonding relationships that takes place as a continuous, concerted reorganization of electrons. This takes place through a single cyclic transition state with no intermediates. Pericyclic reactions require a continuous $\pi$ system in the transition state.

3 Major Categories of Pericyclic Reactions
1) Electrocyclic Ring Closures and Ring Openings

\[
\text{6$\pi$ electron ring closure}
\]

2) Cycloadditions and Cycloreversions

\[
\text{Diels-Alder Cycloaddition (}2\pi\text{ diene }+ 4\pi\text{ dienophile)}
\]

3) Sigmatropic Rearrangement

\[
\text{Cope Rearrangement – A 3,3 Sigmatropic Rearrangement}
\]

Focusing on 3,3 Sigmatropic Rearrangement – Cope and Claisen

\[
\text{Cope Rearrangement}
\]

\[
\text{Claisen Rearrangement – A Cope with an Allyl Vinyl Ether}
\]

The Chair Transition State: Let us examine the Claisen above. Clearly the starting material is chiral. Determine its absolute configuration. Let us now determine the two possible chair conformations of the starting material! KNOW HOW TO DRAW THESE!

\[
The Chair with the Equitorial Methyl Group is more stable and leads to the trans-alkene product.
\]

\[
The Chair with the Axial Methyl Group is less stable and leads to the cis-alkene product.
\]
**Chorismate Mutase**

**Isotopic Labeling** – We can generate chiral methylene (CH$_2$) and methane groups for use in mechanistic analysis. Chorismate mutase was analyzed in this manner by labeling a methylene group. The labeled methylene group in chorismate is shown below red and bolded. One of the hydrogens is protium ($^1$H) and the other is tritium ($^3$T).

Chorismate Mutase Catalyzes a Stereospecific Claisen Rearrangement of the Substrate Chorismate:

**Chair Transition State:**

**Boat Transition State:**

**Important Enzymatic Residues in B. subtilis Chorismate**

Glu78 and Cys75 interact with the hydroxyl group to ensure proper positioning of the chorismate. Additionally, Arg7, Arg90, and Tyr108 interact with the carboxyl residue on the methylene group to ensure fidelity of the transition state. Arg90 further catalyzes the reaction by hydrogen bonding with and thus activating the oxygen of the allyl vinyl ether moiety.

**Inhibitors**

Only transition state analogs will work efficiently because no enzyme-substrate complex is ever made during the course of the reaction. Since it is a pericyclic process, a single transition state with no intermediates is a requisite. A transition state analog can be modeled after the above transition states, and such an inhibitor is discussed on Page 65 of the Sourcebook.

**Antibody Catalysts**

Antibodies can be generated to transition state analogs by injecting transition state analogs into mice and purifying the antibodies from the blood. These analogs can act as modest catalysts of the desired reaction.

**Further Suggested Reading:** Bugg Chapter 10 (233-236), Chapter 8 (202-206)

Typical Test Questions may give you substrates and have you determine whether they have gone through chair or boat transition states. Know how to draw both the chair and boat transition states and find the stereochemistry of the products. Be able to do the arrow pushing for the claisen rearrangement and identify the product given the starting material and starting material given the product.
Pyridoxine, Pyridoxal, and Pyridoxamine – The Chemistry of Vitamin B₆

Although Jeremy Knowles may have called DHQ Synthase a “sheep in wolves’ clothing,” Pyridoxal Phosphate (PLP) could certainly share in the same distinction. PLP is a versatile biological coenzyme involved in numerous amino acid based transformations including decarboxylations, side-chain elimination, β-position and γ-position eliminations, oxidative deaminations, and reductive aminations (PAP), among others. PLP chemistry is the chemistry of the aldehyde (imine formation, imine hydrolysis, enolation, etc.) and of the pyridine ring, to which it owes its unique properties as an “electron sink”. Fortunately, all mechanisms of PLP depend on this property and share a common initiation, the formation of a negative charge at the α position, which is subsequently resolved through various means.

![Pyridoxal Phosphate and Pyridoxamine Phosphate](image)

Vitamin B₆ is ingested as Pyroxidine and is subsequently oxidized enzymatically to Pyridoxal, which is further phosphorylated by pyridoxal kinase, yielding the biologically active form, pyridoxal phosphate. Subsequent reductive amination of the cofactor results in another biological active form, pyridoxamine phosphate.

**Important Chemical Features**

1) Pyridine is modestly basic (pKₐ pyridinium = 5.2). This means at physiological pH, pyridine is found to some degree in its protonated form (2%-8% depending on pH – Remember the Henderson-Hasselbach Equation). The protonated form is the active form of the coenzyme, where the pyridine ring nitrogen in the pyridoxine species is found protonated and hence positively charged. This positive charge allows for these compounds to act as electron sinks.
2) Aldehydes are highly electrophilic species. This electrophilicity is enhanced through intramolecular hydrogen bonding in pyridoxal phosphate (notice above the positioning of the 3’ hydroxyl hydrogen with respect to the carbonyl oxygen.

3) The phosphorylated form of the pyridoxines is cellularly active. The phosphate moiety serves for co-factor recognition. Enzymes will use this as a recognition handle.

Pyridoxal Phosphate (PLP) – Chemical Reactivity

1) PLP utilizing enzymes almost always have a Lysine Residue present in the active site that condenses with the PLP to form an aldimine (Schiff Base) covalent enzyme–co-enzyme linkage. Let’s use this opportunity to review the imine formation mechanism:

Keys to remember: 1) Remember that the aldehyde, though activated by the intramolecular hydrogen bonding, must be protonated in order to become fully activated. This protonation activated the aldehyde $10^6$ (one-million) fold, allowing for efficient progression of the reaction.
2) The lysine ε-amine must be deprotonated in order to attack nucleophilically – though most lysine residues are protonated at physiological pH, it is the deprotonated form which is active in this reaction. 3) Don’t forget all of your proton transfers! Remember – in an acid catalyzed mechanism (which this is), there should not be any negative charges! (Similarly in any base catalyzed reaction, there should not be any positive charges). These charges would be immediately neutralized by the reaction conditions.

2) Subsequently, the pyridoximine (the aldimine of pyridoxal) will condense with the free amino group of an amino acid to carry out its catalysis of various reactions.

3) **The Reactions** – There are many different classes of reactions catalyzed by PLP, but they all fall nicely into a few simple generalizable mechanisms:

a. **α position** – Stabilization of a negative charge at the α position. PLP will stabilize a negative charge at the α position of an amino acid:

   ![Alpha Position Diagram]

   This negative charge may develop through 1) deprotonation of the alpha position, 2) decarboxylation of the amino acid, or 3) side-chain loss. (Essentially one of the three groups at the α position is lost, leaving the bonding electron pair behind. This negative charge can then be resolved in one of two ways: 1) by protonation or electrophile addition at the α position and subsequent hydrolysis, or 2) by protonation at the imino-carbon of the pyridoxal, resulting in oxidation of the substrate and reduction of PLP to PAP. This is also outlined nicely on page 70 of the sourcebook.

b. **β and γ positions** – Once a negative charge has developed, it may also be used for reaction at the β position instead of resolution through protonation. There are two possibilities: 1) Elimination of a leaving group through an E1cb type mechanism; 2) deprotonation of the β position and resolution by protonation of the imine nitrogen to affect further reactivity. Resolution by the second method often results in the expulsion of a leaving group at the γ position. A classic example of this is the enzymatic activity of Methionine γ-lyase, which catalyzes the following process:

   ![Beta and Gamma Positions Diagram]
Biological Amino Acid Synthesis – The Chemistry of PAP

Cellular amino acid synthesis is a key example of PAP chemistry. Here PAP condenses with an \( \alpha \)-keto acid as shown to the left. The co-enzyme-substrate complex then undergoes a reduction at the \( \alpha \)-carbon and a simultaneous oxidation at the benzylic carbon as shown. Subsequent hydrolysis releases the \( \alpha \)-amino acid and PLP. This is a generalizable example of PAP chemistry in the cell.

Inhibitors – The Trojan Horses

PLP inhibitors are always suicide inhibitors which form a covalent linkages to the PLP and then form a stable enzyme-inhibitor complex in the active site of the enzyme. One example of such an inhibitor is \( \alpha \)-difluoromethylornithine (DFMO), an analog of ornithine, the natural substrate of the PLP-dependent enzyme, ornithine decarboxylase. DFMO resembles ornithine and is taken up by the enzyme, but then once it condenses with PLP, it quickly undergoes a series of reactions by which it forms a stable covalent complex with PLP and the enzyme.

Additional Reading

Further Suggested Reading: Bugg (Chapter 9) – You will find this a good summary of PLP and PAP chemistry. Note: This textbook refers to PAP as PMP.

NAD$^+$/NADH and FAD/FADH$_2$ – The Biological REDOX Reagents

A Quick Review of REDOX Chemistry
Reduction-Oxidation, or Redox, reactions involve the transfer of electrons between two compounds. One compound gains electrons and becomes reduced. The other compound loses the same number of electrons and becomes oxidized. The compound that is reduced oxidizes the other compound and is thus the oxidizing agent, while the compound that is oxidized reduces the other and is thus the reducing agent. A reduction is always coupled to an oxidation and vice versa because there can be no gain or loss of electrons from the system. However, each can be separated into a half-reaction: the reduction half-reaction and the oxidation half-reaction. Half reactions are always written by convention in the reduction pathway as shown below and are given a reduction potential, $\xi^\circ$. The more positive the $\xi^\circ$, the more spontaneously will a compound get reduced. Below, you see that oxygen has a very positive $\xi^\circ$ and thus readily gets reduced. Thus is is a strong oxidizing agent!

\[
\begin{align*}
\text{NAD}^+ + H^+ + 2e^- & \rightarrow \text{NADH} & \xi^\circ &= -0.32 \text{ V} \\
\text{FAD} + 2H^+ + 2e^- & \rightarrow \text{FADH}_2 & \xi^\circ &= -0.18 \text{ V} \\
O_2 + 4H^+ + 4e^- & \rightarrow 4\text{H}_2\text{O} & \xi^\circ &= +0.82 \text{ V}
\end{align*}
\]

The $\xi^\circ$ means very little in and of itself because reductions must be coupled to oxidations in order to occur! Let us examine the coupling between the reduction of molecular oxygen and the oxidation of NADH below. Recall that we must reverse the sign of the reduction potential if we examine an oxidation. Also recall that we do not multiply the potential as we multiply a half-reaction. The absolute value of the potential always remains the same:

\[
\begin{align*}
O_2 + 4H^+ + 4e^- & \rightarrow 4\text{H}_2\text{O} & \xi^\circ &= +0.82 \text{ V} \\
2\text{NADH} + & \rightarrow 2\text{NAD}^+ + 2H^+ + 4e^- & \xi^\circ &= +0.32 \text{ V} \\
O_2 + 2H^+ + 2\text{NADH} & \rightarrow 4\text{H}_2\text{O} + 2\text{NAD}^+ & \xi^\circ &= +1.14 \text{ V}
\end{align*}
\]

This is highly exothermic! Remember the equation that relates free-energy ($\Delta G$) changes with the overall potential of a Redox reaction: $\Delta G = -nF\xi^\circ$. Thus, a large positive $\xi^\circ$ means a large negative $\Delta G$. Because entropy does not play a large role in Redox chemistry, this means that this is a large negative change in enthalpy – meaning a large amount of released energy!

Coupling Redox Reactions – The Importance of Energetic Matching
Coupling of multiple matched redox reactions rather than a single mismatched redox reaction allows for the maximization of efficiency in energy usage. The closer the potentials between two reactions, the less energy is released as heat and thus lost to the surroundings. This is very important to a cell which is operating under strict energetic considerations. For this same reason, the electron transport chain in mitochondria involves multiple steps that slowly extract energy from the electron as it moves down its potential gradient. Ultimately, the net reaction of ATP generation is the burning of glucose with molecular oxygen, releasing CO$_2$ and H$_2$O. This is because oxygen has such a large oxidizing potential. However, doing this in one step releases large amounts of unharnessable energy; doing it in multiple smaller energetically matched steps allows for efficient capture of energy. For this reason, coupling of NAD$^+$ and FAD is important.
The chemistry of Nicotinamide Adenine Dinucleotide (NAD) is extremely simple. Like PLP, it has a pyridine ring which can act as an electron sink and source. The ring nitrogen in this case is alkyl substituted, so it is primed for this role. In NAD\textsuperscript{+}, this N is positively charged and primed to accept electron density. It is an electron withdrawing group that places a slight positive charge on the ortho-para positions. It is this positive charge at the para position which enables NAD\textsuperscript{+} to readily accept a hydride to become NADH. Similarly NADH can release a hydride to become NAD\textsuperscript{+}. These are Redox reactions, and for this reason the oxidation potential of NAD\textsuperscript{+} or the reduction potential of NADH must closely match that of the coupled reaction to 1) ensure spontaneity of the reaction ($\Delta G^\circ$\textgreater \textit{0}), and 2) ensure minimal energy loss by coupling reactions with similar energetics ($\Delta G^\circ$ should be small). The most complex aspects are simply the tracking of prochiral protons (labeled above) on NADH and prochiral faces of substrates (the Re face is shown above). Some enzymes utilize the pro-R hydrogen of NADH, while others utilize the pro-S hydrogen. It is simply a matter of analyzing each enzyme individually. One again we can use selective deuteration to determine the specificity of an enzyme.

FAD/FADH\textsubscript{2} Reductions

FAD has a slightly different mode of operation than NAD\textsuperscript{+} – it is also a two electron acceptor, but it accepts along with the two electrons, 2 protons. Generally, a hydride source will add directly to the nitrogen in a conjugate addition, which allows the other nitrogen to pick up a proton from solution as shown. The hydride source may be NADH, in which case it is a NADH/FAD coupling. This is done, as mentioned previously, to allow for appropriate matching of redox potentials.

\textbf{O$_2$/FADH$_2$ Coupling}

One very important role of FADH\textsubscript{2} is the catalysis of oxidation by O$_2$. This can occur either by a series of 2-electron transfer processes (shown here) or by a chain of radical processes (discussed in detail – Bugg pages 131 – 135). The formed peroxide bond is extremely labile and thus extremely reactive. Additionally, peroxide, with a pKa of 11.6 (inductive effects) is much more acidic than water and loses its proton more readily. Thus it can act as a nucleophile. It participates in a wide variety of reactions, including epoxidations (analogous to peracid (mCPBA) epoxidations) and the Baeyer-Villager Rearrangements. In Baeyer-Villager Rearrangements, the large group on a ketone always migrates to give the lactone for simple steric reasons. Construct newman projections for the migration to verify this – come to me to corroborate your reasoning.

\textbf{Further Suggested Reading:} Bugg (Chapter 6) – Focus on pages 121 – 140. You will find this an excellent summary of NADH and FADH\textsubscript{2} chemistry.
Adenosine Triphosphate – The Cellular Currency

The Structure of ATP

Adenosine refers to the base adenine attached to the sugar ribose. The structure of adenosine triphosphate is shown to the left. Notice that the phosphates are attached to the 5’ hydroxyl of the ribose. The three phosphates are attached via phosphoric acid anhydride bonds, which like the bonds of carboxylic acid anhydrides, are extremely labile. Notably, because phosphoric acid is polyprotic, there are also a number of negative charges on the phosphates which repel charged nucleophiles and thus prevent attack. Thus, the bonds are high energy and highly labile but resistant to breakage because of charge repulsion. There are two labile bonds, the αβ linkage and the βγ linkage, and all three of the phosphates could potentially be attacked. Attack at the α and γ phosphates is very common, but attack at the β phosphate is rare because of the excessive charge that a nucleophile must overcome to attack there. Notice that it must repel charge from all three phosphates when attacking the β phosphate! Approximately 7–8 kcal/mol of energy is stored in the Phosphorus-Oxygen bond of ATP.

The Stereochemistry of Attack

Remember that the phosphate-oxygen bonds are actually single bonds. The d orbitals are too high in energy for hypervalency to occur. The phosphate-oxygen bond that is represented as a double bond is actually a dative bond, in which phosphate supplies both bonding electrons! For this reason, phosphate can be approximated as a tetrahedral center, much like carbon, following the rule of inversion of stereochemistry for second-order nucleophilic displacements which for phosphates are called in-line displacements. The use of chiral phosphates, such as the one shown above, allows us to characterize this inversion, which necessarily must take place through an S_N2-like trigonal bipyramidal transition state with a 180° orientation between the leaving group and the incoming nucleophile. Notice above that the starting material has S-configuration and the product has R-configuration (assuming that both the nucleophile and leaving group have 3rd priority according to Cahn-Ingold-Prelog convention).

Leaving Groups

Because there are three phosphates that can be attacked and two potential leaving groups with each attack, there are a total of six possible attack modes. Attack at the α phosphate may either result in the extrusion of a pyrophosphate (very common) or adenosine (never observed). Pyrophosphate is a particularly good leaving group because it can stabilize negative charge very
effectively. **Pyrophosphate is the leaving group in all cellular nucleic acid synthesis.** Attack at the $\beta$ phosphate is rare, again, because of the excessive charge that a nucleophile must overcome to attack there. Attack at the $\gamma$ phosphate is also observed and can result in the extrusion of adenosine diphosphate – ADP (common) or hydroxide (never observed because hydroxide is a very very poor leaving group!). Additionally, there is a seventh mode of attack in which the 5’ carbon is attacked in an $S_N2$ reaction, releasing triphosphate. An example of this is S-adenosyl methionine (SAM), the cellular methylating agent. Where chemists use MeI, nature uses SAM! Synthesis of vitamin B12 also requires attack of the 5’ carbon.

In summary, there are three common modes of attack:

1) **Attack at the $\alpha$ phosphate** and extrusion of a **pyrophosphate** (Observed in all nucleic acid synthesis reactions).

2) **Attack at the $\gamma$ phosphate** and extrusion of **ADP** (Phosphorylation of sugars, proteins, cofactors, etc.)

3) **Attack at the 5’ carbon of ribose** (synthesis of SAM and vitamin B12)

**Biosynthesis of ATP**

ATP is synthesized during glycolysis, as discussed in the section on TIM, through direct phosphorylation of ADP to ATP and also during the electron transport chain in the mitochondria by ATPases in the mitochondria which efficiently catalyze the fusion of ADP and inorganic phosphate.

**Additional Reading**

Bugg (Chapter 5) pages 106 – 109.