Lecture 10: Polyacrylamide Gel Electrophoresis-II

During last lecture we studied about SDS-PAGE (SDS-Polyacrylamide Gel Electrophoresis) also called denaturing polyacrylamide gel electrophoresis. However, some times, we need to separate protein in non-denaturing conditions. This type of polyacrylamide gel electrophoresis is also called native gel electrophoresis because protein remains in native form even after electrophoresis. The basic difference in the native gel electrophoresis (native-PAGE) is the electrophoresis buffer does not contain SDS. Also, loading buffer does not have SDS and reducing agents and samples are not boiled. Moreover, discontinuous pH system is not used as protein focusing by isotachphoresis does not work well here (protein may have lower charge density than glycine).

We shall move ahead with few questions:

Question: What is the role of SDS in the SDS-PAGE?

Answer: It provides uniform negative charge to protein, so protein can migrate towards positive electrode during electrophoresis. In case of native-PAGE, proteins moves towards positive electrode due to its own charge.

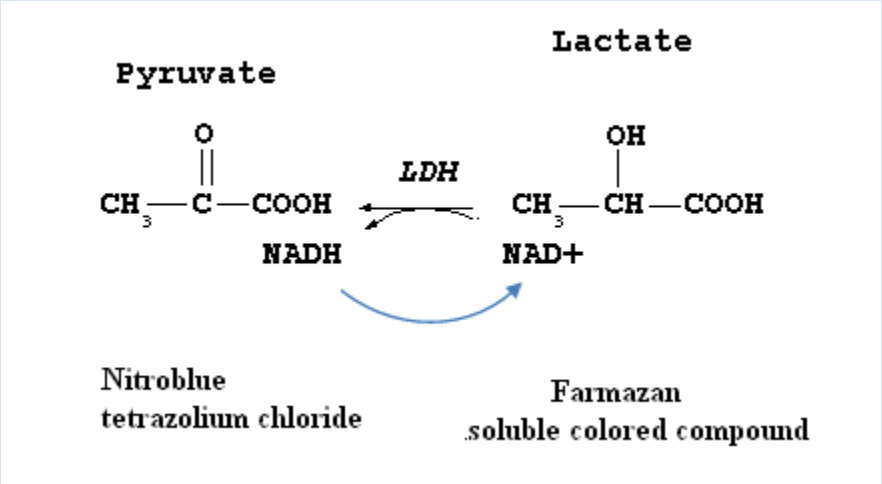
Question: What is relationship between protein isoelectric point, pH and net charge on the protein? What is the pH of electrophoresis buffer?

Answer: pH of the electrophoresis buffer is 8.8. Any protein with isoelectric point above 8.8 will have negative charge and move toward positive electrode. They will be separated based on mass/charge ration. Proteins with isoelectric point below 8.8 may be separated by changing polarity of electrode. As protein focusing in stacking gel does not work well in native-PAGE, resolution of process is not very good (protein bands are not very sharp). However, the biggest advantage is that the proteins are separated in native form.

Use of non-denaturing gel for activity staining/zymogram.

Protein retains their intact native structure when electrophoresis is performed in non-native condition. Thus, it is possible to see activity of protein band in the gel. For example, from a mixture of protein if we have to identify a novel protein showing lactate dehydrogenase activity, what will be experimental process?

Supplement: Lactose dehydrogenas (LDH) enzyme catalyzes reduction of pyruvate to lactate and used NAD^+ as cofactor. Enzyme assay uses NADH made in the process of catalysis. NADH reduces nitroblue tertazolium compound to colored farmazan compound as shown in figure



After native gel electrophoresis, gel is removed from the gel and soaked in enzyme activity buffer. Lactate and nitroblue terazolium compound is spread on the gel and kept for some time. Wherever LDH enzyme is located, it will produce a coloured farmazan product. Since farmazan compound is insoluble it will not diffuse and make a sharp band.

Similar, activity staining methods are developed for several enzymes.

Home assignment: A survey on activity staining of few enzymes, like proteases, lipases etc

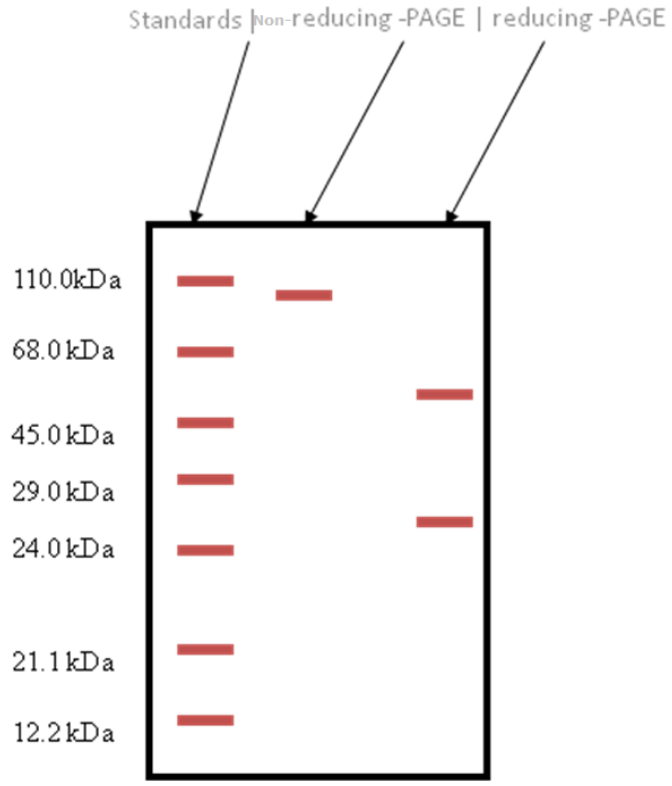
- Native-PAGE is very useful for subunit mass/stoichiometry determination of oligomeric proteins. As in native-PAGE generally oligomeric proteins retains their tertiary structure, we get oligomeric mass. In SDS-PAGE subunits are dissociated when boiled with SDS and reducing agent. Thus, SDS-PAGE gives monomeric mass. A protein shows a band corresponding to 100 kDa in native-PAGE but 25 kDa in SDS-PAGE suggest the protein is an oligomeric protein with four identical subunits.

Question: A protein gives 100 kDa molecular mass in native-PAGE but two bands in SDS-PAGE corresponding to 25 kDa and 50 kDa. How many subunits are there in oligomeric protein. Write molecular mass of each subunits

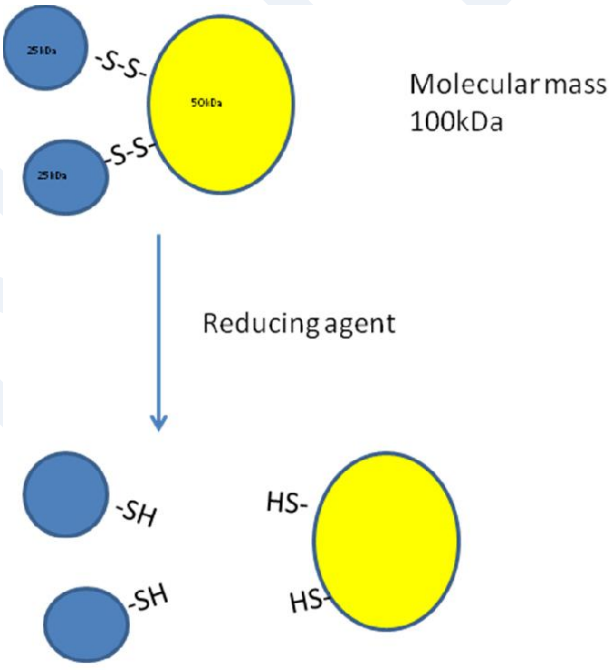
Answer: Total three subunits. Two subunits of 25 kDa and one of 50 kDa

Non-reducing SDS-PAGE: This is a modified version of SDS-PAGE where reducing agents are not used. This provides some information about disulfide bond pattern in oligomeric proteins. Let us try to understand the application by a simple question.

A 100 kDa protein is trimer composed of two molecule of 25 kDa and single poly peptide of 50 kDa. A non reduced SDS-PAGE gives a band of 100 kDa (equivalent to native-PAGE). However, reduced SDS-PAGE gives two bands one equivalent to 25 kDa and other 50 kDa . What information do you get from the experiment?



Answer: 25kDa subunits are attached to 50kDa subunits by disulfide bond.



Another form of non denaturing SDS-PAGE is called diagonal electrophoresis: Peptide joined together by disulfide bond can be detected by diagonal electrophoresis. The mixture of peptide is subjected to electrophoresis in single lane in vertical direction (without treatment with reducing agents). After first run, gel is treated with reducing agent and run in second direction (horizontal). Peptide joined by disulfide will not come in diagonal line in second direction as their molecular mass changes due to reduction of disulfide bonds (peptides are separated)