Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 19

Electrophoresis

and Thiol-Disulfide Chemistry

22 March 2022

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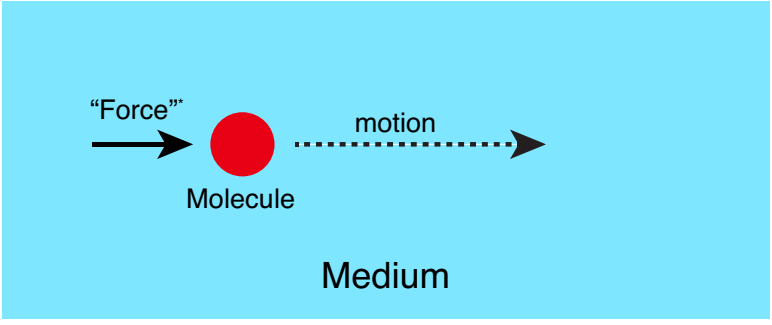
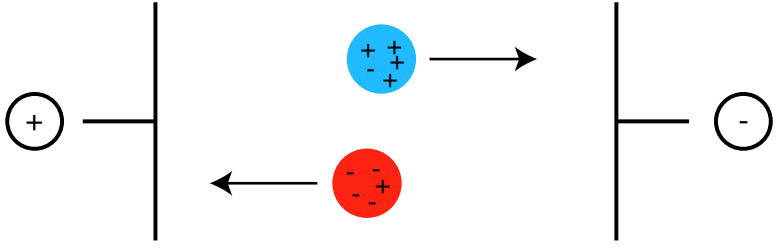
Separation Methods: The General Idea



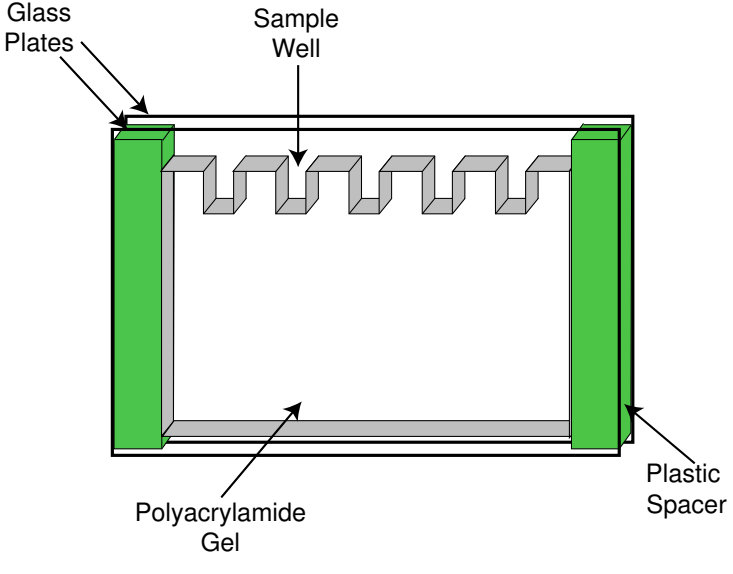
- Something (a “force”*) causes molecules to move through a medium.
- The rate of motion depends on the strength of the force and the interactions of the molecules with the medium.
- Different kinds of molecules move at different rates, allowing them to be separated.

* “Force” is used rather loosely here to describe anything that causes motion of the molecules.

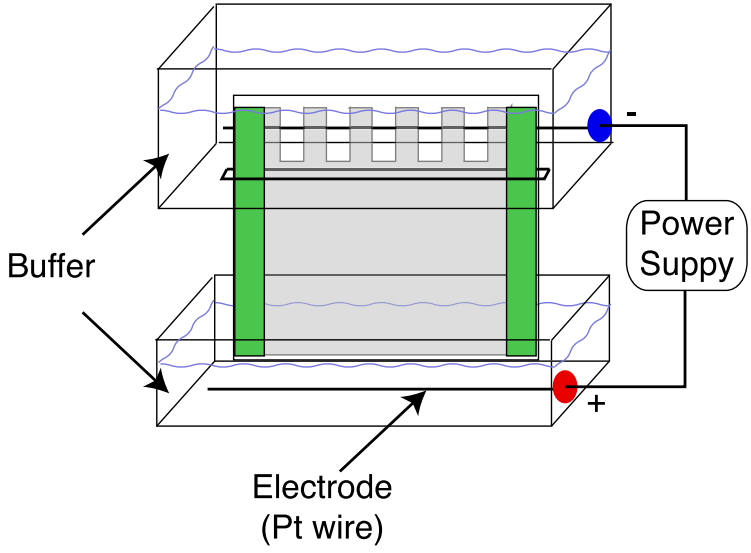
Electrophoresis:
Separation based on movement in an electric field



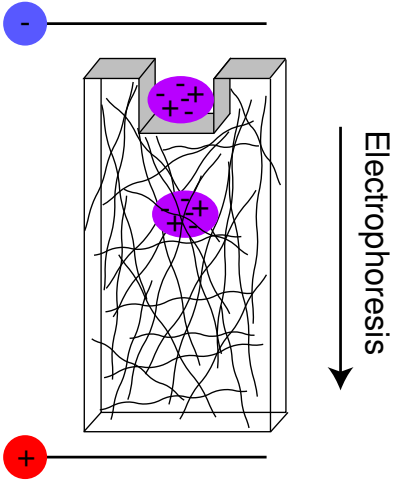
A Gel “Sandwich” for Electrophoresis



Apparatus for Gel Electrophoresis



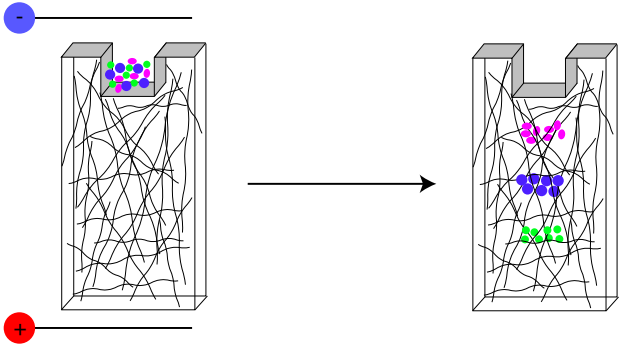
Electrophoresis Through a Gel



Rate of migration through the gel depends on:

- Strength of the electric field.
- Net charge of the protein.
- Size and shape of the protein.
- Density of the gel matrix

Separation of Proteins by Electrophoresis

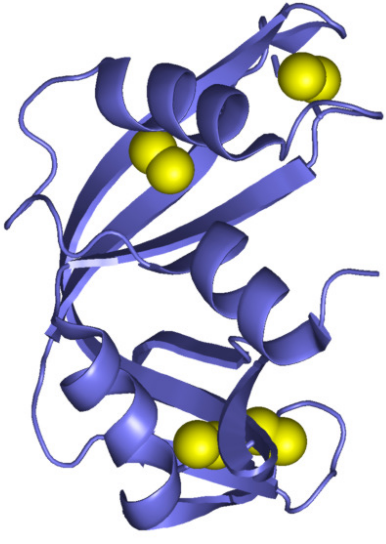


- Proteins with different mobilities migrate as “bands” in the gel.
- Various ways of detecting the proteins in the gel.

Two Major Variants of Gel Electrophoresis for Proteins

1. Non-denaturing (“native”) electrophoresis.
   - Carried out in the absence of denaturants, though sometimes relatively low or high pH values are used.
   - Protein migrates through the gel on the basis of its intrinsic net charge, size and shape, and the sieving effect of the gel.
2. SDS gel electrophoresis
   - Proteins are denatured in the presence of sodium dodecyl sulfate (SDS), a detergent that denatures proteins and complexes.
   - Mobilities reflect molecular weights of polypeptide chains.
   - Very useful for analyzing complex samples and macromolecular complexes composed of multiple polypeptides (*e.g.,* viruses, organelles, membranes).

Ribonuclease A: A “Classic” Protein Stabilized by Disulfide Bonds

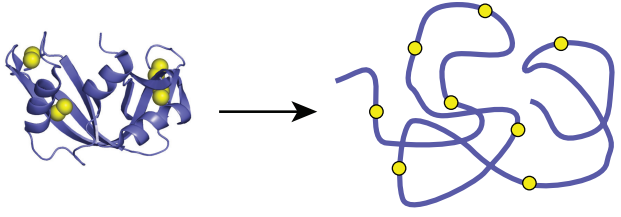


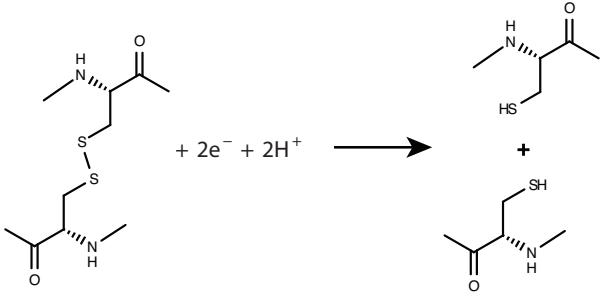
- Hydrolyzes RNA, much as trypsin hydrolyzes proteins.
- Like trypsin, made in pancreas.
- A favorite protein for chemical, enzymatic and structural studies in the 1950s and 1960s. Two Nobel prizes (4 awardees).
- Produced in large quantities (kilogram) by the Armour Meat Packing Company near the end of World War II, and provided at very low price to scientists.
- Close relatives are cytotoxic and are being explored as anti-cancer agents.
- Presence of 4 disulfide bonds allows some neat chemical manipulations of the protein.

Outline of Experiment 5

- Day 1:
  1. Preparation of modified RNase A
- Day 2:
  1. Non-denaturing gel electrophoresis of native and modified RNase A
  2. Trypsin treatment of RNase A forms
- Day 3:
  1. SDS gel electrophoresis of trypsin-treated RNase A samples
  2. Image capture of non-denaturing gel
- Day 3+1 (first day of experiment 6):
  1. Image capture and quantitation of SDS gel

Unfolding RNAse A by Reducing its Disulfides

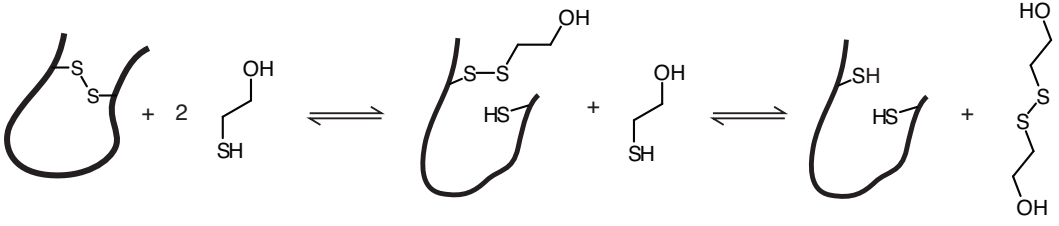




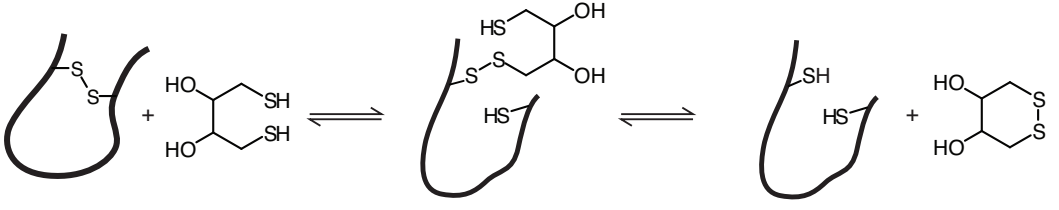
- Without disulfides, the folded protein conformation is unstable.
- Unfolded protein is a broad ensemble of rapidly interconverting conformations.
- Reaction is shown here as a reductive half-reaction.
- There are a variety of ways to promote the reduction reaction.

Reduction of Protein Disulfides by Thiol-Disulfide Exchange

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



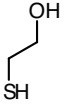
- With dithiothreitol (DTT, Cleland's reagent)



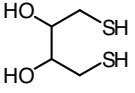
Clicker Question #1

Which reagent (at equal concentrations) reduces protein disulfides more rapidly:

A) 2-mercaptoethanol



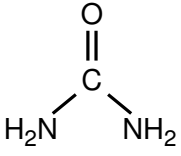
B) Dithiothreitol



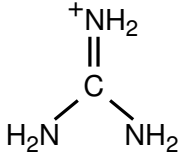
All answers count for now.

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

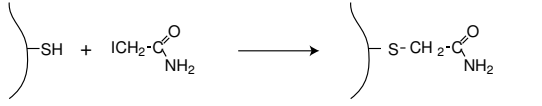


Guanidinium

- Urea and GuHCl destabilize folded proteins. Why?
  ~~Probably by weakening the hydrophobic effect~~
  Probably by interacting with the polypeptide backbone (as of 2022).

Reformation of Disulfides Can be Prevented by Alkylating the Cys Thiols

- Reaction with iodoacetamide



Protein thiol | Iodoacetamide | Carboxyamidomethylated protein thiol

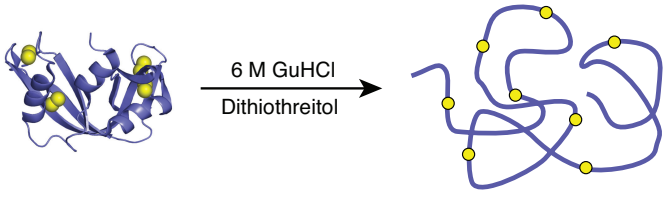
- Reaction with iodoacetic acid

Protein thiol | Iodoacetic acid | Carboxymethylated protein thiol

- Reactions are essentially irreversible.
- Same chemistry is widely used to modify proteins with other groups, such as fluorescent labels.

Reduction and Alkylation of Ribonuclease A

- Unfolding and reduction of RNAse A:



- Carboxyamidomethylation with iodoacetamide

- Carboxymethylation with iodoacetic acid

Three Forms of RNase A for Electrophoresis Experiment

1. Native RNAse A (N). Compact, net positive charge.
2. Reduced and carboxyamidomethylated (RCAM). Less compact than native, same net charge as native.
3. Reduced and carboxymethylated (RCM). Less compact than native, decreased positive charge.

How will they behave upon electrophoresis?

Clicker Question #2

Which form of RNAse A will migrate most rapidly through a non-denaturing gel?

A) Native RNAse A (N). Compact, net positive charge.

B) Reduced and carboxyamidomethylated (RCAM). Less compact than native, same net charge as native.

C) Reduced and carboxymethylated (RCM). Less compact than native, decreased positive charge.

All answers count for now.