CHAPTER 20 - AMINO ACID METABOLISM

Introduction
Amino acid catabolism constitutes a significant contribution to the generation of metabolic energy of an organism. Clearly, carnivores will derive a greater fraction (up to 90%) than herbivores.

Amino acid degradation also occurs as a result of normal protein turnover (see Table 20-1 for half-lives of some enzymes). Proteins, for example, can be damaged and must be replaced. Also, controlling the levels (as well as the activities) of enzymes constitutes an important regulatory mechanism. Accordingly, these enzymes and proteins must be broken down as well as synthesized.

Lysosomes are cellular organelles that degrade extracellular substances that the cell takes up via endocytosis, including proteins, and also cellular substances within vacuoles by fusing with them. They contain a variety of proteases for this purpose, known as **cathepsins**. The internal pH of a lysosome is acidic (~ 5), and lysosomal proteases have optimal activities in this pH range. Presumably, the cell is protected from damage associated with accidental lysosomal leakage because their enzymes have much lower activities at higher cellular pH values. Lysosomes also selectively degrade proteins containing the amino acid sequence, Lys-Phe-Glu-Arg-Gln, or a closely related sequence. The pathway by which such proteins are selectively degraded is only activated after a prolonged fast, thereby protecting other essential proteins and enzymes from being degraded in starving cells.

Proteins can also be degraded in cells in an ATP-dependent process by a large, multiprotein cellular complex known as the **26S proteasome**. Such degradation requires that proteins be tagged covalently to a protein known as **ubiquitin**. This is also an ATP-dependent process involving other enzymes (E1, E2, E3) that ultimately result in the formation of an amide linkage between the carboxyl terminus of ubiquitin with the \( \text{\text{-amino group of a lysine residue} } \) on the protein selected for degradation (isopeptide bond).

During starvation or uncontrolled diabetes, body proteins are broken down when carbohydrate is unavailable (recall ketone body formation under similar conditions).

The 20 commonly-occurring amino acids all have an alpha amino group in common. The removal of this potentially toxic group is thus common to all amino acids and will be considered in some detail.
The carbon skeletons remaining after amino group removal are broken down by multiple mini-pathways that all converge on the central metabolic pathways, the citric acid cycle or glycolysis (pyruvate).

**Protein degradation**

A variety of proteases break down dietary protein in the stomach (pepsin) and intestine (trypsin, chymotrypsin, carboxypeptidase, aminopeptidase) into amino acids. These digestive enzymes are typically stored as inactive precursors (zymogens). Activation of these zymogens is typically hormone-initiated.

**Amino group removal**

Dietary amino acids enter the liver where their amino groups are removed via **transaminations:**

Transaminations require the coenzyme **pyridoxal phosphate** (PLP):

This phosphate group of this versatile coenzyme was seen to function as a general
acid/base in the glycogen phosphorylase-catalyzed phosphorolysis of glycogen. The “business end” of PLP in amino acid metabolism is the pyridine nitrogen, which serves as an electron sink to stabilize a variety of bond cleavages. The amino acid attaches to the PLP via Schiff base formation. Subsequently, decarboxylations and reverse aldols can occur as well as transaminations, as indicated below:

Note that flow of charge into the ring system requires that C$_{\text{alpha}}$ N CH be coplanar with the ring system for charge to flow into the coenzyme. The six-membered ring depicted above, completed by the hydrogen bond indicated, favors co-planarity of these groups (see Figure 20-7 for a complete mechanism).

There are different transaminases for different amino acids, but the amino group acceptor is typically alpha-ketoglutarate. The result of these transamination reactions is that amino groups wind up on glutamate. The glutamate then enters the mitochondria where it undergoes oxidative deamination, catalyzed by glutamate dehydrogenase:
Note that one of the products of the glutamate dehydrogenase reaction, alpha ketoglutarate, is a citric acid cycle intermediate. One of the main causes of ammonia’s toxicity is that excess ammonia will shift the reaction to the left, thereby lowering alpha ketoglutarate, hence depressing citric cycle activity, causing energy depletion. The brain is particularly sensitive.

Amino acids degraded in muscle donate their amino groups to pyruvate via a similar transamination as depicted above. Alanine, the amino acid produced, then enters the blood, then liver during the glucose-alanine cycle, depicted in Figure 21-6, p. 671. Note that glucose enters the muscle cell as a result of this cycle, thereby providing energy via glycolysis, in addition to amino acid breakdown.

Note that alanine serves as the amino group carrier operating between muscle and liver. Free ammonia is also transported to the liver from extrahepatic cells, but not in this form. Instead, it is combined with glutamate to form glutamine, which acts as the carrier of free ammonia:
This reaction is carried out by glutamine synthetase. The glutamine is transported through the blood to the liver where it is stripped of its amino group in the mitochondria (glutaminase). The amino group then enters the urea cycle. To summarize then, amino groups enter the mitochondria of a liver cell either as glutamate or glutamine. Glutamate receives the amino group from either alanine or dietary amino acids via transaminations in which alpha ketoglutarate is the amino group acceptor. Both glutamate and glutamine are stripped of their amino groups in the matrix, which are then converted into urea by the catalytic action of the urea cycle, then excreted.

**Urea cycle**

This is the third catalytic cycle we have encountered this semester, the first two being the citric acid and Calvin cycles. The catalytic event in the urea cycle is the formation of urea from its amino group donors, carbamoyl phosphate and aspartate. The catalyst is ornithine, not one of the common twenty amino acids, but similar to lysine, from which it differs by having three instead of four methylene groups. As urea is assembled on the amino group of the side chain of ornithine, receiving portions of the molecule first from carbamoyl phosphate, then aspartate, the ornithine is successively converted to citrulline, then arginino succinate. The arginino succinate then casts off the carbon skeleton of aspartate (fumarate), forming arginine. Arginine then splits off urea, thus reforming the catalyst:
Formation of the first amino group donor, carbamoyl phosphate, comes at the expense of two ATP:

$$2 \text{ ATP} + \text{HCO}_3^- + \text{NH}_3 \rightarrow \text{H}_2\text{N}-\text{C}=\text{O}-\text{PO}_3^2^- + 2 \text{ ADP} + \text{P}_i$$

Carbamoyl Phosphate

The second amino group donor, aspartate, is an amino acid, which forms fumarate when stripped of its amino group (see Figure 20-8). Aspartate is reformed, thus acting as a catalyst itself. Since aspartate is formed from oxaloacetate via transamination, and since the link between fumarate and oxaloacetate involves the citric acid cycle, the urea and citric acid cycles are linked:
The ATP cost per urea is 4, 2 from the formation of carbamoyl phosphate and the energy equivalent of two for the formation of arginino succinate. This is because ATP is cleaved to form AMP + PP\(^i\), which is cleaved to form 2 Pi.

Regulation occurs at two levels in the short run. Glutamate dehydrogenase is inhibited by the energy-rich indicator, GTP, and stimulated by the energy-poor ADP. Carbamoyl phosphate synthetase (version I) is allosterically activated by N-acetylglutamate, the formation of which is stimulated by glutamate, which is logical since glutamate feeds amino groups into the urea cycle. Urea cycle is subject to long-term regulation at the level of transcription, by which the amounts of urea cycle enzymes are controlled.

It has been estimated that animals use about 15% of the energy they derive from amino acid metabolism in urea formation. Microorganisms in the rumens of cows recycle urea back to amino acids in order to reduce the net investment of energy, thereby reducing their protein intake. Urea is sometimes added to cattle feed as in inexpensive nitrogen supplement.

Various genetic defects occur in humans involving the five enzymes required in nitrogen excretion. Such individuals cannot tolerate a protein-rich diet. In these individuals the 10 essential amino acids are given as their alpha-keto acid analogs, which can then obtain their amino groups from non-essential amino acids via transamination.

**Breakdown of amino acid skeletons**

Amino acids are categorized as either **glucogenic** or **ketogenic**, depending on whether they can be converted to glucose. Glucogenic amino acids whose mini-pathways converge into the metabolic mainstream as either pyruvate or any citric acid intermediate, since these metabolites can be converted into glucose via gluconeogenesis. Ketogenic amino acids are those
that form either acetyl CoA or acetoacetyl CoA, since neither of these can be converted into glucose (see Figure 20-11).

The amino acids alanine, glutamate and aspartate are brought into the metabolic mainstream via simple transaminations to form pyruvate, alpha ketoglutarate and oxaloacetate, respectively.

Arginine is a glucogenic amino acid that is converted into alpha ketoglutarate by a scheme depicted in Figure 20-4.

Threonine is also a glucogenic amino acid, being converted to pyruvate in a scheme depicted in Figure 20-12. Note that this mini-pathway requires the coenzyme tetrahydrofolate (THF), a one-carbon carrier that can carry carbon in various oxidation states. We have already seen that biotin is a one-carbon carrier that carries and transfers carbon in a highly oxidized state (carboxyl). Also, S-adenosylmethionine (Figure 20-16) can act as a methylating agent (i.e., transfers one carbons in a highly reduced (CH$_3$) state).

THF is derived from its vitamin precursor, folate, or folic acid (acidic functionality in R group)

NOTE: The R group of THF contains a para-amino benzoic acid group. Sulfa drugs are antibiotics that are structural analogs of p-amino benzoic acid.

The one-carbon units bond to either or both of the nitrogens indicates with asterisks
Methionine is of interest because of its connection with homocysteine and atherosclerosis. In 1969 McCully made a clinical observation linking elevated plasma homocysteine levels with vascular disease. Subsequent investigations have confirmed McCulley’s hypothesis. Mild hyperhomocysteinemia occurs in about 5 to 7 percent of the general population. Such patients typically develop premature coronary artery disease in their twenties or thirties. Elevated homocysteine levels have been found in 20-30% of patients with premature atherosclerosis. The link with heart disease may be due to the fact that homocysteine is rapidly oxidized when added to plasma, forming the disulfide, mixed disulfides, etc., which also forms many potent reactive oxygen species in the process. These include hydrogen peroxide and superoxide anion radical which are known to lead to heart disease. Also, homocysteine has been shown to stimulate vascular smooth muscle proliferation, a hallmark of atherosclerosis. Vitamin supplementation with folic acid, pyridoxine and B₁₂ is generally effective in reducing homocysteine levels. This is because THF, pyridoxal phosphate and B₁₂ are involved in methionine metabolism.

Methionine is metabolized by one of two pathways, a salvage, or remethylation, pathway and a transsulfuration pathway, both of which are depicted in Figure 20-16, page 630.

Problems: 2, 3