Separation of histone protein

For estimating protein mixture qualitatively most widely used method is SDS-Polyacrylamide gel electrophoresis (SDS-PAGE). According to size of the protein, this SDS-PAGE is separate the protein and purification of protein is to be monitored by this method, and relative molecular mass of protein can also be determined. In this SDS-PAGE anionic detergent is SDS. Before loading the sample, the samples are boiled for 5minutes, that contain s SDS and ï)¢ï€mercaptoethanol in the buffer. While boiling the sample the SDS act to denature the protein and where ï)¢ï€mercaptoethanol decrease the disulphide bridges of the protein that are holding tertiary structure of protein . by this denature process the protein get fully denatured and form a rod shape structure with negatively charged molecules of SDS throughout polypeptide chain. Every couple of amino acids binds with one SDS molecule on average. Due to the negatively charge SDS the structure remains as rod like. So repulsion take place between the negatively charge on proteins and no folding occurs and remains rod shape. In the sample loading buffer, contains bromophenol blue and sucrose or glycerol. The bromophenol blue is helpful in monitoring the sample, when electrophoresis running and glycerol give density to the sample that can settle at the bottom of the well on stacking gel. The samples are loaded on the electrophoresis gel, which is made up of two gels . the lower gel is main separating gel and upper gel is stacking gel. This stacking gel helps in loading the sample into wells and had large pore size. Where protein sample moves freely and makes the protein sample concentrate and forms sharp band and enters into main separating gel with effect of electric field. Here isotachophoresis take place.

The glycinate ion which is negatively charge has lower mobility than SDS-proteins molecule in running buffer than cl- ion in stacking and loading buffer. At the higher field strength both cl- and glycinate travel at same speed. So these ions and protein adjust those concentrations. The separating gel has higher PH environment, once glycine receives it become highly ionised state and mobility increases. By this the cl- and glycinate leaves the SDS-protein molecule. Now the SDS-Protein molecule moves towards the anode in separating gel by the effect of electric field. Here the protein having smaller size moves faster and reaches to the bottom of the gel than protein having larger size, with the help of bromophenol blue dye we can indicate the electrophoresis front because smaller particle unretarded the dye colour. When dye comes bottom of the gel then turned off the current, remove the gel from the sandwich properly and stained with coomassie brilliant blue and then by using destaining solution, gel is washed. Depending on the protein size the preparation of polyacrylamide gel is used like 15%, 10% and 7. 5%. By the help of the standard protein the mobility of unknown can be calculated by using calibration curve. In SDS-PAGE the protein should give single band, then that protein is said to be pure. So for purification protein process SDS -PAGE is most widely used.

To the cluster of eight histone protein (H1-H8) DNA is wounded around. By the help of histone and DNA chromatin is made. The regulation of expression of genes and organisation of DNA is done by the help of histone proteins. Due to histone protein modification we can keep the genes active or silent and modifications are like methylation and acetylation. The transcription factors take place by the modulate accessibility of DNA by histone

modification. DNA access might blocked by histone methylation to transcription factors. Electrostatic interaction might change due to histone acetylation in chromatin and allows transcription after opening up DNA. In blood cells development in chicken the principle of the histone modification is clearly demonstrated. In the transition the structural and functional role is played by histone protein between the states of active and inactive chromatin. high degree of conservation consists in histone . This is due to structural maintained constraining the entire nucleosomal octameric core. In the gene regulation and epigenetic silencing the diverse role play by a histone proteins. DNA replication, repair, transcription and recombination are influenced by the post translational modification, interactions with chromatin remodelling complexes and histone variants. DNA is packed in the nucleus and forms a complex called chromatin. The first level of chromatin organization is represented by the nucleosome core particles. The octameric core is composed of 146-147 bp of DNA that are tightly wrapped around two copies of histone H2A, H 2B, H3 and H4. Nucleosome cores are associated with linker histone H1 and separated by variable length of linker DNA. Core histone internucleosomal interactions are mediates by composing packed nucleosome arrays to start helical model. Due to the presence of histone fold domain the core histone are characterised and variable lengths of N-TerminaL tails are extensive subjects for post translational modifications. The epigenome are the component of post translational modifications hence that includes protein connected to its gene and changes in DNA occur. For regulation of gene expression the epigenetic mmodifications are act as switches. DNA and histones are its chemical modifications . which does not disturb the sequence changes to DNA. The organisms reveal a variety of

striking similarities despite histone tail and core variation due to characterization of structural nucleosome core particles. Using structural information they reanalysed histone fold domain variably sequence in a novel fashion. The variable pair of histone protein are H2A and H 2B and the conserved one are H4 and H3. In eukaryotes histone proteins are associated with DNA and are positively charged, this is due to presence of positively charged amino acids like lysine and arginine . H 1, H2A, H 2B histone are rich in lysine and H3 , H4 are rich in arginine. Each nucleosome consists of 8 histone proteins. Around one nucleosome to another nucleosome 200bp is present in DNA. In a circle of 1 nucleosome 146 bp are present. Where 54 BP are present in connection link of DNA between 1 nucleosome to another nucleosome. In nucleosome H1 histone is absent. here linker DNA connects two nucleosomes and H1 protein present in linker DNA. H1 protein takes an active role in formation of eukaryotes and heterochromatin.

Genetic and epigenetic changes both involved in breast carcinogenesis and it is a multi step process. Epigenetic is a change that observed in gene expression in both reversible and heritable by the gene sequence without alteration. In cancer that influence the two major epigenetic changes are DNA methylation and histone modification interactions is well orchestrated. Malignant and premalignant breast neoplasm is methylated by involvement of several genes in metastasis, proliferation and antiapoptosis. In breast cancer treatment with other systemic therapies, histone deacetylase inhibitors become synergistically an important class of drugs. Potentially reversible processes are epigenetic changes and for finding novel therapies

and refined diagnostic of breast cancer many efforts has been done for understanding the mechanism.

MATERIALS AND METHOD:

30% W/V Acryl amide /Bis acrylamide

Tris Hcl 3. 0M, PH = 8. 8 (lower gel)

Tris Hcl 0. 5M, PH = 6. 8 (upper gel)

Bio-rad mini protean tank

TEMED

Ammonium persulphate (APS 25%W/V)

Running buffer

Bromophenol blue

Sample buffer

Coomassie blue stain

Human recombinant proteins H4, H3. 3, H2B, H2A

EXPERIMENTAL PROCEDURE:

SDS -PAGE GEL PREPARATION:

PREPARATION OF GEL CASSETTE SANDWICH:

The casting frame is taken and place on the flat surface.

Select the glass plates to make a sandwich and place the short plate on the spacer plate and fix the casting frame to make sandwich.

Fix the casting frame to the stand and the sandwich glass plates on the gray rubber gasket.

Then checked the sandwich plates with distilled water to ensure any leakage occur.

Prepare the resolving gel into a beaker without adding TEMED and APS.

Add TEMED and APS into the prepared resolving gel and mix the solution homogenously and immediately pour the mixed solution into the sandwich plates, more than half of the glass plates.

Allow the resolving gel for 35-40 minutes to get gel polymerised.

Wash the resolving gel with distilled water and discard the water from sandwich, dry the inner surface by using filter paper.

Prepare the stacking gel into another beaker without adding the TEMED and APS.

Added TEMED and APS and mix equally and pour it on the top of the resolving gel and gently place the comb on the top of the stacking gel.

Then leave the stacking gel overnight for its polymerization.

RESOLVING GEL AND STACKING GEL PREPARATION:

Resolving gel: acrylamide/bis-acrylamide 10. 0ml, 3. 0M Tris /Hcl (PH= 8. 8) 3. 75ml, dH20 15. 8, 10% SDS 0. 3ml, TEMED 0. 015, Ammonium Per sulphate 0. 15.

Stacking Gel: Acrylamide/bis acrylamide 2. 5ml, 0. 5M Tris /Hcl (PH 6. 8) 5. 0ml, dH20 12. 26ml, 10% SDS 0. 2ml, TEMED 0. 015ml, Ammonium persulphate 0. 04ml.

Separation of H2A/H2B/H3. 3/H4 Human Recombinant Protein using 1D SDS-PAGE Gel .

After overnight polymerisation taken out the comb carefully and well are washed with running buffer.

Remove the gel sandwich from the casting stand and allow to place them in the electrophoresis tank placing short plate facing inwards.

Fill the gel electrophoresis tank with running buffer up to halfway between inner chamber i. e. 125ml and in the mini tank add 200ml of running buffer.

SAMPLE PREPARATION AND LOADING:

Taken the sample of histone protein of ï€±ï€ ïl and added into the sample buffer of 20ïl eppendorf tube.

The protein samples are labelled to each tube.

The histone protein samples are heated to 100o c for 2 minutes in hot block and at room temperature allow cooling down.

Now samples of histone proteins are allowed to load into the well of 20ï€ ïl of each sample with the help of loading gel tips and while loading, load the sample carefully and slowly without air bubbles and allow the sample to settle down at the bottom of the wells.

Taken molecular marker of 2ï€ ïl and loaded in another well for the identification of the proteins migration.

GEL ELECTROPHORESIS :

Cover the mini tank with lid properly by using colour code present on the banana plugs.

Connect the gel electrophoresis tank to power supply by using 200volts of constant current for about 35-40 minutes until samples runs more than 3/4th of the gel.

Stain and de-stain gels:

After reaching the sample nearly bottom of the gel turn of the power supply to the gel electrophoresis tank and disconnect the electric leads.

Discard the running buffer to avoid splitting and carefully remove the gel sandwich, gently separate the gel from plate by using sharp wedge, separated gel is placed in coomassie blue stain solution of 20-30 ml for 30 minutes on shaker for constant shaking.

After the 30 minutes discard the stain solution and wash the gel with distilled water for 4- 5 times for constant time intervals and incubate at room temperature for overnight by placing on shaker.

Finally rinse the detained gel with distilled water till the protein bands can clearly visible.

Taken the pictures by using camera.

RESULTS:

By observing the obtained result after running the histone samples H4, H3. 3, H2B, H2A in 1D SDS-PAGE . The separation of samples has been seen on the gel by using a dye coomassie blue stain solution. While observing the samples are run on gel according to their molecular mass and get separated from each other. The sample histone protein H4 shows little bit difference in observation which is present near to bottom of the gel , that shows it run little bit faster than other histone samples due to its smaller size. While observing the other histone samples like H3. 3, H2B, H2A they has no lot of difference in separation to differentiate from each other.

DISCUSSION:

The present experiment explains the isolation of human recombinant protein H4, H3. 3, H2B and H2A by using the 1D SDS-PAGE. By observing the obtained result that found the histone protein H4 migrated little bit faster than the other samples . where as other histone samples H3. 3, H2B, and H2A are observed , there is no lot of difference in the migration to differentiate from each other. According to Kornberg, R. D when they performed the experiment on histone protein of human recombinant, found the histone protein H4 migrate faster than other protein and appears to be at 11 kDl . Where H3. 3 appears near 15kDl, H2B appears near 14 kDl , H2A appears near 12 kDl with these result we expect to be the same result but

according to the above result that H3. 3 , H2B, H2A does not show much separation in migration of protein sample. So for getting such result may have many reasons that might have not loaded the samples with equal volume or properly loaded in the wells or power supply to the electrophoresis tank is not adjust properly or one sample over float into other wells while loading.

The separation of the histone proteins that observed by different authors are histone proteins which undergoes non-acetylase that migrates faster than the protein that undergoes monoacetylation and acetylated derivatives. In this sequence the histone proteins are clearly separated in the core histone protein by using 1D SDS-PAGE. The retarded mobility are shown when the histone protein is highly acetylated compared with non acetylated parent compared. The variants are observed in histone protein due to differing of amino acids in the sequence. Histone proteins undergoes different biological conditions and form to be post synthetically modified like ADP-ribosylated, phosphorylated and acetylated . by using SDS-PAGE the purity of isolated proteins are identified. In the present experiment if the mixture of four histone proteins would need to be separated by the same technique. I would preferably take the three consequent results of same and expected as follows molecular size of H4 has less kDl than H2A, H2B and H3. 3 in kDl. Few journals and reviews found to be support my hypothesis like Kornberg, R. D(1977) and Herbert and Linder(1992).