BL4820 BASIC BIOCHEMICAL TECHNIQUES

Lecture 7 - Polyacrylamide Gel Electrophoresis (PAGE) on GOT -- Expt 4 Part A

Read in Text: p. 43-45; 48-49

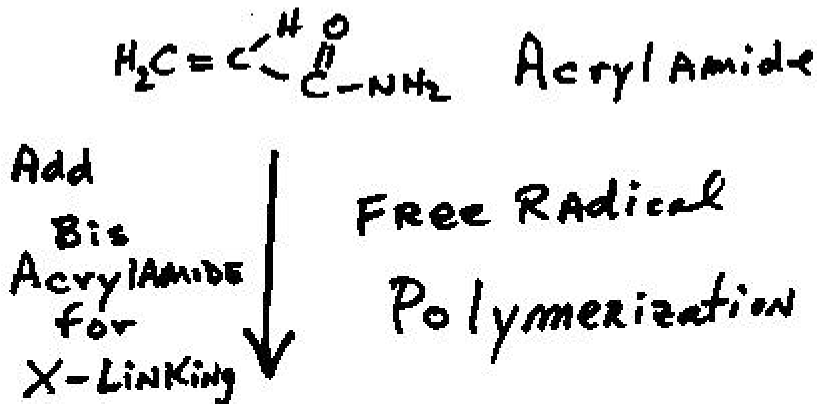
Polyacrylamide Gel Electrophoresis (PAGE) for Proteins

There are two types of PAGE which you will do:

- 1) Native PAGE - in Week 7
- 2) SDS-PAGE (Denaturing PAGE) - in Week 8

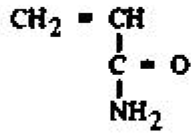
1. Native PAGE

PAGE is probably the most highly resolving electrophoretic method yet developed for separating proteins. In electrophoresis, proteins are separated by charge-density. In gel electrophoresis, the support media (the gel) also contributes to the separation power of the method. Using the organic monomer, acrylamide, to make the gel by free radical polymerization results in very uniform pore sizes which can be reproduced each time a gel is poured without a lot of variation.

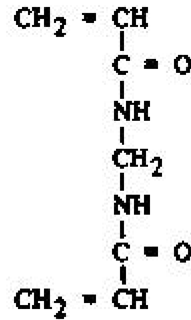


How to make a PAGE Gel.

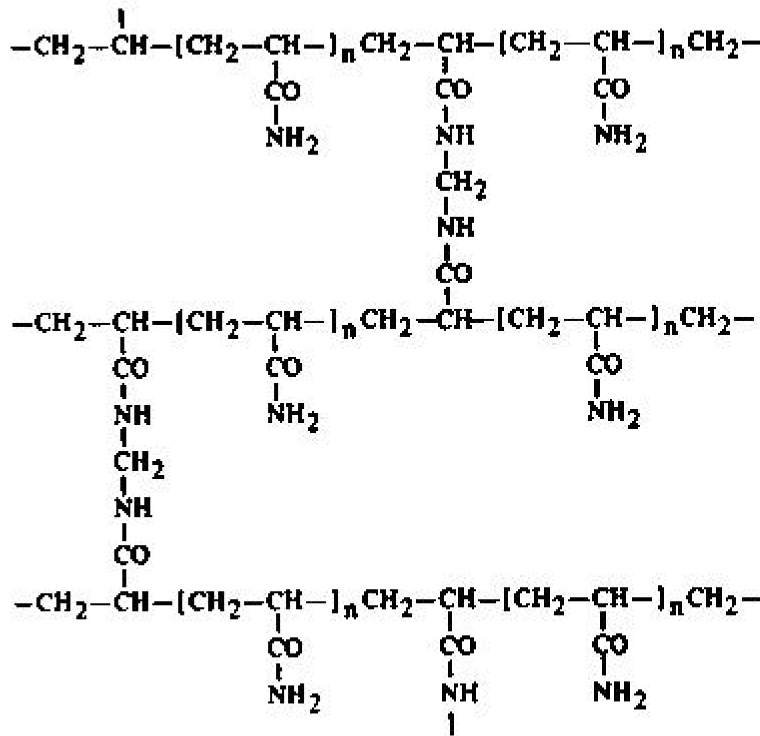
Chemistry:



Acrylamide



N,N'-methylene bisacrylamide



Polyacrylamide gel

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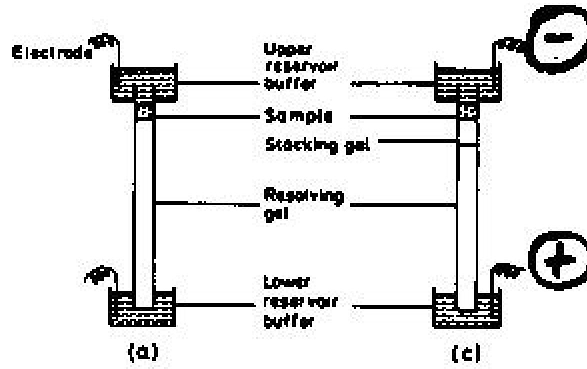
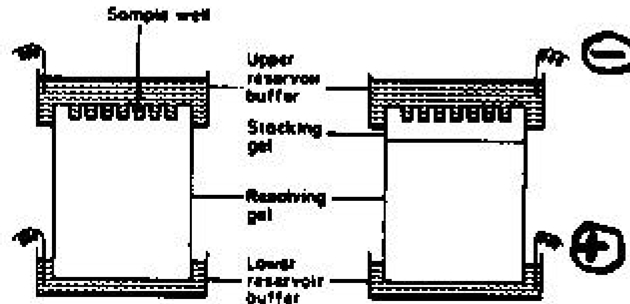
Since the pores in a PAGE gel are the size of proteins, molecular sieving contributes to the resolving power of PAGE. Consequently, PAGE is a high resolution method and one of the best available for separating complex mixtures of proteins, while using simple equipment.

Models of PAGE Gel and Setups:

Tube Gels



Slab Gels



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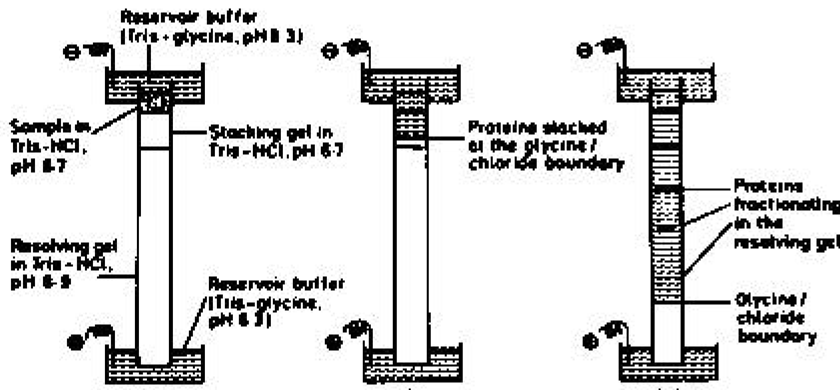

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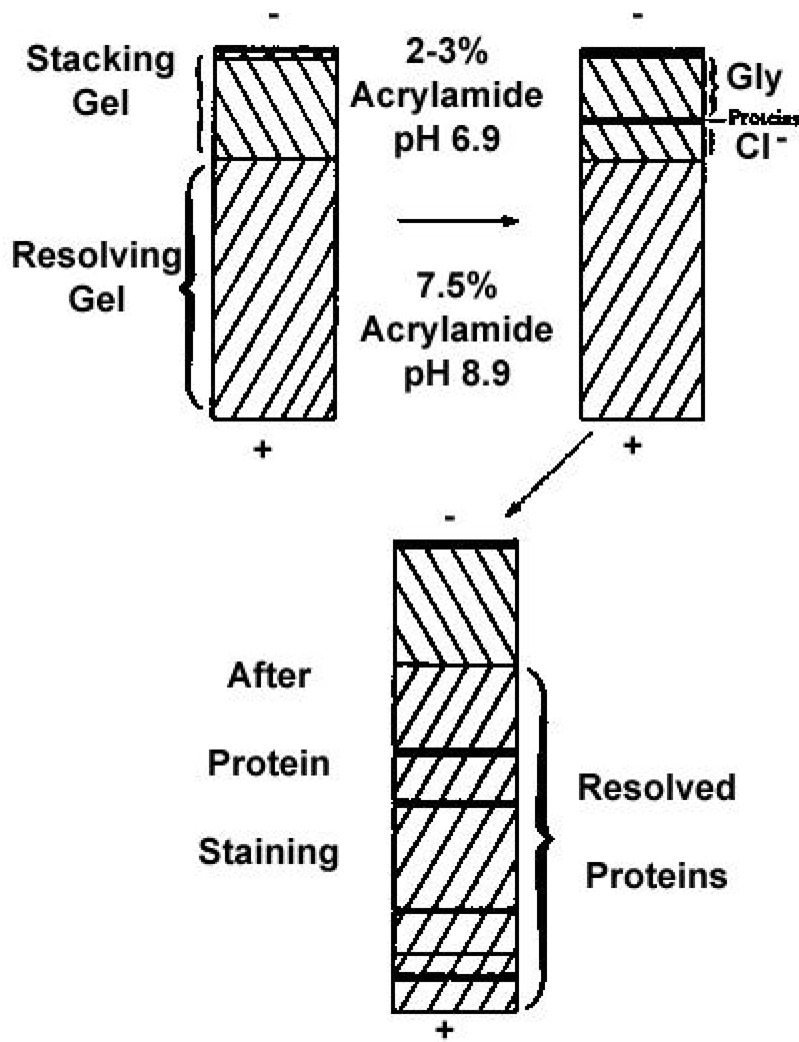
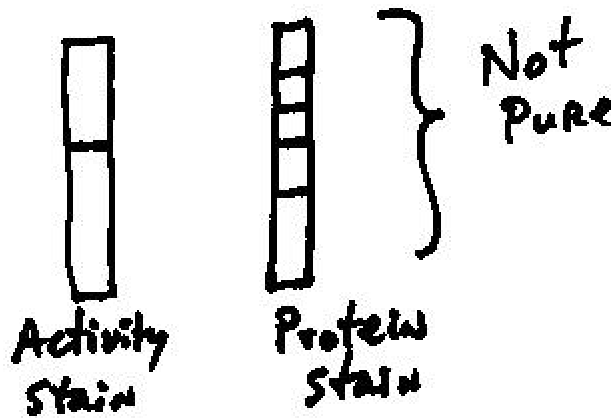
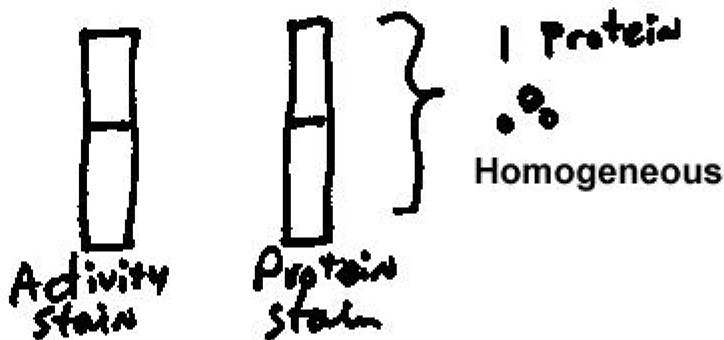


Native PAGE is used to determine the purity of a protein or enzyme.

After DEAE-Cellulose



After Affinity Chromatography



This method of gel electrophoresis allows one to separate native proteins according to difference in their charge density. The buffer in the gel is suitable for maintaining the protein in its Native state. Thus, for enzymes their activity can be assayed after the electrophoretic separation. All proteins present in the gel can be visualized using a general protein stain. By comparison of identical gels stained specifically for the enzyme of interest and gels stained by the general protein stain, you can evaluate the purity of an enzyme preparation. The evaluation can be done in a quantitative manner by comparing relative mobilities of the enzyme-stained protein band and the protein stained protein bands. Relative mobility is defined as the distance moved by the protein band of interest as compared to the distance moved by the dye front (a low molecular weight dye which is highly charged is used to mark the electrophoretic front).

NOTE WELL:

- NATIVE PAGE is NOT a method for determining native molecular weight. Proteins do not separate according to molecular weight under the conditions of the native gel. Thus, native molecular weight of a protein can not be obtained by doing a single native PAGE gel.

2. Native PAGE for GOT

For native PAGE in Week 7, the gel is cast between glass plates to form a "Mini-Slab Gel". After the "running or resolving gel" is cast using a Tris-Cl buffer of pH 8.9, a "stacking gel" is cast on top of it using a Teflon comb in order to create wells for loading the protein samples. The stacking gel is made using a lower percentage of acrylamide than the running gel using a pH 6.7 buffer. Thus, after loading your samples collected over the last 3 weeks in the GOT purification from pig heart, the proteins run rapidly through the stacking gel which is highly porous and then "stack" up at the interface between the two gels since the running gel has much smaller pores. Then pH difference between the stacking and running gels also contributes to the stacking effect at the gel interface. The net effect is that sharper protein bands are produced during electrophoresis. The running buffer for the gel overall is Tris-Glycine, pH 8.3.

Prior to your class this week, the TA will have run a native gel on your GOT samples and stained it for protein using the standard PAGE gel stain, Coomassie Brilliant Blue G. The TA will also have prepared a virtually identical gel for electrophoresis of the same GOT samples to be stained for enzyme activity. The activity gel will be run and stained during class. The activity stain is based on a dye which will react with the product of the GOT catalyzed reaction, oxaloacetic acid (OAA). So the activity stain for the gel is set up in a 0.2 M Tris-Cl buffer at pH 8.0 with 30 mM aspartic acid and 14 mM 2-oxoglutaric acid (alpha-keto-glutarate) and 3 mg/ml of Fast Blue BB. During the reaction catalyzed by GOT, OAA is formed and then it reacts with the Fast Blue dye to form a blue/red precipitate at the site where the GOT band is in the gel.

You should measure the mobility of all the protein stained bands and the dye front on the Coomassie Brilliant Blue stained gel and also do the same for the activity stained gel. To assist you in identifying the GOT band in these gels, a sample of commercial GOT (purchased from the Sigma Chemical Co.) will also be electrophoresed along with your GOT fractions. The final result from this week's experiment should be a schematic drawing of the two gels and a table of the relative mobilities. By evaluating, the relative intensity of the protein bands in Coomassie Brilliant Blue stained gel, you can make an estimate of the amount of GOT in your most purified sample (ie CMC fraction) as compared to the amounts of protein in all the other bands. Of course, this will only be a semi-quantitative estimate of the success of your purification, but it does give you a way to compare the result from the NATIVE PAGE gel to your calculation of specific activity carried out in the report of the GOT purification.

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