Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 20:

More on Disulfides and Protein Folding:

The Anfinsen Experiment

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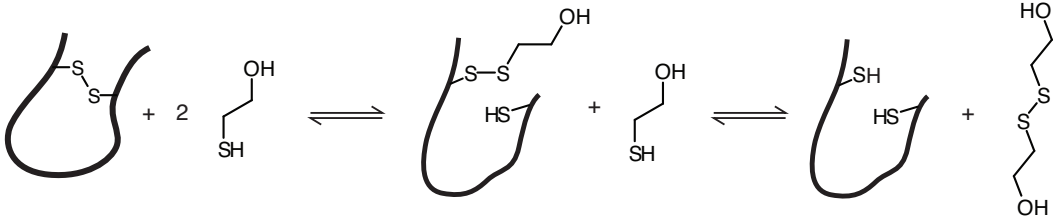
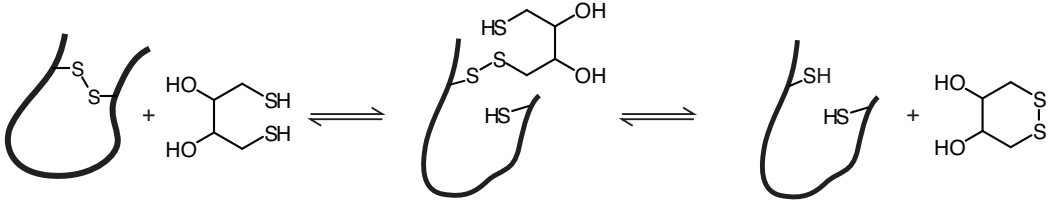
Reduction of Protein Disulfides by Thiol-Disulfide Exchange

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

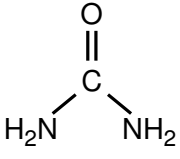


- With dithiothreitol (DTT, Cleland's reagent)

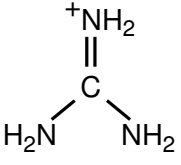


Reduction of Disulfides in RNAse A

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



Urea

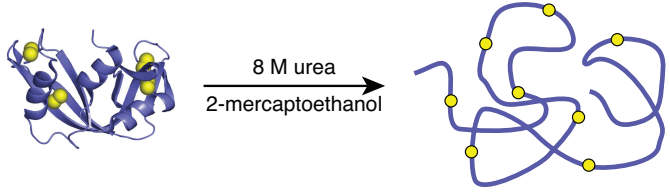


Guanidinium

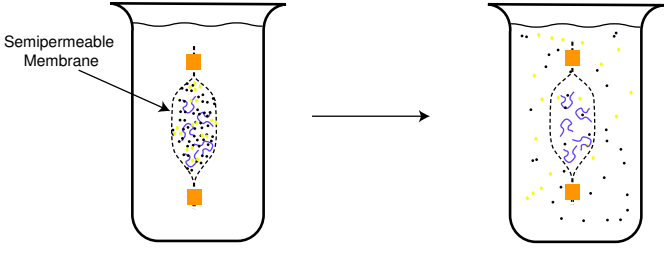
- Urea and GuHCl destabilize folded proteins. Why?
  Primarily by weakening the hydrophobic effect. ~~Probably by weakening the hydrophobic effect~~
  Primarily by interacting with the polypeptide backbone (as of 2018).

The Anfinsen Experiment

- Unfolding and reduction of RNAse A:



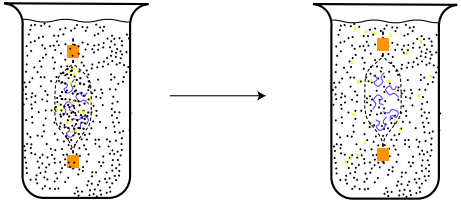
- Removal of urea and 2-mercaptoethanol by dialysis in the presence of O_2:



- 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.