

Biochemistry 401 Lecture 31

Today we're going to start off with the degradation of dietary and cellular proteins, and then we're going to talk about general amino acid catabolism and then we're going to finish with inborn errors of metabolism and to do this we're going to talk about the catabolism of specific amino acids. So let's get started.

The catabolism of dietary proteins begins in the stomach through the action of pepsin, which is an enzyme that hydrolyzes proteins in a nonspecific manner. Pepsin is the active enzyme but it is synthesized and released into the stomach as the inactive zymogen, pepsinogen. And so pepsinogen is released into the stomach, and is activated by the low pH environment of the stomach, and then it cleaves dietary proteins nonspecifically, in a random manner that is not tied to the specific sequence of the protein itself. The low pH environment of the stomach helps this process out not only by activating pepsin, but also through the denaturation of proteins to form random coils, and in this way gives the proteases access to the proteins.

It is an autocatalytic enzyme in that when the zymogen unfolds, it can clip 44 residues out of the inactive zymogen to form the active pepsin enzyme. Now the other thing about pepsinogen is that it actually becomes denatured in a pH that's five or higher. This will come into play a little bit later on.

Catabolism of dietary proteins continues in the duodenum, the first area of the small intestine. This occurs through the action of pancreatic proteases that are produced as zymogens in the pancreas and then are released into the duodenum. Now unlike pepsin, pancreatic proteases cleave proteins at specific residues as part of a proteolytic cascade. This cascade begins through the action of enteropeptidase that is found on the surface of the enterocytes, the epithelial cells that line the lumen of the small intestine, and so enteropeptidase activates the pancreatic protease cascade turning zymogens into active enzymes. These enzymes cleave proteins to short oligopeptides and since these are still too big to get into the cell quite easily, they're further cleaved through the action of amino peptidases to form dipeptides and tripeptides, which are then internalized. On the other hand, free amino acids can enter the cell quite easily.

This is an overview of the gastric and pancreatic zymogens that are secreted by the stomach and the pancreas, and below we see the proteolytic cascade that's

initiated by enteropeptidase. You do need to know this pathway, and you do need to know the names of the enzymes. In the stomach, pepsinogen is produced and is activated through the low pH of the stomach contents to form pepsin. The pancreas secretes chymotrypsinogen, trypsinogen, procarboxypeptidase, and proelastase, and these are activated through proteolysis to form the active enzymes, chymotrypsin, trypsin, carboxypeptidase, and elastase. Now before we go any further, let's talk about some names. We saw aminopeptidase previously, and here we see carboxypeptidase. Those two names mean something.

Aminopeptidases cleave at the amino-terminus. They attack at the very amino end of the protein to release amino acids, one after another, after another.

Carboxypeptidases, on the other hand, cleave proteins at the carboxy terminus.

Okay back to the cascade. So this cascade is activated by enteropeptidase. This is an enzyme that's found on the luminal surface of enterocytes, epithelial cells that line the small intestine. Enteropeptidase cleaves trypsinogen to form trypsin. Now this active protease can go back and activate more trypsinogen, and can also activate chymotrypsin, proelastase, procarboxypeptidase, and can even activate pro-lipase to form active lipase. Now we know that lipase is not going to cleave proteins, it's going to cleave triacylglycerols.

Pancreatic proteolytic zymogens enter the duodenum along with bicarbonate, and mix with the dietary contents just leaving the stomach. What effect does this bicarbonate have?

Bicarbonate ion raises the pH of the stomach contents as they enter the duodenum.

Now, pepsin is active only at low pH. At a pH Level of 6.5 and greater, pepsin becomes inactive. Now why is this characteristic of pepsin important for proteolysis in the duodenum?

Pepsin cleaves proteins randomly. By increasing the pH and inactivating pepsin it is prevented from proteolyzing the zymogens and enzymes present in the small intestine.

Endogenous proteins are also catabolized, and this happens for many reasons, three of which are shown here, the regulation of protein activity through turnover, quality control, and as a source of amino acids. The activities of some proteins

are regulated through turnover. By getting rid of the protein, you can get rid of the activity. This can either have an activating or inhibiting effect on pathways as a whole. If you get rid of an activator, you're going to decrease the activity of a pathway, but, on the other hand, if you get rid of an inhibitor you're going to increase the activity of the pathway. Think about this for a minute; make sure you understand it. Additionally, some proteins are just the wrong shape. This can happen through mis-translation, or misfolding, or the protein can become oxidized. Proteins like this can be trouble. They can lead to aggregation and diseases such as Huntington's or Alzheimer's. If a protein is misshapen and can't do its job well, then it's important to get rid of it.

Finally the catabolism of proteins offers a source of amino acids. They can either be used as intermediates in anabolic reactions, or can be broken down further as a source of fuel.

If it's time for cellular proteins to be degraded, this is where they go. They're tagged by the ubiquitin pathway and degraded by the proteasome.

Ubiquitin binds to proteins through the formation of isopeptide bond This is a different kind of peptide bond. It's still formed between an alpha carboxy group and an amino group, but in this case it's an epsilon amino group from a lysine. Several ubiquitin proteins can be linked together through the formation of these isopeptide bonds, and the more ubiquitins that are added, the stronger the signal is to deliver this protein to the proteasome.

Here we see the whole proteasome, 26S. It's comprised of three regions. Two 19S regions that serve as caps at the top and the bottom, and an intervening 20S region and this is actually the catalytic region that breaks down the proteins.

The ubiquitinated protein binds to the 19S cap, and isopeptidases in the 19S cap remove the ubiquitin. ATPases in the 19S cap unfold the protein and allow it to enter into the 20S region. In the 20S region, threonines attack the protein backbone, and so the proteolysis of peptides in the proteasome is carried out by catalytic threonines at the N-terminal ends of the subunits within the central core of the enzyme. The peptide fragments created in this way are not released until the peptide is sufficiently cleaved. Peptides are cleaved into short fragments that average 7-9 amino acids in length.