Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 20:

More on Disulfides, Denaturants and Protein Folding:

The Anfinsen Experiment

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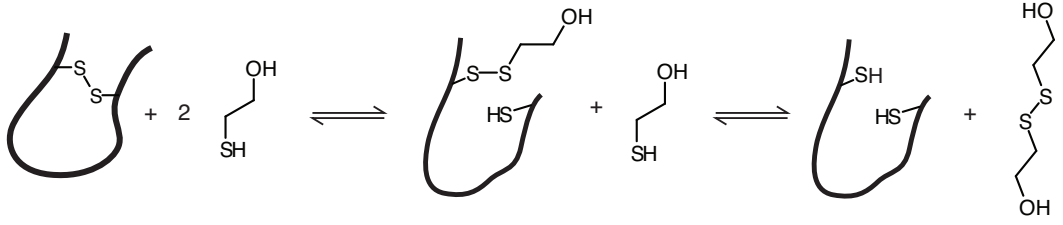
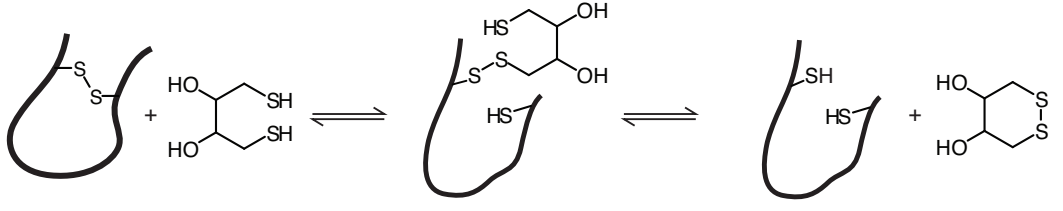
Reduction of Protein Disulfides by Thiol-Disulfide Exchange

- By 2-mercaptoethanol (β-mercaptoethanol, BME)

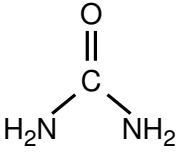


- With dithiothreitol (DTT, Cleland's reagent)

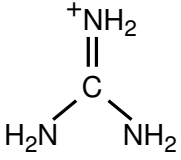


Reduction of Disulfides in RNAse A

- Reaction is very slow in native protein.
- Rate is much higher in presence of strong denaturants, such as 8 M urea or 6 M GuHCl (guanidinium chloride).



Urea



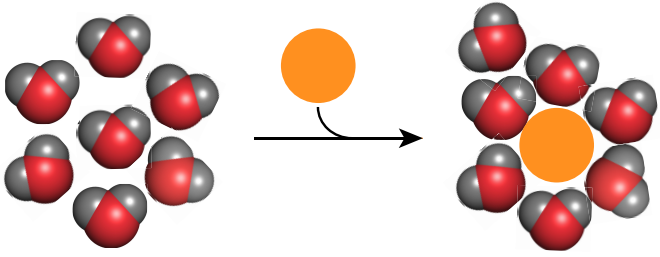
Guanidinium

- Urea and GuHCl destabilize folded proteins. Why?
  Partially by weakening the hydrophobic effect.
  Primarily by interacting with the polypeptide backbone (as of 2022).
- Agents like urea and GuHCl are sometimes called chaotropes.

The Hydrophobic Effect

- The basic observation: Water and oil don't mix!
- A confusing and still controversial subject, partly because of terminology.
- Non-polar molecules are poorly soluble in water.
- Are non-polar molecules afraid of water?
- Urea and GuHCl increase the solubility of non-polar molecules in water.
- What happens when a non-polar molecule does dissolve in water?

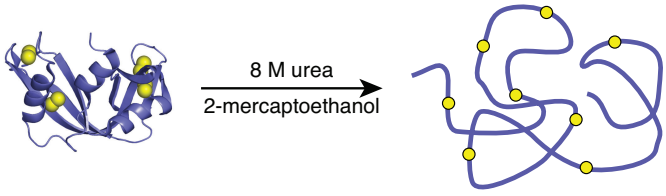
The “Iceberg” Model



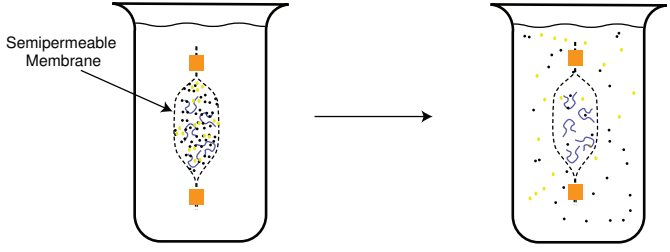
- Introduction of a non-polar molecule causes water molecules to become more ordered.
- The poor solubility of non-polar molecules is largely due to the loss of entropy of the water.
- Urea, GuHCl and similar compounds reduce the energetic cost of dissolving non-polar molecules, thus the term “chaotrope.”
  But, it is hard to say that these agents increase or preserve disorder of water.
- It’s complicated!

The Anfinsen Experiment

- Unfolding and reduction of RNAse A:



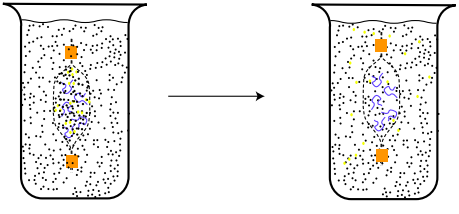
- Removal of urea and 2-mercaptoethanol by dialysis in the presence of O_2:



- Recovery of active RNAse A, with properly formed disulfides!

Anfinsen Experiment: Part II

- Reduce and unfold RNAse A, as before.
- Remove 2-mercaptoethanol and form disulfides, without removing urea.

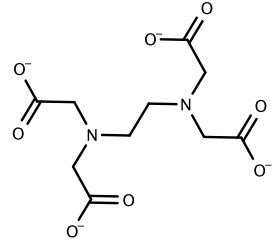


8 M urea in the dialysis buffer.

- Recover only about 1% RNAse A activity.
- Conclusions:
  - Information to specify the native structure is contained within the amino acid sequence and its interactions with solvent.
  - Disulfides and non-covalent interactions act together to stabilize the native structure.
- Nobel Prize in Chemistry to Christian B. Anfinsen, 1972.

What Oxidized the Thiols Back to the Disulfide State?

- Probably molecular oxygen, O_2.
- But, this reaction requires a divalent metal ion as a catalyst. Cu^{2+} is particularly effective, and only trace amounts are necessary.
- We *don't* want the thiols to re-oxidize after we reduce them in our experiment! To minimize oxidation, we add ethylenediaminetetraacetic acid (EDTA):



Very strongly chelates divalent cations, including Cu^{2+}.