Chemistry 5.07SC Biological Chemistry I

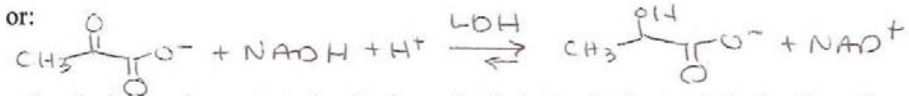
Fall Semester, 2013

Lecture 15/16 Endings to Glycolysis: how to regenerate NAD^+ and reversible conversion of P to alanine.

I. There are three endings to glycolysis. We will look at each transformation and the energetic consequences.

1. Homolactate fermentation which occurs in muscle under anaerobic conditions.
2. Ethanol production which occurs in yeast under anaerobic conditions and is central to the beer and wine industry.
3. AcetylCoA production in aerobic metabolism and a segway into the TCA cycle.
4. We will also discuss conversion of pyruvate to alanine using Vitamin B6, pyridoxal phosphate. PLP is central to all amino acid metabolism and reversible conversion of α-ketoacids and amino acids is central to feeding intermediates into/ and removal of intermediates from the TCA cycle.

1. **Lactate dehydrogenase (LDH**) catalyzes the conversion of pyruvate (P) to lactate (L) using NADH as the cofactor:



This ending of the glycolysis pathway is utilized when the demand for ATP is high and the O_2 supply is low. Anaerobic glycolysis can continue at high rates for 1 to 3 min. Generation of ATP by this mechanism is much faster than the O/P pathway. In muscles, lactic acid is the end product produced and is transported through the blood to the liver, where it can be converted back to pyruvate and used to make glucose. Lactic acid requires a transporter in muscles and is coupled to H^+ transport. Contrary to general belief, it is not lactic acid buildup that causes muscle fatigue and soreness; this phenotype occurs on too long a time scale.

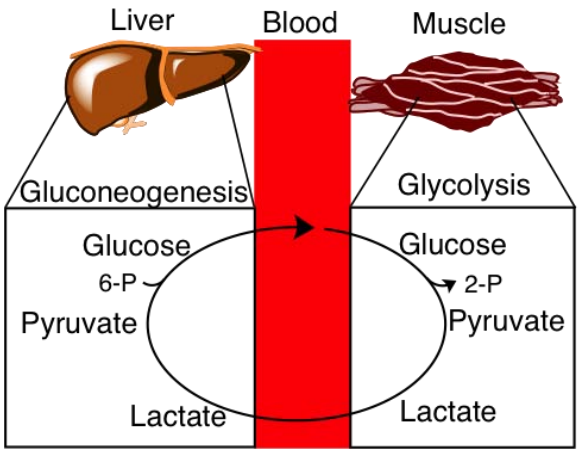
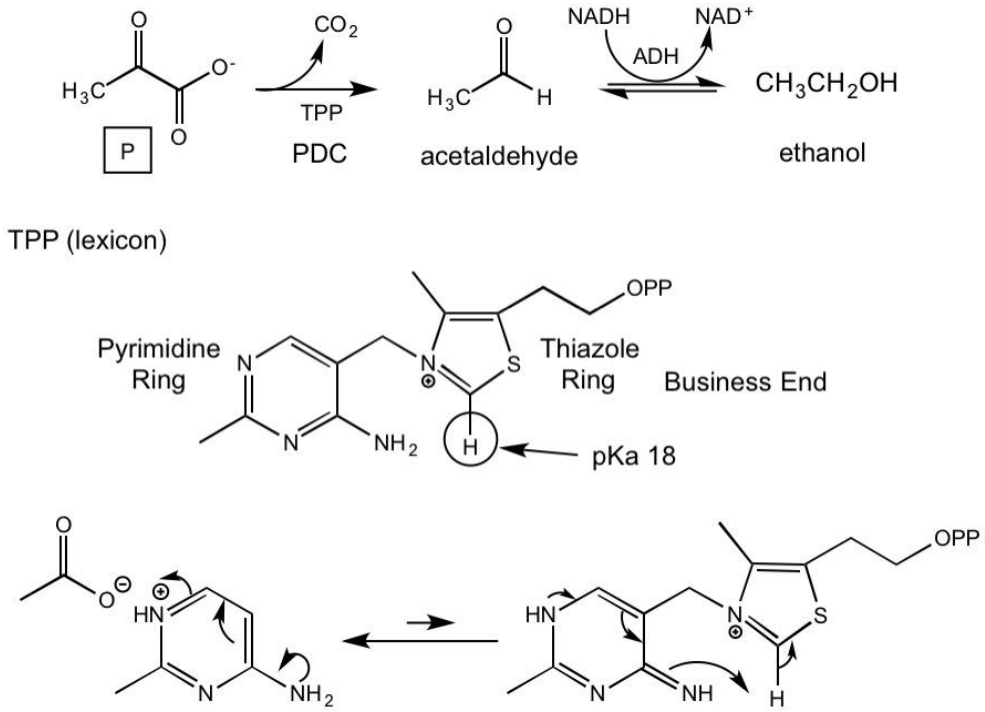


Figure 1. The Cori cycle. Glycolysis in active muscles convert glucose to pyruvate and then to lactate, which is released into the blood and carried to the liver where gluconeogenesis occurs. This process shifts some of the metabolic burden to the liver from active muscles.

Aside: Yogurt is produced by bacterial fermentation of milk. Lactose (a disaccharide is broken down to G and is converted to L by anaerobic glycolysis. Lactate gives yogurt its texture and tang.

2. **Ethanol formation or alcohol fermentation occurs in two steps**. The first step is the non-oxidative decarboxylation that converts pyruvate to acetaldehyde and CO_2. This process requires the cofactor thiamin pyrophosphate (TPP) or Vitamin B1. Deficiency in TPP results in the disease Beriberi. The acetaldehyde produced is then reduced to EtOH by NADH regenerating NAD^+. Since we have not yet encountered this cofactor, we will examine its mechanism in detail.



From recent structures of TPP requiring enzymes, it is now known that the amino group of the pyrimidine ring of TPP plays a key role in formation of the ylid of the thiazolium ring, the first step in all TPP requiring enzymatic reactions.

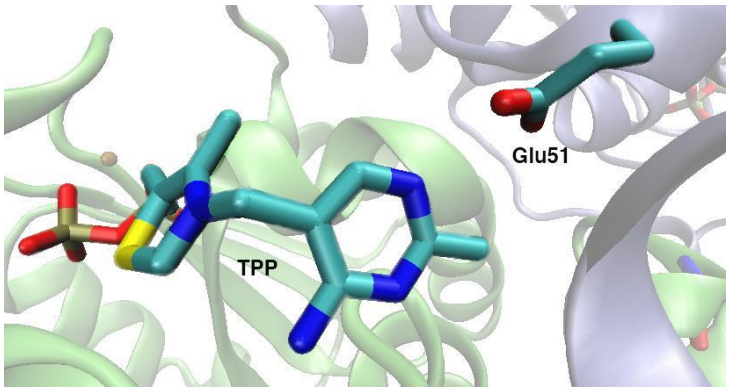
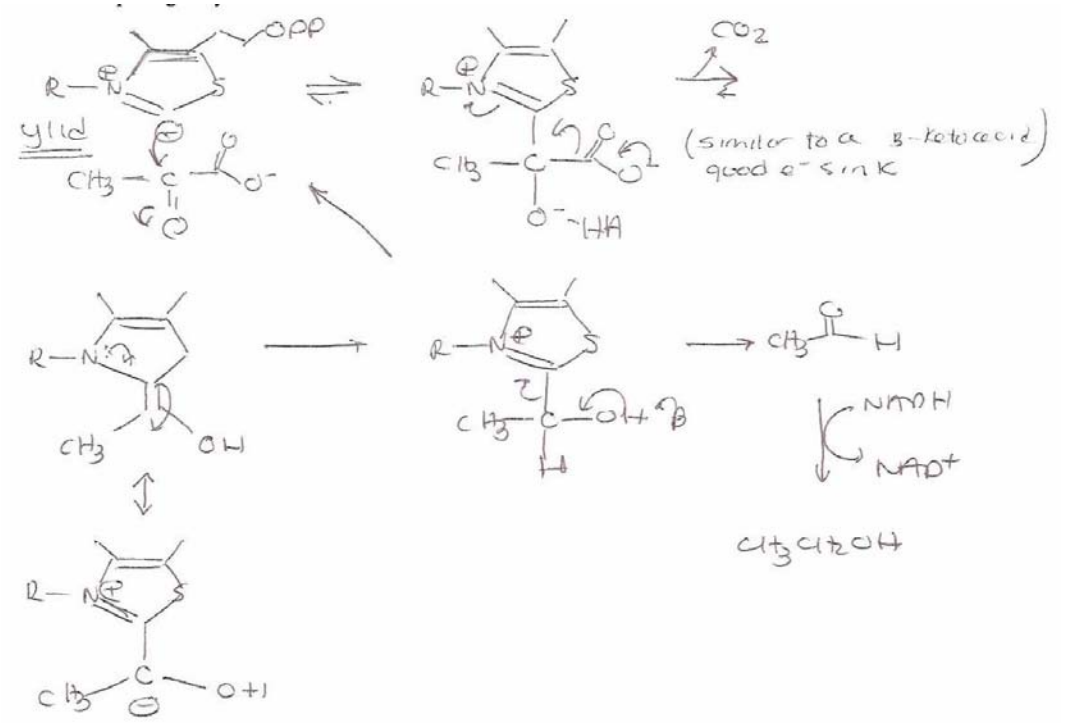


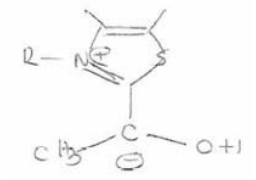
Figure by O'Reilly Science Art for MIT OpenCourseWare.

PDB: 1PVD

Arjunan, P., T. Umland, F. Dyda, S. Swaminathan, W. Furey, M. Sax, B. Farrenkopf, Y. Gao, D. Zhang, and F. Jordan. "Crystal Structure of the Thiamin Diphosphate-dependent Enzyme Pyruvate Decarboxylase from the Yeast Saccharomyces cerevisiae at 2.3 Å Resolution." *Journal of molecular biology* 256, no. 3 (1996): 590-600.

Figure 2. The active site of pyruvate decarboxylase with glutamate 51 adjacent to the amino group of the TPP pyrimidine.

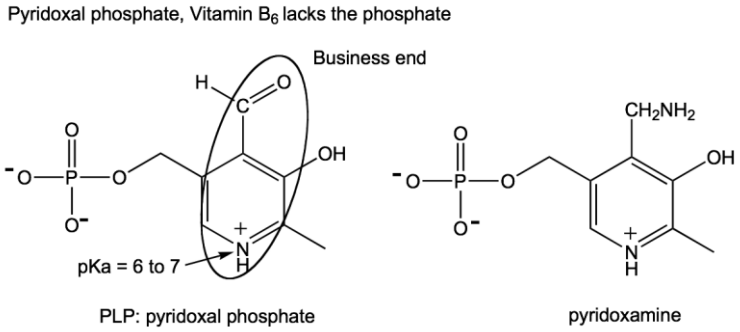



can be oxidized, an oxidative decarboxylation of pyruvate, and leads to the aerobic ending of the glycolysis pathway in the pyruvate dehydrogenase enzyme (PDH) discussed below.

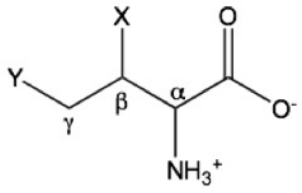
Pyruvate to Alanine: the role of Vitamin B6, pyridoxal phosphate (PLP) in reversible amino acid formation from α-ketoacids such as pyruvate. The enzymes that catalyze this reaction are transaminases (or transiminases since PLP is always covalently bound to a K in the active site of the protein). A similar conversion of oxaloacetic acid (OAA) and α-ketoglutarate (α-KG), intermediates in the TCA cycle, to aspartic acid and glutamic acid also requires PLP and a transaminase.

I. Structure of the cofactor. The entire molecule is the business end. The cofactor comes in two forms pyridoxal phosphate and pyridoxamine phosphate. The Vitamin is not phosphorylated and

must be metabolized to form the cofactor. PLP is always bound to the enzyme through a Schiffs base or imine (remember class I aldolases discussed earlier).

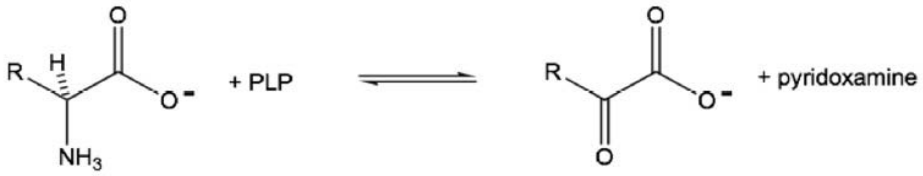


II. Scope of the reactions. The first step in the metabolism of most amino acids involves a PLP dependent enzyme. These enzymes can catalyze reactions that occur at the α, β and γ positions.



Since we are not going to discuss amino acid catabolism or anabolism, we will focus on just the reactions that occur at the Cα position of the amino acid and feed into the TCA cycle or gluconeogenesis pathways. As indicated below chemistry at Cα can result in transaminations, racemizations, decarboxylations and not shown, reverse aldol reactions. In the transamination reaction the pyridoxamine is converted back to PLP via reaction with an α-keto acid such as α -ketoglutarate which is converted to an α amino acid such as glutamate.

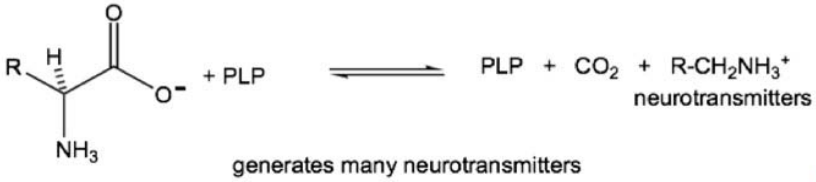
i. Transaminations: first step in breakdown of all amino acids



amino acid α-ketoacid

ii. Racemization: R(aa) ⇌ S(aa)

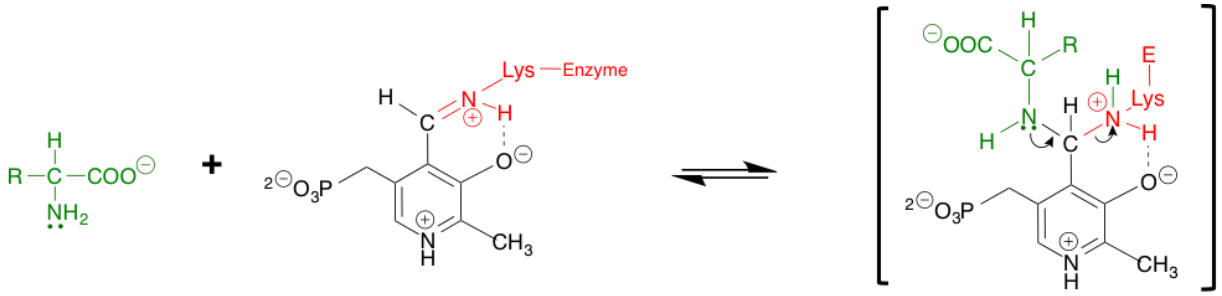
iii. Decarboxylations



generates many neurotransmitters

III. Mechanism of PLP reactions.

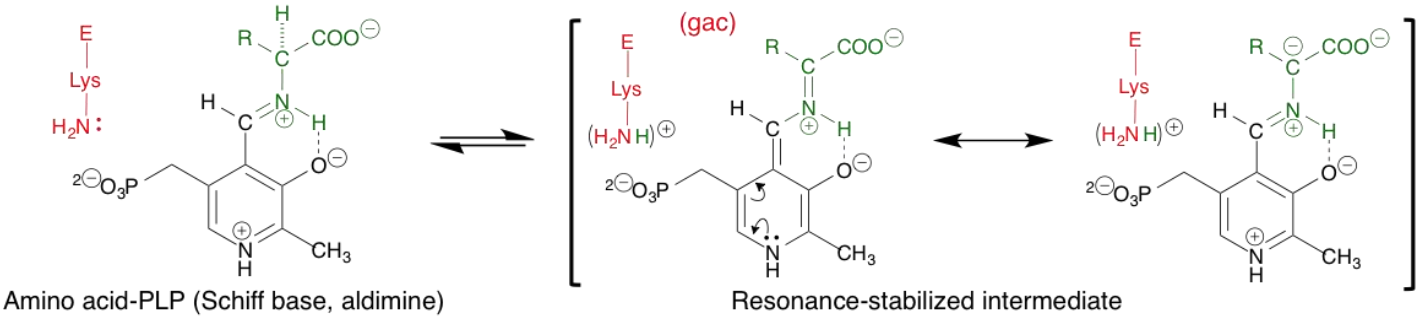
i. The first step in all reactions is a transimination. PLP is always bound covalently to the active site of an enzyme as an aldimine (also called a Schiffs base, the imine of an aldehyde). Note initially, that the proton from the NH_3^+ group of the amino acid is transferred to the imine to form a protonated imine. Now the NH_2-group of the amino acid is ready to carry out chemistry.



α-Amino acid Enzyme-PLP (Schiff base) Geminal diamine intermediate

ii. Formation of the aldimine increases the acidity of the α-hydrogen of the amino acid. This labilization is in fact, with the exception of decarboxylation reactions, the second step in all PLP dependent reactions. It also plays a key role in activating the β and γ positions for chemistry as well. The lysine that was originally in the Schiffs base with PLP is most likely to be the base

and becomes protonated. Again with these enzymes they have figured out how to minimize the number of GBC and GAC groups in their active sites.



iii. The bond to be cleaved in the second step is placed by the enzyme active site groups into a plane perpendicular to the plane of the π pyridine-imine system to maximize electron overlap. This strategy was first identified by Dunathan and is called appropriately Dunathan's hypothesis (Figure 3). The cartoon below should have the α hydrogen removed and the π orbital remaining should contain the pair of electrons.

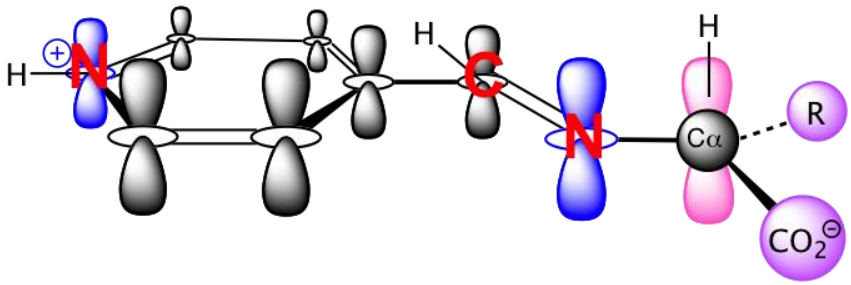
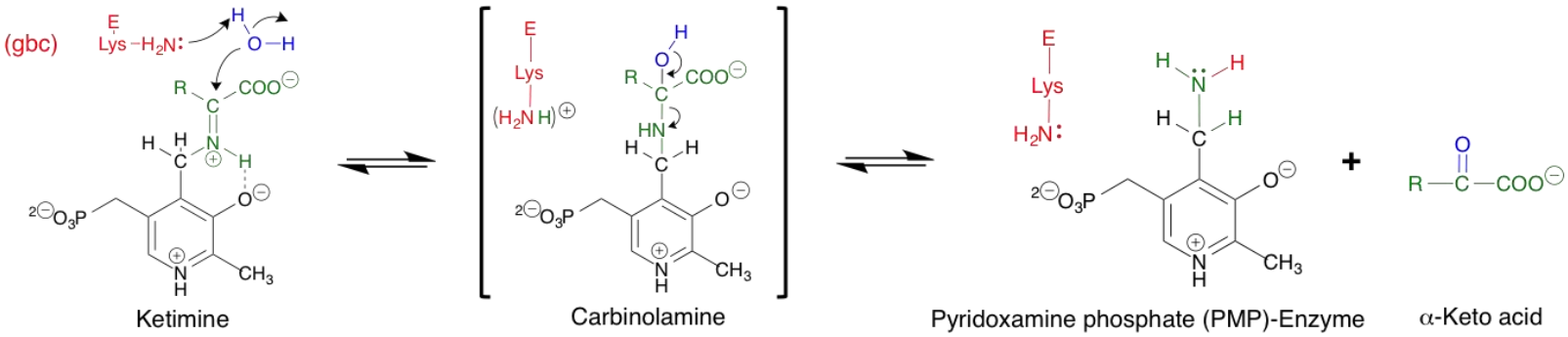


Figure 3. Dunathan's hypothesis. The bond to be cleaved, in this case the Cα C-H bond, is placed perpendicular to the plane of the π aldimine by the enzymatic binding of R and CO_2^- (purple balls). When the bond is cleaved, the electrons in the p orbital of the resulting anion overlap to form a resonance stabilized structure.

iv. The last step in the PLP dependent reactions varies depending on the product. When trying to describe the chemistry, carefully look at the END product and it will help you define where appropriate GAC and GBC need to be positioned.

In the case below that occurs when an amino acid is converted to an α-keto acid, protonation occurs at the CH_2 group adjacent to the pyridine ring leaving the K deprotonated. The last step would thus be that water attacks the Cα carbon to form a carbinolamine that then collapses to form pyridoxamine and α-keto acid.



To convert the pyridoxamine back to the Schiffs base of PLP, the pyridoxamine reacts with an α-keto acid (such as α-ketoglutarate, an intermediate in the TCA cycle, which is then converted to glutamate). Alanine conversion to pyruvate can then be converted to glucose in the liver by gluconeogenesis (Figure 4).

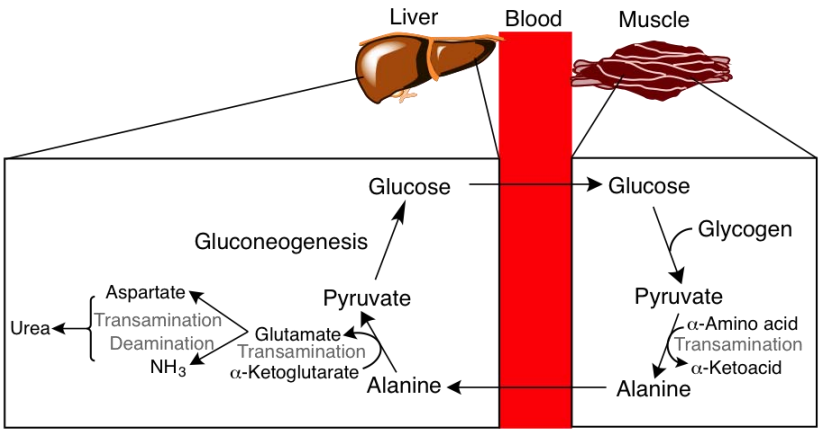


Figure 4. Glucose/alanine cycle.

You have already encountered an important PLP-dependent reaction in an earlier lecture involving the biosynthesis of the cell wall (peptidoglycan) of all bacteria. Each muramic acid in the polysaccharide has a pentapeptide attached to it. PLP is required for the racemization of L-ala to D-ala that is a part of this pentapeptide. Fluoroalanine is a potent, time dependent inhibitor of alanine racemase that was rationally designed by Merck many years ago. It still might be pulled from the shelf because of resistance problems we currently face. You should think about why fluoroalanine might be a potent inhibitor.

Energetics of the reactions involved in the endings of the glycolysis pathway.

1. $G + 2ADP + 2Pi + 2H^+ \rightarrow 2$ lactates $+ 2$ ATP $+ 2H_2O$ $\Delta G^{o\prime} = -196$ kj/mole

2. $G + 2ADP + 2Pi + 2H^+ \rightarrow 2EtOH + 2CO_2 + 2ATP + 2H_2O$ $\Delta G^{o\prime} = -235$ kj/mole

In the overall pathways (1, 2 above), NAD^+ is used by GAPDH and must be regenerated. In these reactions 2ATPs are produced. Recall that the synthesis of ATP requires $\Delta G^{o\prime}$ of + 30 kj/mole. The overall efficiency is thus 31% (26%). Note efficiencies can be much higher under certain conditions, modulated by the concentrations of metabolites. In the case of ATP production in muscle, ATP production is rapid and 100 x faster than production through oxidative phosphorylation (O/P) that will be discussed subsequently.

Medical Digression

Skeletal muscle is mostly devoid of mitochondria and anaerobic glycolysis occurs in these cells that are called "slow twitch" or type I muscle cells. These cells are used by a sprinter. A second type of muscle cell, "fast twitch" or type II, have mitochondria and are used to supply a slow steady source of ATP. Mitochondria are rich in heme (think about metal based redox reactions) and the high heme content causes the muscles to be red. Chickens, birds that do not fly very much, have white breast meat, while other birds that migrate each year and must fly over very long distances, have red breast meat.

End medical digression

Let us return to the **aerobic ending of the glycolysis pathway** in which P is converted to acetylCoA by the pyruvate dehydrogenase (PDH) pathway.

Respiration is the metabolism of all fuels (carbohydrates, lipids, amino acids) to CO_2 with generation of ATP in the presence of O_2 or a comparable oxidizing agent (NO_3^-, SO_4^{2-}, Fe^{3+}). There are three steps: 1. pyruvate dehydrogenase (PDH); 2. the TCA cycle or the Krebs cycle; 3. the electron transfer (ET) chain coupled to generation of a proton gradient that allows production of ATP. PDH is the aerobic ending to the glycolysis pathway. The glycolysis pathway occurs in the cytosol, the PDH reaction and the TCA cycle occur in the mitochondria, and the ET chain occurs within the inner mitochondrial (IM) membrane. See Figure 5 for the big picture. Compartmentalization is a key regulatory mechanism in eukaryotes. We will see that there are a number of metabolic shuttling systems required for metabolites to move between the cytosol and the mitochondria. Pyruvate will be the first example. It is generated in the cytosol through glycolysis and the PDH multienzyme complex exists in the mitochondria. There is a pyruvate transporter that requires a H^+ gradient to transport pyruvate. The genes for this important protein were only discovered in 2012 (Herzig, Sébastien, Etienne Raemy, Sylvie Montessuit, Jean-Luc Veuthey, Nicola Zamboni, Benedikt Westermann, Edmund RS Kunji, and Jean-Claude Martinou. "Identification and functional expression of the mitochondrial pyruvate carrier." *Science* 337, no. 6090 (2012): 93-96). The transporter is composed of two 15 Kda proteins, but the size of the transporter is 150,000 da.

Big Picture:

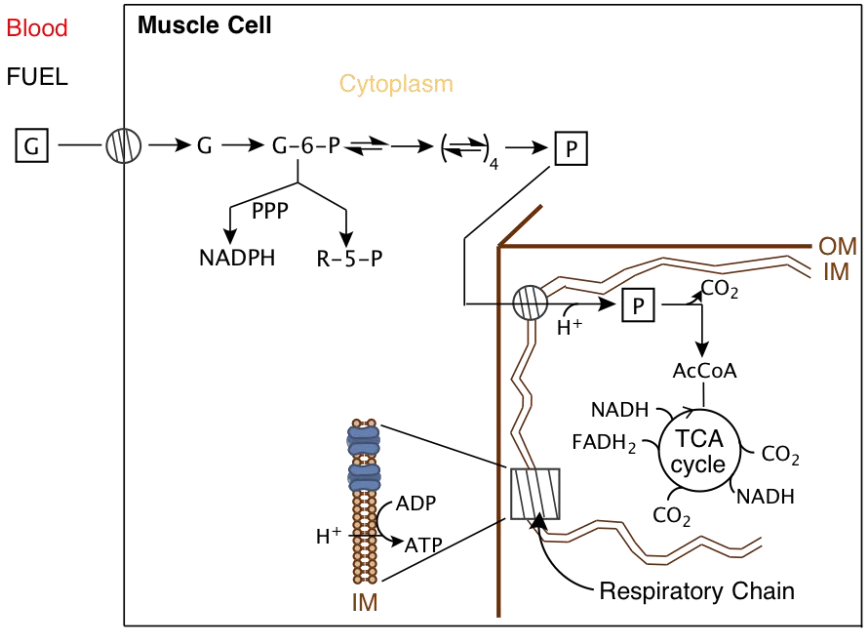
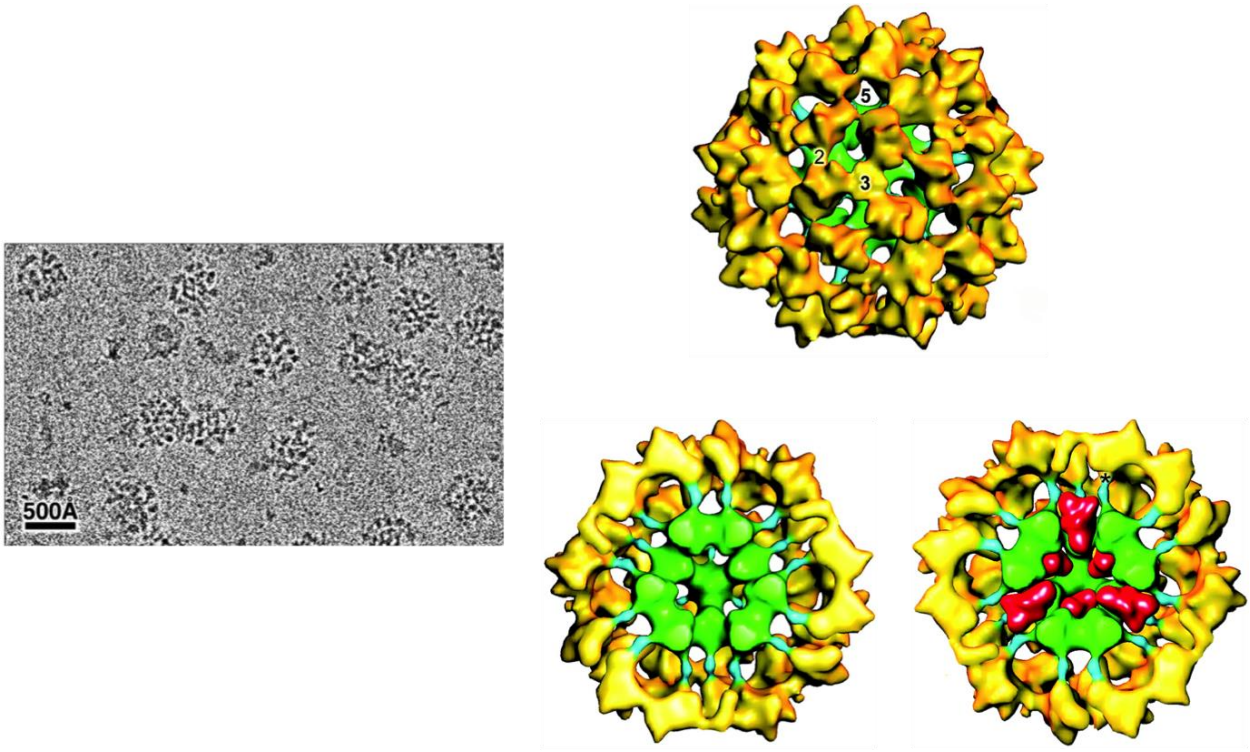


Figure 5. Overview of the aerobic endings to the glycolysis pathway.

PDH has been isolated from many sources. The *E. coli* PDH is 4.5 million molecular weight and is your first introduction to a macromolecular machine. You will see many of these machines in Chemistry 5.08 and learn the methods that have been developed to study these complex multienzyme machines. PDH uses five cofactors: TPP, CoA, lipoic acid, FAD and NAD. You have already been introduced to all of these cofactors.

The overall reaction is: $CH_3CO{*}CO_2^- + {}^-SCoA + NAD^+ \rightarrow CH_3COSCoA + {*}CO_2 + NADH$ (* is a radioactive label that allows one to follow the fate of the carbon of the carboxylate of pyruvate; * is ^{14}C rather than $^{12 \text{ or } 13}C$ (these isotopes of C are not radioactive)). The PDH complex is composed of three types of proteins: E1 is a pyruvate dehydrogenase (PDH) that contains 24 subunits and requires TPP. E2 is the dihydrolipoyl transacetylase (DHLT) that also contains 24 subunits and requires lipoate covalently tethered in an amide linkage through a lysine in E2, and E3 that is the dihydrolipoyl dehydrogenase (DHL-DH) that has 12 subunits and a

covalently bound flavin and a binding site for NAD^+. See the cryoelectron micrograph of PDH at 35 angstroms resolution (Figure 6).



Source: Zhou, Z. Hong, Diane B. McCarthy, Catherine M. O'Connor, Lester J. Reed, and James K. Stoops. "The remarkable structural and functional organization of the eukaryotic pyruvate dehydrogenase complexes." *Proceedings of the National Academy of Sciences* 98, no. 26 (2001): 14802-14807. Copyright © 2001 National Academy of Sciences, U.S.A

Figure 6. Cryo-EM (left) and 3D reconstructions (right) of bovine pyruvate dehydrogenase complexes. Yellow sections are E_1, green are E_2, and red are E_3.

E1 is the first step in the PDH catalyzed reaction, an oxidative decarboxylation (we have just studied the TPP-dependent non-oxidative decarboxylation in the anaerobic ending of the glycolysis). E2 is covalently bound to lipoic acid through the ε amino group of one of its lysines. Note that the arm on lipoic acid to E2 is 14 Å and allows movement of lipoic acid between E1 where TPP mediates the decarboxylation and E3 where the reduced lipoic acid becomes reoxidized (Figure 7).

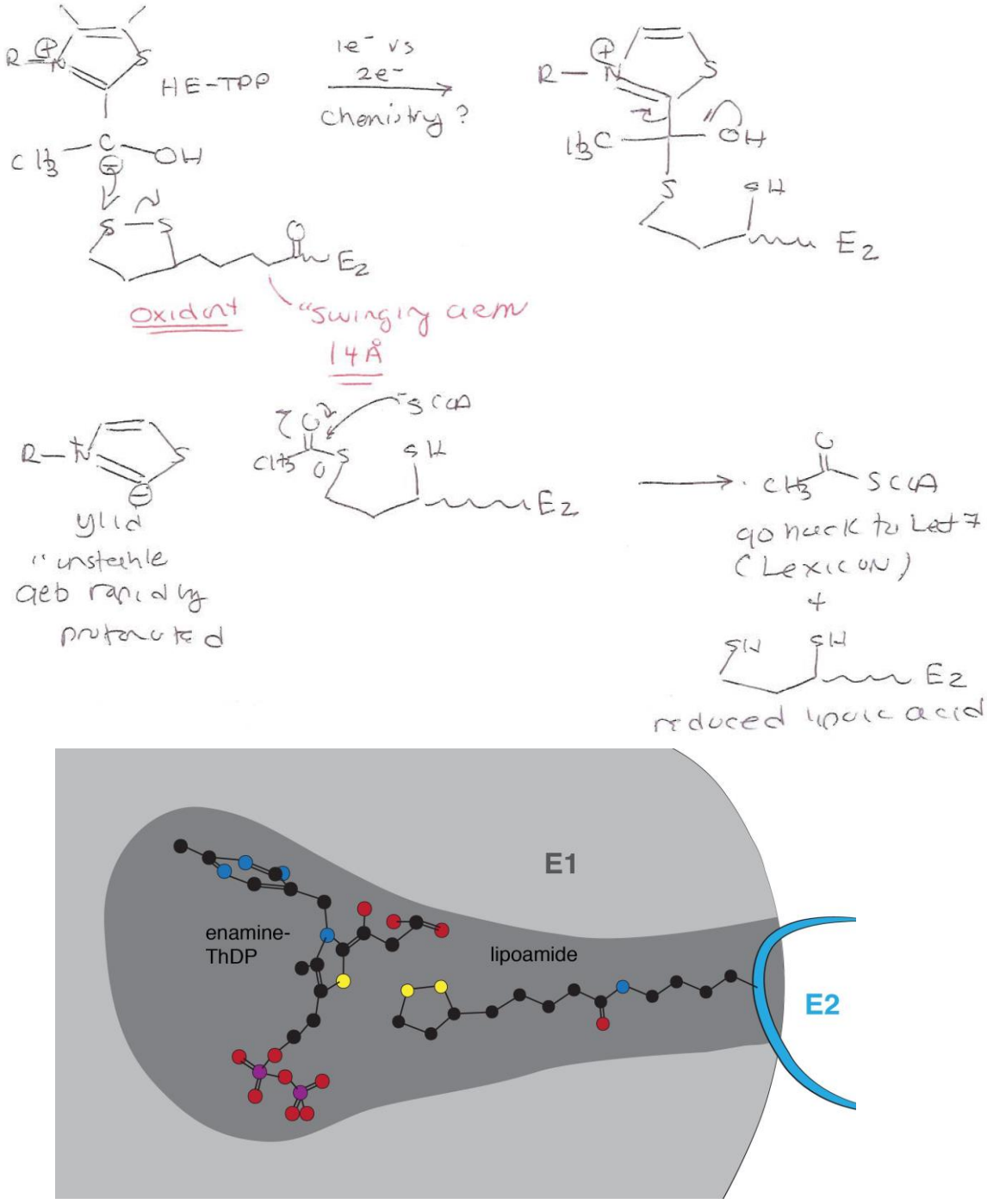
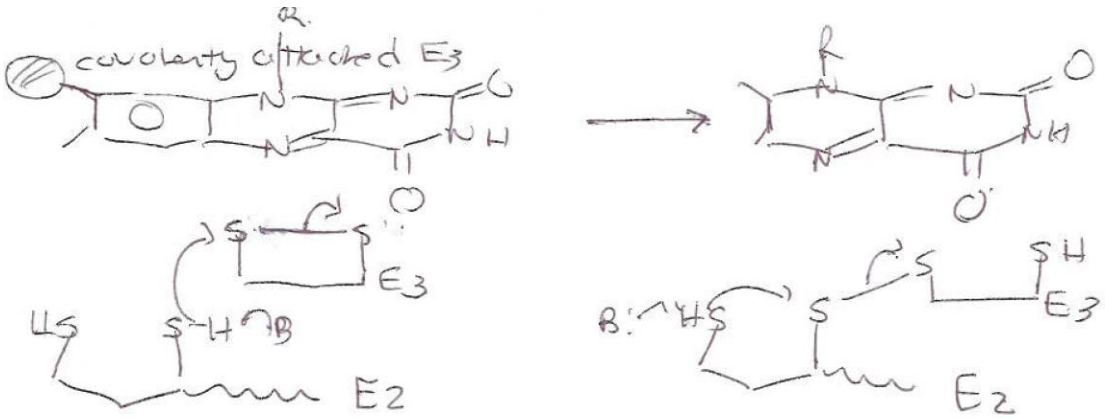


Figure by O'Reilly Science Art for MIT OpenCourseWare.

Figure 7. α KGDH with a lipolate arm from E2 extending into the active site of E1.

Lipoic acid needs to be reoxidized and this reaction is catalyzed by E3. You have been introduced to flavins in Lecture 9/10 and were told that flavins react at N5 and C4a. Both of these mechanisms are used in the reoxidation of lipoic acid. In the DHL-DH the enzyme also contains a disulfide adjacent to the flavin which is involved in the reaction (Figure 8).



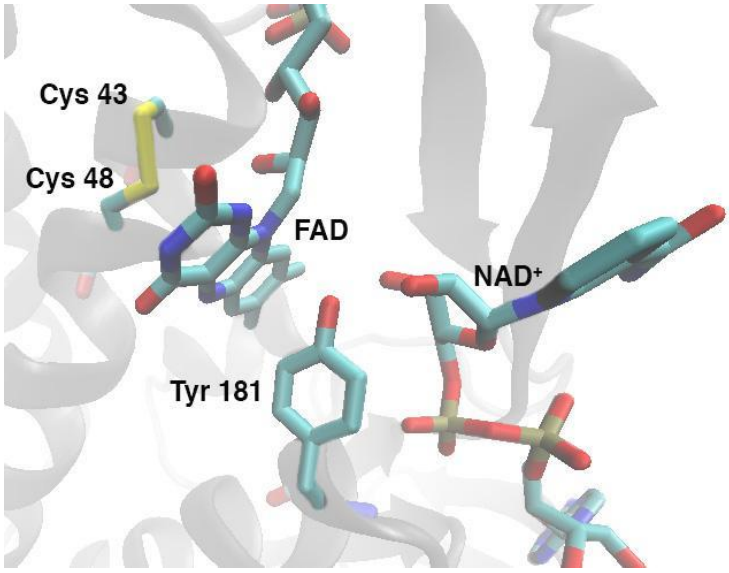
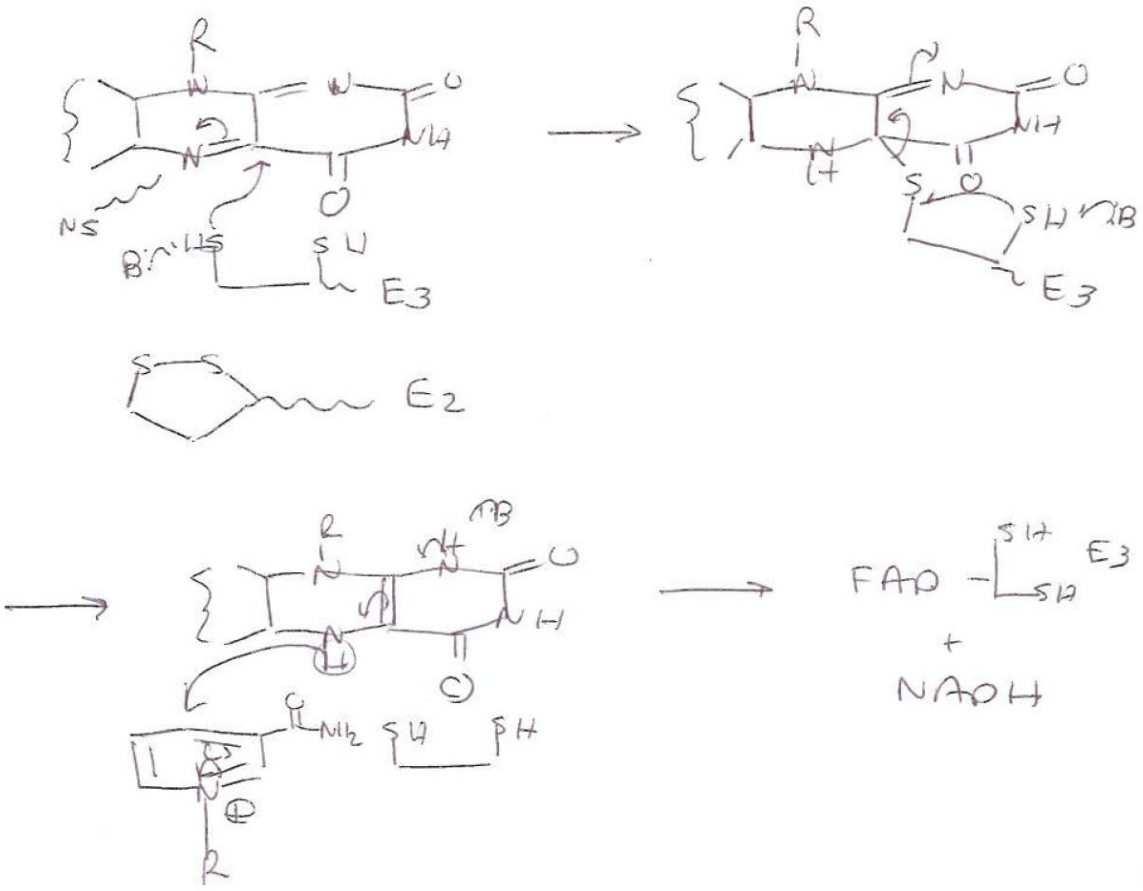


Figure by O'Reilly Science Art for MIT OpenCourseWare.

PDB: 1LVL
Mattevi, Andrea, Galya Obmolova, John R. Sokatch, Christian Betzel, and Wim GJ Hol. "The refined crystal structure of Pseudomonas putida lipoamide dehydrogenase complexed with NAD+ at 2.45 Å resolution." *Proteins: Structure, Function, and Bioinformatics* 13, no. 4 (1992): 336-351.

Figure 8. The *Pseudomonas putida* lipoamide dehydrogenase active site. Note in this figure that the NAD^+ is on the opposite face of the disulfide.



Medical Digression

Arsenic is a poison and was used to treat syphilis. $AsO_3H_2^-$ has also long been used as a reagent to detect vicinal thiols in an active site of enzymes. Without the vicinal thiols in E3, the lipoic acid could not be reoxidized.

End medical digression

Note: A similar type of complex machine will be observed again in α-KGDH reaction that you will encounter in the TCA cycle and in branched chain amino acid biosynthesis that you will not encounter in this course. In these two cases as well the enzymes are huge machines.

We examined the energetics of anaerobic glycolysis above. We will now examine the energetics of aerobic glycolysis. Aerobic glycolysis that occurs in the cytosol, regenerates NAD^+ by the

electron transfer pathway in the inner mitochondrial membrane and ultimately by reduction of O_2 to H_2O.

$$G + 2\ ADP + 2\ Pi + 2\ H^+ + 2\ NAD^+ \rightarrow 2\ pyruvates + 2\ ATP + 2\ H_2O + 2\ NADH + 2\ H^+$$

We will see in the next few lectures that NADH can be converted into 2.5 to 3 ATPs and that

Glycolysis → TCA cycle → ET, together can produce 32 to 38 ATPs.

What is the efficiency of ATP production for anaerobic versus aerobic glycolysis? How much energy is actually available from the fuel glucose?

$$C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \Delta G^{o\prime} = -2870\ kj/mole$$

If you look at anaerobic glycolysis: G → lactate or EtOH → 2 ATPs are generated. Since ATP requires +30 kj/mole, the efficiency is 30 x 2/2870 = 2% efficient.

If you turn to aerobic glycolysis: G → pyruvate + 2 ATPs + 2 NADH, since 2 NADHs can give rise to 6 ATPs (given information above), your efficiency is 8 ATPs x 30 kj/mole = 240 kj/mole. The efficiency is 240/2870 = 8%. You will see that this calculation is not quite correct in that there are NO transporters for NAD+/NADH to get into the mitochondrial and it is unable to diffuse across lipid bilayers. Cytosolic reducing equivalents (NADH) need to be transferred into the mitochondria by a shuttle system that John will describe.

Now if you couple glycolysis to the TCA cycle and ET: G → CO_2 + H_2O and 38 ATPs the efficiency is 30 x 38/2870 = 40%.

Recall that conditions in the cell are never standard, that is the concentrations are far removed from standard concentrations and in reality the efficiency can vary.

5.07SC Biological Chemistry I

Fall 2013