Amino Acid and Nitrogen Metabolism I: Overview; Elimination of N-waste

Date: Wednesday, September 21, 2005*  
Time: 8:10-8:50 AM*

Location: G-202 Biomolecular Building

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Office Hours: by appointment, pretty much anytime; send an email to schedule a time

*Be sure to consult the online schedule for this course for the definitive date and time for this lecture.

Note: This is the first of three closely related lectures on amino acid metabolism (the others are on Friday, September 23 from 8:00-8:50, and 10:45-11:50*). Everything you need to know is in these notes and/or the associated .ppt slide set on our Molecules to Cells homepage, although coming to class and paying attention is always a good idea too. If you want more detailed information and/or a better understanding of these topics, look at Chapters 19-21 in Lippincott’s Illustrated Reviews: Biochemistry (3rd ed) or Chapter 20 in Voet, Voet, and Pratt, Fundamentals of Biochemistry.

Objectives: By the end of this lecture, you should:

a. know the sources and general uses of amino acids in the body
b. understand the importance of protein turnover, and the involvement of the ubiquitin-proteasome and lysosomal degradation pathways
c. understand the concept of nitrogen balance (negative and positive) and its clinical relevance
d. have a general understanding of amino acid catabolism, both with respect to NH₃ groups and the remaining C-skeletons
e. know the sources of ammonia and how it can be transported safely to liver and kidney
f. understand the role of transamination and deamination reactions in amino acid synthesis and catabolism, and in excretion of waste nitrogen atoms

g. understand the general idea of what’s going on with the urea cycle

Important Overall Concepts:

1. Body proteins turn over (dynamic), usually in the “steady state.” Lysosomes are responsible for “non-specific” degradation, while the ubiquitin-proteasome system takes care of “targeted” protein degradation.
2. There is always some amino acid catabolism – quite a bit during prolonged fasting (mostly from muscle protein) to support gluconeogenesis in liver.
3. N-waste travels to liver (and kidney) as glutamine, glutamate, and alanine.
4. Waste NH$_3$ is eliminated mostly as urea (liver). Aminotransferases trade NH$_3$ among amino acids/α-keto acids, and importantly, funnel waste NH$_3$ to glutamate. Glutamate dehydrogenase releases this waste NH$_3$, which is used to synthesize urea (aspartate supplies the 2$^{nd}$ NH$_3$ group of urea). Urea then travels in blood to the kidneys for excretion.

5. C-skeletons of amino acids (α-keto acids) can be oxidized for energy, serve as gluconeogenic substrates in liver during fasting, or be stored as triglycerides for later energy use. More on this in the next lecture.

1: Introduction and General Overview

There are over 150 amino acids (biomolecules that have at least one carboxylic acid group and at least one amine group) in our bodies, but sadly we will only be able to concern ourselves with about 20 or so. Amino acids provide the building blocks for proteins (chains of amino acids linked by amide or peptide bonds), and they also supply the nitrogen required for the nucleotide building blocks (e.g., ATP, GTP) for RNA and DNA. These nucleotides also have very central roles in metabolism as well. Other important stuff like neurotransmitters is made from amino acids too.

In addition, the carbon skeletons of amino acids are important energy sources in some dietary situations. Use of these carbon skeletons requires proper disposal of ammonia (NH$_3$), a toxic by-product of amino acid catabolism, so we need to know about how our bodies transport, handle, and eventually excrete nitrogen waste (this involves the urea cycle in liver, and ultimately your kidneys and bladder).

Refer to the diagram on the next page whenever you want to see how amino acid metabolism (including the urea cycle), or any other metabolic pathway, fits into the overall scheme of metabolism. Amino acid metabolism is in green, but the most important concept is how this relates to all the other metabolism that occurs in the cell.
Look for a great big metabolic map here – coming soon!
2: Protein Turnover & Degradation; Nitrogen Balance

Most amino acids are either in proteins, or on their way to or from being in proteins. Protein metabolism is dynamic - virtually all proteins turn over (are degraded and replaced), although the turnover rates for individual proteins varies greatly - from minutes to the life of the organism. In general, the rate of synthesis equals the rate of degradation (steady-state). However, some amino acids are also used for energy production and storage and for synthesis of non-protein molecules, so there is a constant need for dietary intake of protein (or essential amino acids). In addition, there are situations where protein synthesis must exceed protein degradation, such as during growth, pregnancy, and recovery from illness.

After chewing and swallowing, dietary proteins enter the stomach where the low pH denatures them, rendering them more amenable to digestion by proteases such as pepsin in the stomach and trypsin and chymotrypsin in the small intestine (all of these are synthesized and stored as inactive zymogens). Liberated amino acids are absorbed into the hepatic portal blood, pass through the liver where some are taken up, and then go everywhere else.

The metabolic energy requirements for synthesis of proteins to replace those turning over (about 400 g/day) and those excreted as digestive enzymes or lost from cells lining the small intestine (about 20 g/day) is considerable, as much as 20% of the total daily energy requirement.

General “non-specific” protein degradation takes place in lysosomes - specialized organelles that operate at low pH (to denature proteins) and contain proteases for proteins, lipases for lipids, and many other hydrolases (~ 50 total) - remember the Lysosomal Storage Disorders Case Conference and the Complex CH₂O/Glycolipids lecture?). Both internal proteins (enclosed in vacuoles that fuse with lysosomes) and external proteins (obtained via endocytosis) are transported to lysosomes where proteins are degraded and the resulting amino acids either recycled for new protein synthesis or degraded for energy production or storage. In normal healthy cells, lysosomal protein degradation is nonselective. However, lysosomes in tissues that waste away or atrophy during starvation (e.g., liver and muscle, but thankfully not brain and testes) have a
special degradative pathway activated only during prolonged fasting - this involves selective degradation of cytosolic proteins containing the pentapeptide sequence Lys-Phe-Glu-Arg-Gln, thus sparing more essential proteins from increased non-selective degradation. There are no truly non-essential proteins - all are there for a good reason, and we don't “store” amino acids as proteins. Many normal and pathological processes involve increased lysosomal activity, including disuse atrophy of muscles and regression of the uterus after childbirth - the latter muscular organ's mass is reduced from about 2 kg (2000 g) to about 50 g in just nine days. Chronic inflammatory diseases such as rheumatoid arthritis involve extracellular release of lysosomal enzymes, which attack surrounding tissues.

Although most control of metabolic activity and cell function is at the level of post-translational control or action of regulatory molecules, some control is at the actual level of the proteins themselves - by synthesis and/or degradation. This controlled or programmed protein degradation involves the Ubiquitin-Proteasome system.

Proteins destined for proteasome degradation are marked by addition of ubiquitin molecules, a small (~8.5kd) protein that is attached to the ε-amino groups of lysine on target proteins. Three different enzymes add progressively more Ub molecules, in tandem chains, an energy-requiring process (ATP). The more Ub molecules attached, the more rapid the degradation.

What controls the rate and degree of ubiquitination and thus the rate of protein degradation? Factors include the N-terminal residue (Met and Pro = very slow; Arg, Leu = very fast), as well as “cyclin-destruction boxes” and PEST sequences (Pro-Glu-Ser-Thr) in proteins.

Ubiquitinated proteins enter the proteasome, where they are digested, ultimately all the way to free amino acids. The ubiquitin is recycled.

Use the animated .ppt slide version (AA Met I online slide set) of the figure to see the steps involved.

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**Lysosomal Protein Degradation**

1. Protein destined for degradation tagged with ubiquitin molecules (tandem links)
2. Ubiquitinated proteins are recognized by proteasome, which unfolds and transports protein to its proteolytic core
3. Peptide fragments produced by proteasome degraded to amino acids by non-specific proteases
3: Catabolism of Amino Acids

A. Overview: Ammonia (NH$_3$) is toxic. Waste NH$_3$ groups (mostly from amino acids, but also from other sources) are safely incorporated into urea (2 HN$_3$ groups hooked onto an oxidized C atom) by the liver for disposal by the kidneys in the urine. The deaminated C-skeletons can be oxidized for energy, stored as triglyceride until needed, or used for synthesis of new glucose (gluconeogenesis, mostly in liver). Both waste NH$_3$ groups and C-skeletons for gluconeogenesis travel from tissues to liver as glutamine, glutamate, or alanine. Alanine carries both C-skeletons and waste NH$_3$, both derived from skeletal muscle proteolysis, to liver where it serves as a major substrate for gluconeogenesis during fasting (more on this during the next lecture).

Glutamate is a central molecule in amino acid and N metabolism. Transaminases funnel waste NH$_3$ to glutamate ($\alpha$-ketoglutarate accepts the NH$_3$ group from all other amino acids to form glutamate). Glutamate can be oxidatively deaminated to release free ammonia, which enters the urea cycle (liver). Aspartate, formed by transamination of oxaloacetate, provides the other NH$_3$ group for urea; the C-skeleton of what used to be aspartate is eventually recycled to oxaloacetate, then transaminated to aspartate. The newly formed urea travels in the blood to the kidneys, where it is excreted in the urine. Some NH$_3$ groups also come from deamination of glutamine in liver (for urea synthesis) and in kidney (for excretion into urine; involved in acid-base balance).

B. Transamination and Deamination of Amino Acids: Transamination reactions serve two purposes - first, they help maintain adequate levels of non-essential amino acids required for protein synthesis. Importantly, they also funnel amino groups from catabolized amino acids to glutamate and aspartate for eventual excretion as urea. There are at least 17 different transaminases, nearly all of which use $\alpha$-ketoglutarate as the NH$_3$ acceptor, producing glutamate and an $\alpha$-keto acid. They all require pyridoxal phosphate (comes from vitamin B$_6$).

These reactions are freely reversible, with $K_{eq}$ near 1.0 - there is amino acid degradation to keto acids following a protein-containing meal, and synthesis of amino acids from keto acid skeletons when needed.

Aminotransferases are named after the amino acid donor. The two most important are ALT (alanine aminotransferase; alanine is converted to pyruvate), and AST (aspartate aminotransferase; an exception to the rule that amino acids transfer their amino groups to form glutamate). During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate to form aspartate, which is used as the source of one of the NH$_3$ groups for urea syntheses.

Levels of ALT and AST in serum are of diagnostic value, in particular for liver disease (and sometimes for muscle disorders and myocardial infarcts). Aminotransferases are normally intracellular enzymes, with normal low levels in serum indicating release of
cellular contents during normal cell turnover. Elevated levels in serum indicate damage to tissues rich in a particular enzyme (trauma or disease causes cell membrane leakage and eventually lysis, with release of cellular contents into blood).

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\begin{align*}
glutamate + pyruvate & \rightarrow alanine + \alpha\text{-ketoglutarate} \\
& \text{alanine amino-transaminase (ALT)} \\
glutamate + oxaloacetate & \rightarrow aspartate + \alpha\text{-ketoglutarate} \\
& \text{aspartate amino-transaminase (AST)}
\end{align*}
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C. Oxidative deamination of glutamate - In contrast to transaminase reactions, oxidative deamination yields an \(\alpha\)-keto acid with release of the amino group as free ammonia. Glutamate dehydrogenase in liver is the most important enzyme involved. Glutamate is the only amino acid that is rapidly deaminated - remember \(\alpha\)-ketoglutarate collects amino groups on glutamate. Glutamate dehydrogenase then produces ammonia, regenerating \(\alpha\)-ketoglutarate. The released ammonia provides one of the two NH\(_3\) groups for urea synthesis (coming soon).

The direction of the glutamate dehydrogenase reaction depends on levels of substrates, including the ratio of oxidized/reduced coenzymes. After a protein-containing meal, the reaction proceeds in the direction of amino acid degradation and ammonia production, but the reverse reaction can also be used to synthesize glutamate (and then other amino acids via transaminase Rx).

Glutamate dehydrogenase is unusual in that it can use either NAD\(^+\) or NADP\(^+\) - it usually uses NAD\(^+\) for oxidative deamination and NADPH\(_{-}\) for reductive amination, but doesn’t have to.

ADP and GDP (energy-poor signals) allosterically activate glutamate dH, while ATP and GTP inhibit. When energy levels are low, amino acid degradation is high, facilitating energy production from the carbon skeletons of amino acids.

There are lots of \(d\)-amino acids in plants. We can’t use them for protein synthesis, but we can burn the carbon skeletons for energy. \(d\)-amino acid oxidase is especially active in liver, where it deaminates dietary \(d\)-amino acids; the resulting keto acids can either be catabolized for energy or reaminated to \(l\)-amino acids usable for protein synthesis.
D. Transport of ammonia to the liver (and kidney): All tissues produce some ammonia from a variety of compounds. The level of ammonia in blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (a little more on this in an upcoming lecture). There are two major mechanisms to transport ammonia to liver for its conversion to urea and ultimate excretion in the urine. **Glutamine synthetase** in many tissues puts free ammonia on glutamate to form glutamine, an energy requiring process. Think of glutamine as a non-toxic transport form of ammonia (carries 2 NH$_3$, actually). Glutamine travels in the blood to the **liver**, where **glutaminase** releases free ammonia which can enter the urea cycle. The kidneys can also form ammonia from glutamine by action of **renal glutaminase**. This NH$_3$ is excreted into the urine as NH$_4^+$, an important mechanism for maintaining whole-body acid-base balance.

The second mechanism involves the **glucose-alanine "cycle."** Amino acids derived from skeletal muscle protein breakdown are converted to alanine, which is transported to liver where it is deaminated to form pyruvate. Waste NH$_3$ groups enter the urea cycle, while pyruvate is used for gluconeogenesis – more details on this pathway in the next lecture.

Where does waste ammonia come from? Deamination of amino acids is the major source (lots in liver and muscle). Amines in the **diet** and “monoamines” (hormones and neurotransmitters like dopamine, serotonin, epinephrine and norepinephrine) are also significant sources - **monoamine oxidases** catalyze the oxidative deamination of these molecules. Amino groups on **purines and pyrimidines** are also released as NH$_3$ during nucleotide catabolism (Dr. Chaney on this soon).
E. **Urea Cycle.** The urea cycle is how mammals get rid of excess nitrogen arising mostly from metabolism of amino acids (it accounts for about 90% of N-containing compounds in urine). In the liver, ammonia, produced by oxidative deamination of glutamate (which collects amino groups from other amino acids) and the amine group from aspartate combine with carbon dioxide (actually a $\text{HCO}_3^-$ ion) to form urea. The urea is carried through the blood to the kidney, which sequesters it for excretion in the urine.

The initial reaction (actually a prelude to the urea cycle itself) is catalyzed by carbamoyl phosphate synthetase, the major control point in urea synthesis. You can think of carbamoyl-P as “activated ammonia.” Overall, 4 $\sim$P (equivalent to 4 ATP) are required to form urea, which gets rid of 2 amino groups. You are not responsible for the structures of intermediates in the urea cycle, but you should have a good understanding of what’s going on, including key intermediates and the enzymes shown in the figure below. If you can stand it, look once at a diagram in a biochemistry textbook with actual structures (Figure 20.8, page 621 in Voet, Voet, and Pratt, or Figure 19.14, page 252 in Lipincott’s Illustrated Review of Biochemistry, 3rd ed. are pretty good) so you can see what’s really going on. Hopefully you will find this informative and reassuring.

A “Key Points” summary for all of amino acid metabolism (3 lectures) is at the end of the notes for lecture #3 (Sept. 23). Some additional practice questions are there as well.