Lecture 22A • 06/11/12

[lab directions][lab quiz – Merrified resin synthesis; DCC]
[exam 3 – amino acids; d versus l; pI; acid-base properties; electrophoresis; ion exchange chromatography; ninhydrin; synthesis of amino acids; protein structure; Edman degradation; methionine]

[structures of methionine (sulfur containing), tyrosine (hydroxylated phenylalanine), indole, tryptophan (not really enough in turkey to make you sleepy, related to seratonin)]

Strecker synthesis

We start out with an aldehyde and then from an imine. We use only ammonia because the amino groups, except for proline, they don't have any attachments on the nitrogen. If we take quite a potent combination – sodium cyanide and hydrochloric acid [a lethal combination] – this going to end up being across the equivalent of a carbonyl, [as if we] were making the cyanohydrin. We're doing the same style reaction, its just that we happen to have an imine instead. Push that open, you're going to end up with the amino group, plus then we have a nitrile. Turn up the heat and hydrolyze. If we hydrolyze a nitrile, we're going to end up with a carboxylic acid. We're effectively adding one carbon, and it's going to end up making that nitrogen that was part of the imine a nitrogen that's at the alpha position – in other words, we turned it into an amino acid. Like the phthalimidomalonic ester synthesis, [you] don't have control over stereochemistry; I'm showing this product with just a plain line.

[need to do HVZ reaction]

Enantiomeric resolution

The problem with enantiomers in separating them is that they are identical to each other, except for this issue of optical rotation [and new property]. They have the same boiling point, melting point, freezing point, index of refraction, all the other things that you might try to pull tricks with in order to separate these compounds out – so you don't try. One approach is to take a chiral auxiliary, a separate molecule that you react with the one that you're trying to isolate. For example, if we had an amino acid that we were somehow able to attach to some other chiral molecule [keeping identities of molecules generalized]. I'm going to put a star here – technically, stars are often used in the discussion of mechanism to show that you have a stereocenter. Let's say that we had some other molecule that we were going to be able to react with the amino acid – maybe not react, maybe make some kind of salt out of it. The amino acid only has one stereocenter to it; let's say that the chiral auxiliary also has only one stereocenter to it. If both of the molecules had just the potential to be enantiomers beforehand, if somehow we make a complex, even if we were to use just the R version of the chiral auxiliary, but if the amino acid could be R or S, that means we're going to end up with diastereomers, not enantiomers. Here's the amino acid; here's the auxiliary. When we do the synthesis, we actually end up with two amino acids; let's say that one's in its R form, and one's in its S form. Let's say that we use the auxiliary only in its R. When we make the complex, one of them is going to be RR, one of them is going to be SR. The amino acids couldn't be separated because they're enantiomers, which means all of their physical properties are identical. Once we made a complex that's diastereomeric, diastereomers have different physical properties, which means one of them we'll be able to take advantage of in order to separate the two complexes out. If this was a salt formation, for example, something very easily reversible, then after we separate the complexes, we decompose them again, and we get back to the amino acid.

Since amino acids are enantiomers, the can not be easily separate, since their physical properties are identical – except optical rotation [and new property] which cannot be used to separate the complex. If an amino acid forms a complex with some other compound that has one stereocenter, if a mixture of enantiomeric amino acids, this mixture is reacted with some other compound that has a stereocenter, diastereomers are produced. Since diastereomers have different physical properties, they can be separated, and then the amino acid can again be reseparated from the molecule it reacted with. The enantiomers have effectively been separated.

[enzymatic approaches – not shown because not presentable mechanistically]

Protein structure

There's four forms of structure. Primary, we've already done – that is the sequence, which is the order of amino acids.

We need to talk about methionine. Let's say that we're in the middle of a peptide chain, and here's a methionine unit. This methionine can be decomposed by this molecule – bromine directly attached to what looks like a cyano group, this is called cyanogen bromide. Turns out that it can react with the sulfur in methionine, so you have a Sn2 reaction. Sulfur becoming positively-charged in this way is not nearly as unusual as oxygen becoming positively-charged.

[Swern oxidation] This sulfur group ends up being a leaving group. Nitrogen strongly is conjugated with its carbonyl neighbor, so it turns out that that carbonyl can be forced to open can kick that sulfur leaving group off. I'm going to drop stereochemistry because it doesn't particularly matter. I made a five-membered ring. When you hydrolyzed this, you have, effectively, an imine trapped in the same thing that looks like part of an ester; this will hydrolyze. Notice that it's got the rest of the amino acid chain that's going to go away. [presented one derivative right after] Edman degradation, we had learned previously, allows to pull one amino acid off at a time. This is a technique used to chop up an amino acid chain just at this one methionine group; it just happens to work.

Secondary structure. What I'm trying to show is what you can get when you have two different amino acid chains that line up with each other. You can get quite a bit of hydrogen bonding in between two strains. Imagine this: if you were to allow those amino acid chains to start to form some kind of twist, then maybe within that twist you'd also get these inter-chain interactions as well. It's on the basis of these kinds of intramolecular hydrogen bonding that we form different kinds of structures. One type of structure is an alpha-helix; it is clockwise for l-amino acids, which means from the top, as you go down, if you're looking from above, then it's going to appear to be clockwise as you go further and further and further down the helix. There are beta-pleated sheets. Essentially what this is is you have these two chains of amino acids that are kinda crinkling back and forth as you go from one amino acid to the next to the next to the next; that's where this pleated term comes from for the sheets. There are various forms of coils that can form. The difference between those and an alpha helix: an alpha helix is very predictable pattern that's got a fairly fixed distance between the different loops of the helix; the coil can be a larger structure, maybe less well-defined in structure. [different between types of sheets] Secondary structure is based on hydrogen-bonding interactions.

Then there's tertiary structure, which is the minimization of energy. This is not due just to hydrogen bonding, but hydrophobic-hydrophilic interactions in solution. Think about all these different amino acids that we have, with all the different R groups. Some of the R groups that are more likely to be ionized, something like aspartic acid, might be more likely to turn so that its R group is facing the water phase. If you have something like phenylalanine, which is quite clearly non-polar in its R group, would be much more likely to be pointed towards something that's not aqueous – on other words, if you have fat molecules floating around, they'd want to turn in that direction. If all these proteins are floating around in cells and other structures in the body, often where you have interfaces between an aqueous phase – the blood stream, for example – or a non-aqueous phase – fat linings, for example – exactly which sequence of R groups that you have on amino acids is going to cause them to fold up in such a way that, as an entire structure, that protein is able to orient as many of those hydrophilic groups toward the water phase that it can, and as many of the hydrophobic groups away from way. Other terminology that's often used is lipophilic, which means fat-loving, which would be hydrophobic, or lipophobic which would be fat-fearing which would be hydrophilic. That's what happens in tertiary structure – the protein attempts to adopt a lower-energy structure. Aside from hydrogen bonding, the configuration of a protein is caused by hydrophobic-hydrophilic – or lipophilic-lipophobic – interactions of the amino acid side chains with their surrounding environment. It is largely because of this tertiary structure that proteins have whatever function that they do – because the various alpha-helicies and beta-sheets, those might be features buried within this larger structure that. Having all the complex folds that occur in a protein is more due to what happens once it gets into solution. Destroy that order, and that to remove its special functionality, which would be to denature a protein. When we cook things, we're denaturing because we're unraveling this tertiary structure. The secondary structure, because it involves more localized interactions, might be tougher to break. Primary structure requires stomach acid, hydrolyzing all those amide bonds. Denaturing is destroying the tertiary structure of a protein.

Quaternary structure is macromolecular structure, which is the assembly of large protein units into a complex. Hemoblogin is a molecule that has four protein units, each one of which has an iron center – it's the center at which oxygen-carbon dioxide exchange occurs, and four of these things that are not necessarily bound to each other, just extensive interactions with each other, that hold four of these protein units into one structure of hemoglobin.

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A few more amino acids:

Synthesis of amino acids
Strecker synthesis

Since amino acids are enantiomers, they cannot be easily separated since their physical properties are identical (except optical rotation, which cannot be used to separate the compounds). If a mixture of enantiomeric amino acids is reacted with some other compound that has one stereocenter, diastereomers are produced. Since diastereomers have different physical properties, they can be separated. If the amino acid can be released from the complex it forms, the enantiomeric amino acids can then be separated.

Protein structure : 1°, 2°, 3°, 4°
1° – sequence – order of amino acids

2° structure – features of a protein caused by H-bonding interactions

alpha-helix – clockwise helix for l-amino acids
beta-pleated sheets
coils

3° structure – Protein attempts to adopt a lowest-energy structure. Aside from hydrogen bonding, the conformation of a protein is caused by hydrophobic/hydrophilic (or lipophilic/lipophobc) interactions of the amino acid side chains with their surrounding environment.

denaturing – destroying the 3° of a protein

4° structure – macromolecular structure – the assembly of large protein units into a complex –> hemoglobin

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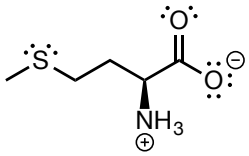
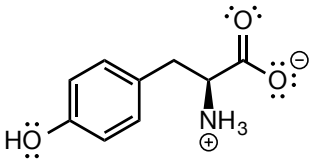
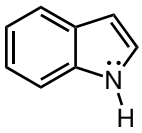


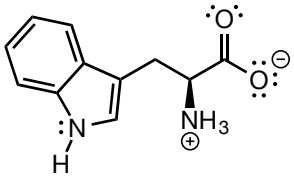
L-methionine



L-tyrosine



indole



L-tryptophan

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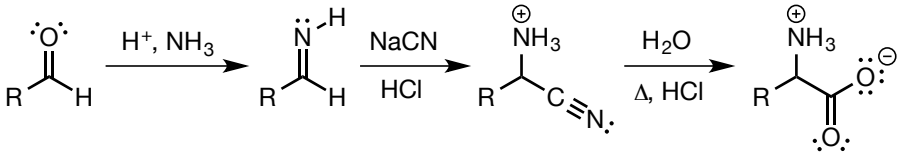

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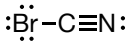

cyanogen bromide

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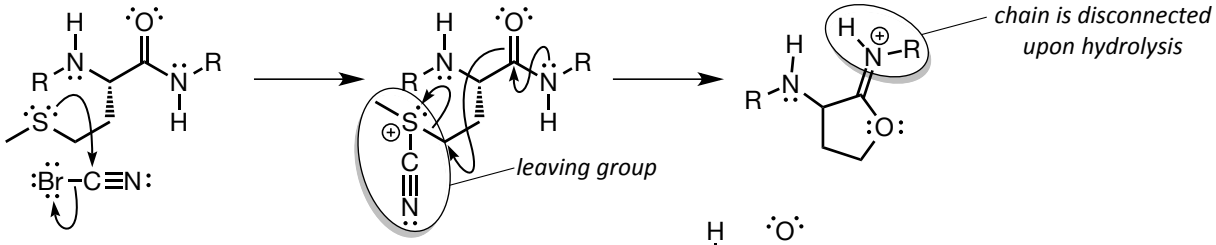

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