

Lecture #24

Lecture 24
12 April 2004

Remember:

- Review session, April 13, 7:00 – 8:30
- Exam, April 14, 7:30 – 9:30

Today's Lecture Topics

- Peptide bond formation
- New technologies using the ribosome

PEPTIDE BOND FORMATION

Approach

- We'll look at the proposed mechanism of peptide bond formation; this model has changed dramatically since 2000!
- Use proteases as a mechanistic model system.

Proteases

- Proteases have served as the “precedent” for peptide bond formation. We understand the mechanism of peptide bond breakage in proteases quite well, and the reactions are reversible, proteases can make peptide bonds if the proteins are placed in organic solutions.
- Proteases accelerate their rates of reaction as much as 10^{12} fold! Where does this huge difference come from?
 - Mostly from *binding energy* (correct orientation, desolvation, freezing out entropy, transition state stabilization).
 - Enzymatic catalysis can also result from:
 - General acid/general base catalysis (This occurs with proteases.)
 - Covalent catalysis (This does NOT occur with proteases.)
 - Binding Energy and Transition state stabilization
- Remember that the chemistry involved in peptide bond formation and cleavage is not particularly difficult.
 - For peptide bond formation, no energy is required! (Energy is only used for the fidelity steps that we've already discussed.)

Chymotrypsin

- Refer to handout taken from Voet (labeled “Figure 14-23”).
 - The handout depicts the generally accepted mechanism for the action of chymotrypsin.
- Chymotrypsin is a serine protease with a catalytic triad.
- Perhaps a metalloprotease would have been a better model, since there are magnesium cations present in the peptide bond forming region, but chymotrypsin is what Steitz and Moore used to think about peptide bond formation in the ribosome.
- How to perturb the pK_a of serine? **THINK ABOUT THIS AT HOME!**
 - Remember that enzymes don't work by magic – it's all just chemistry.

- pK_a perturbations of 5-6 are well-documented in the literature; but changes of 10-12, as suggested by some, are not possible or have not been experimentally documented!
- Key steps of the mechanism:
 - Step 1: nucleophilic attack of serine on carbonyl carbon of the peptide bond.
 - Asp102 is a key player, but does not change protonation state, it simply orients the His57 so that its pK_a can be perturbed and it can function as a general base catalyst (GBC). The proton is NOT transferred to Asp102.
 - His57 is the GBC that can “remove” the proton of Ser195, activating it for nucleophilic attack on the carbonyl of the amide bond to be cleaved.
 - The product of this step is a tetrahedral transition state or high energy intermediate.
 - Step 2: The tetrahedral transition state (intermediate) breaks down to form an acyl-enzyme intermediate in a general acid catalyzed reaction using protonated His57 as the GAC.
 - Step 3: water is used in the hydrolysis step; The hydrolysis process is essentially the reverse of step 2, it must be activated by GBC with His57 so that the "OH⁻" can attack forming a second tetrahedral intermediate.
 - Step 4: breakdown of the tetrahedral intermediate (GAC) by His57 to form the product and active enzyme.
- Points to remember:
 - Each step is reversible!
 - Use GAC/GBC, and binding energy (specifically TS stabilization) for catalysis.

Berg's mechanism of peptide bond formation

- Refer to hand-out.
- A-2451 (pK_a of 7.6) removes proton from terminal amine cationic group of tRNA (pK_a of ~8); the resulting amino group is a good nucleophile that can attack the carbonyl group of the ester linkage to the tRNA, forming a tetrahedral intermediate. This intermediate then decomposes and a proton transfer step yields the final product.
- This working hypothesis centers on the GBC to deprotonate the attacking amine, GAC facilitating cleavage of the leaving group and potential stabilization of the transition state.
- Chemically, this mechanism is much more reasonable than that put forth by Steitz and Moore.

Steitz & Moore's (wrong) mechanism

- Refer to handout; figures taken from *Science* 289 from 2000.
- Models are all based on GAC, GBC, TS stabilization, and chiefly, binding energy.
- The researchers used crystals of the 50S subunit for their study.
 - Must take results with a grain of salt, because the 30S subunit was missing; in addition, the crystallization took place at pH 5 (non-physiological; will change protonation states!)

- They then diffused in various compounds. One of these compounds was the Yarus inhibitor, a transition state analog.
 - In this inhibitor, the phosphorus is tetrahedral; this helps the molecule to serve as a potent TS analog, as it mimics the tetrahedral intermediate. Not the ts analog is chemically stable, the td P does not undergo any chemistry.
 - Also note that the Yarus inhibitor is a deoxy form, which is more stable than the hydroxy form. (A hydrogen replaces the 2'-hydroxyl group.)
 - Refer to “Fig.1” on handout for structures of the Yarus inhibitor and the tetrahedral intermediate.
- In a subsequent study (refer to last page of translation hand-out), they used the antibiotic sparsomycin to “plug up” the A site – this will ensure that what you’re loading only gets in the P site (otherwise it will be 1/2 in A site and 1/2 in P site).
- Their model is constructed from four different structures.
 - There is close proximity of the carbonyl group of the ester-tRNA in the P site and amine group of the charged tRNA in the A site, so the amine is in attacking range of the carbonyl.
 - Residues around the active site: ABSOLUTELY NO PROTEIN!!!
- Major conclusions of paper:
 - The two substrates are oriented in a position capable of catalysis.
 - Binding energy plays a key role in catalysis.
 - No protein, so the ribosome is a ribozyme!
- Still don’t know many things.
 - Do know from the structures that there is a “P loop” that hydrogen bonds with 3'-ACC end of the tRNA. There is also an “A loop” also participates in H-bonding to 3'-ACC end of tRNA in the A site.
 - Remember that this position of tRNA is just “flapping in the breeze” – i.e., not hydrogen bonding with any other part of the tRNA.
 - We really don’t know the mechanism just yet.

USE OF THE RIBOSOME TO PUT IN UNNATURAL AMINO ACIDS

Despite all the fidelity issues we’ve been discussing, we can trick the ribosomes into inserting unnatural amino acids into peptide chains. We’ll discuss three methods, two of which are *in vivo* (preferred) and one of which is *in vitro*.

Use of auxotrophic organisms

- These organisms require a specific compound (amino acid in our case) for growth. (All the genes used for producing that amino acid have been knocked out.)
- Technique:
 - Grow up organism; get all machinery ready for making polypeptides (EF-Tu, ribosomes, etc.)
 - Then replace media with a minimal media having none of the required amino acid but plenty of an unnatural amino acid to act as a substitute.

- The unnatural amino acid gets recognized by the RS and put into every site where the required amino acid would be.
- Example:
 - Tyrosine vs. **fluorinated tyrosine**.



- The new media in this case would have lots of fluorinated tyrosine (in addition to the other compounds required for growth) but no regular tyrosine.
- The fluorinated tyrosine will be recognized by the RS^Y and put into every site in proteins where Y would normally be.
- Fluorinated tyrosine is not that much different from normal tyrosine:
 - pK_a differs only slightly (~8.5 instead of ~10)
 - van der Waals radius of F not much bigger than that of H.
- This mechanism is nice but is completely non-specific!