Gel filtration chromatography: ligand protein study

In this experiment our purpose was to observe the ligand binding of the serum albumin and phenol red and observe it by using the gel filtration chromatography.

Ligand is a substance, usually a small molecule that is able to bind to a biomolecule. This binding produces a complex and serve as a biological purpose; activation, inhibition or act as a neurotransmitter. It binds to a site of the target protein. This binding of the ligand to protein occurs by intermolecular forces such as ionic bonds, hydrogen bonds or van der waals force. Because the binding is takes place by intermolecular forces and not with covalent bonding which is a strong bond, ligand binding is reversible. Ligand binding changes the conformation of the receptor protein, the three dimensional shape of the protein.

Serum albumin:

Serum albumin is a plasma protein in humans and other mammals. This protein is important for maintaining the osmotic pressure which is needed for the distribution of body fluids properly. Albumin has six binding sites and it is soluble. It acts as a transport protein for hemin and fatty acids. It also acts as a plasma carrier.

Gel filtration Chromatography:

gel filtration is a form of column chromatography technique that separates proteins, peptides and oligonucleotides on the basis of their size. In the column chromatography the stationary phase is solid and the mobile phase is liquid. The stationary phase is put into a column which is usually a glass tube and the mobile phase that is a buffer or solvent is allowed to flow through the solid phase. Here molecules will move through a porous

substance. This molecule while passing through will interact with the stationary phase and this interaction and the size of the molecule will effect the time that they leave the column. The degree of interaction between stationary phase and proteins depends on the properties of the protein, propeties of the stationary phase and the composition of the mobile phase.

EQUIPMENT AND CHEMICALS

Equipments:

- Chromatography column
- Plastic cuvettes
- Beakers
- Clamps
- Pipette
- Tips

Chemicals:

- Distilled Water
- BSA (bovine serum albumin)
- Acetate buffer
- Phenol red
- NaOH
- Sephadex G25

PROCEDURE

Preparation of the chromatography (prepared before):

0. 1 g phenol red was dissolved in 10 ml acetate buffer.

Chromatography was washed with buffer and then sephadex was poured into the column.

After gel was polymerized the gel was washed with buffer again.

Preparation of the samples:

6 samples were prepared (before the experiment) with different concentration of phenol red solution by mixing the same acetate buffer and albumin. (each group run 2 of the prepared samples in the chromatography.)

250 µl of the first sample was poured into the gel and then 50 ml of acetate buffer was added slowly.

Samples that passed through the chromatography was collected in 40 cuvettes. In to the each cuvette only 10 drops of the sample was added.

2 ml distilled water was added to the cuvettes.

200 µl 1 M NaOH was added to each cuvette.

Absorbance values of the all samples were measured at 520 nm.

steps were repeated for the second sample

DISCUSSION

In this experiment our purpose was to observe the ligand binding of the serum albumin and phenol red and observe it by using the gel filtration chromatography.

In this experiment we used albumin because different ligands can bind to it easily. We used sephadex as gel in the experiment. Before using it, it was waited in water for 3-4 hours so that it could swell.

During the experiment after collecting 10 drops of the sample from chromatography we added NaOH and 2 ml water to the cuvette. The reason why we added NaOH was because we needed to obtain a colored solution so that we could measure absorbance values with spectrophotometer. In the acidic medium samples were yellow. The reason why we added 2 ml water was also because to be able to obtain absorbance values. It is needed approximately 3 ml sample in the cuvette in order to be able to have results.

Mobile phase was the acetate buffer and stationary phase was the sephadex gel. We were careful when we added our samples and buffer adding into the chromatography column. When we first poured the sample we added it really slowly and when we added buffer first we added a little amount of the water waited for it to go in to the column and then we continued to add the buffer. We were careful when we added the buffer the solution which was at the top of the column not to go up.

When we look at the absorbance values we obtained from the sample 1 and 2 we see that they are different, because we used different amounts of phenol red and buffer in each sample. We were expecting that in the samples after the chromatography there would be protein-phenol red complex and phenol red alone. In the first cuvettes we would expect that protein amount would be higher because big molecules pass through chromatography, faster whereas small molecules go into porous gel and run slower in the column. Our graphs show that result but with some errors. Some absorbance values were unexpected. It might be due to the wrong handling of the cuvettes. Most of them were not clean, while doing the

experiment reagents that we used spread around it and also bubbles were occurred.

We were expecting that two peaks would occur in the graphs. We can observe that in the sample 2. They represent the serum albumin-phenol red complex and only the phenol red.

We were able to observe what was in the cuvettes also by the color change in them. The first cuvettes that we filled were colorless than slowly they became pink. Pink cuvettes were containing high amounts of phenol red and colorless ones high protein.