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Biomarker Discovery in Bisphenol A Stimulated Human Breast Cancer Cells

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Abstract: **Separation of any kind of protein from a complex mixture can be achieved by numerous methods. In this research experiment, SDS-PAGE technique was used. Samples of H2B and H3.3 protein were loaded in calculated manner and band images obtained after 1D electrophoresis were analyzed. Biomarker was taken as a reference to explore the migratory pathway followed by the sample protein. Basically, the impact of Bisphenol A (BPA) on protein in stimulated human cancer cells was governed. It was concluded that BPA induce mutation in histone protein by altering its molecular mass which depicts a correlation between BPA and cancerous cells.**

Keywords: **Biomarker, Bisphenol A, human cancer cells, gene profiling, breast tissue mutation.**

1. INTRODUCTION

Separation of a protein, comprises of processes and methodologies invented to isolate a particular type of protein from a complex mixture. It is a very important aspect for studying the characteristics of protein like function, composition, physical structure and its interaction with protein of interest. The initial material is a biological tissue to obtain desirable protein from it. Isolation of one protein from others is usually the most laborious aspect of protein purification. These techniques may exploit its properties like protein size, biological activity, binding affinity and physio-chemical properties. The separation can be analytical for research quantification or preparative for preparing commercial products [5].

Methods of protein separation are numerous and involve the step of extraction as foremost, as depending upon sources; the protein has to be brought in contact with solution. Repeated freezing and thawing, homogenization by high pressure and sonication can be done. In protein purification involving large amount precipitation involving large amount precipitation and differential solubilisation has to be performed. Centrifugation is a technique that uses the centrifugal forces to separate the mixture of particles of different masses or densities within a liquid. In this process the mixture of proteins is kept in a vessel and rotated at high speeds the angular momentum yields an outward force to each particle that is equivalent to its mass [12].Electrophoresis is a method used to separate complex mixtures of protein to detect subunit composition and for the purification of protein for subsequent applications. In the case of polyacrylamide gel electrophoresis, protein migration occurs due to an electric gel matrix as acrylamide concentration becomes higher pore size reduces. The protein migration is determined by protein charges, gel pore size, and shape. In 1-D electrophoresis, denaturing condition prevails (0.1% SDS present) isolates protein results in giving identical charge densities. In 2-D, separation is done first by isoelectric focusing focussing (pH gradient established by ampholytes) and then in second dimension SDS-PAGE is done [11].Western blotting is used to separate protein along with identification,exploting efficiency of SDS- PAGE and also ability of immunochemical reagents to associate specifically with a given protein antigen. Various kinds of chromatography's can be performed like column chromatography to fractionate proteins. Ion-exchange chromatography (based on charge), gel filtration (base on size) and affinity chromatography, HPLC are feasible for isolation [6]

Biomarker discovery is the process through which biomarkers are discovered. The newly emerged interest in biomarker discovery is due to new molecular biologic techniques promises to reveal relevant markers quickly. The screening of various biomolecules, by the use of genomics and proteomics can be done. In the pharmaceuticals sector, blood test or the biomarkers pose a great opportunity for clinical trials and possible drug targets. Proteomic technologies along with protein arrays and new advancement in MS are giving the necessary tools for discovery and identification of diseases

related to biomarkers [8]. The emerging field of biomarkers has resourceful utility in the staging, diagnosis, and prognosis, in progression of disease, clinical reactions of a therapeutic intervention. Biomarkers have good impact on health economics [9]. Recent enhancements in bioinformatics links and statistical tools have contributed a lot in revolutionising the approach for the discovery of biomarkers. Due to integration of different technologies for analysis development of biomarkers in validation has been improved in a novel direction [3].

2. METHODS

Firstly we prepared (SDS-PAGE) Gel cassette sandwich during the first session of our experiment to set the apparatus as shown below.

Fig-1a apparatus of 1D electrophoresis

Fig-1b apparatus of 1D electrophoresis

We placed the casting frame upright with the pressure cams in the open position. We selected spacer plate and placed short plate on the top. When the glass plates were in place, the pressure cams were engaged to secure the glass cassette

sandwich. The gel cassette assembly was placed on the grey casting stand gasket. Insuring that horizontal ribs on the back of the casting frame are flushed against the face of the casting stand and the glass plates are in perpendicular position then, the lever pushes the Spacer Place down. For a second gel, all the above steps were repeated. We then added water, for the security and accuracy, also for ensurity that no leakage occurs. We placed a comb completely in to the assembled gel cassette marking glass plate 1 cm below the comb teeth to know for pouring resolving gel. Removing the comb is important. After that we prepared the resolving gel solution in a tube by mixing all reagents except TEMED and APS as listed in table 1 below.

Table 1: Preparation of resolving and stacking gels (x 2) for SDS-PAGE (15%)

Solution	Resolving gel (ml)	Stacking gel (ml)
Acrylamide/bis-acrylamide	10.0	5.0
1.5M Tris/HCl (pH8.8)	5.0	
0.5M Tris/HCl (pH6.8)		2.5
dH2O	4.8	2.4
10% SDS	0.2	0.1
TEMED	0.01	0.01
Ammonium persulphate	0.1	0.05

We in the end, added TEMED and APS to the solution and poured to the mark smoothly. Within no time we overlayed the solution with water slowly and evenly. We allowed the gel polymerisation for 30-45 mins. While waiting we prepared the stacking gel solution. After 30-45 mins of polymerisation we rinsed the gel surface completely. We took care not to touch the surface of the gel. We inserted the comb between the spacers starting at the top of the spacer plate. Like before we added TEMED and APS to the stacking gel solution and poured the solution between the glass plates till the short place is reached. Then, allowed it to stand for polymerisation. After removing the comb we filled the wells with running buffer. Then we rotate the cams of the casting frames inward for releasing gel cassette sandwich. After placing gel cassette sandwich in to electrode assembly we lifted gel cassette sandwich into place against the green gaskets and slided into the clamping frame. Then, we pressed down on the electrode assembly to form the inner chamber. Then, we lowered inner chamber assembly into the mini tank. Then we filled it with running buffer, by taking caution of not over-filling it. Also, we added approximately 200ml of running buffer to the mini tank.

The second session of experiment comprised of performing with the gel prepared previously. Sample loading was done by; heating all samples in a hot block (at 100° C) for 2 mins and the cooled them down then. We loaded each of the samples into the wells using a gel loading tip without puncturing the bottom of the well. Then placed the Lid on the Mini Tank, by making sure there is correct positioning banana plugs and jacks on the electrode assembly. A suitable power supply with the proper polarity was chosen for insertion of electrical leads. Then we applied power to the mini-PROTEAN 3 cell and began electrophoresis. 200 volts constant was set for SDS-PAGE gel for about 35 mins, till the bromphenol blue dye-front had migrated 0.5-1cm to the bottom of gel. After electrophoresis was completely done we turned off the power supply. The tank lid was removed and the running buffer was discarded before opening the cams to avoid spillage. The gel cassette sandwiches were also removed along with the gels. The gel was then submerged with 20-30ml instant-blue stain solution for 15 mins with slight constant shaking. To see the protein bands clearly, we rinsed gel with water thoroughly and then clicked the photos of the gel for result and analysis.

3. RESULTS

Through the band image be obtained after the completion of experiment, we can analyse lots of facts. When we loaded the samples in the wells using gel loading tip we followed the following table sequence:-

Table 2:- Gel loading sequence

Lane	Sample detail
L1	5 µl of pre-stained molecular marker
L2	20µl H2B histone protein
L3	20µl H3.3 histone protein
L4	20µl(8µg) total protein sample(control)
L5	20µl(8µg) total protein sample(treated)

We got the below shown band image by performing 1D- electrophoresis

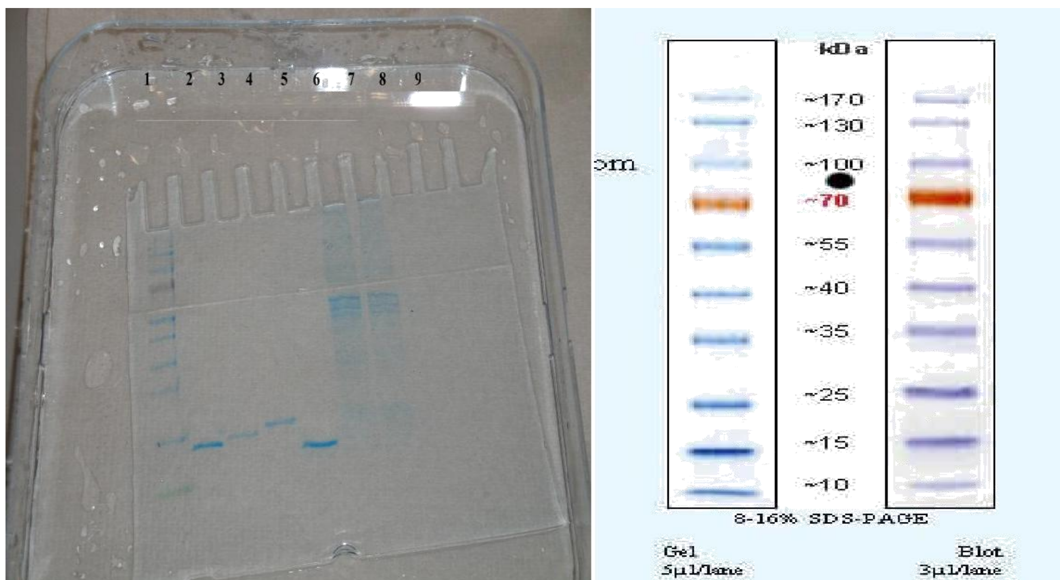

Figure N: - a) Image of the band after 1D electrophoresis b) Page ruler image

If we compare the bands obtained in the lane 1 we have the biomarker for checking the migration of protein. Then, in lane 2 H2B histone protein band is present which when compared with biomarker has around 20kDa molecular mass. Similarly we can do approximation for lane2 having slightly more molecular mass than lane2 of about 23kDa. The molecular mass of protein sample taken as control comes out to be 25kDa. We can observe very evidently, that the molecular of the treated protein sample is altered. It is about 15kDa according to my result.

This shows that treatment with bisphenol A (BPA) has an impact on the protein present in stimulated human cancer cells. BPA treated cells have different molecular weight due to BPA applying its action. BPA introduce mutation in histone protein by altering its molecular mass [4]

4. DISCUSSION AND CONCLUSION

Expression profiling of BPA responses on treated cells reveals that there is some association of BPA with tumors and cancers. The data obtained has relevance that exposure to BPA has contribution to establishing and/ or maintaining of breast tumors [14].

There are many other publications regarding breast cancer cells stimulated by BPA. Differential gene profiling has also been reported. Previous researchers have proved that when BPA was added to cultures of breast tissue mutation occurred. In prior studies a major criticism was given that when we use higher doses of BPA they don't act on human exposure levels to this compound by this experiment, we have overcome this short coming of prior publications by taking a

calculated amount of BPA. Experiments with rats before showed that at low level exposure of BPA lead to breast cancer and higher sensitivity of chemical resulting in breast cancer [2]. The data analysis shows that BPA at relevant concentration provides chemo resistance and antagonizes multiple anti-cancer drugs. Clinical trials are still going on that whether action of BPA is mediated through genomics versus non genomics mechanisms. In this experiment, we can confer that BPA can modulate tumor cell proliferation, causes mutation in histone protein present in cells. Altogether, this data reveals that environmental exposure to BPA may pave the way for advances in conversion to therapeutic resistance in cancer [7]

For over several decades, scientists have been investigating importance of application of biomarkers in disease reduction and protection of persons from harmful chemical exposure. Just the availability of biomarkers is not enough for studies but proper validation to the point of efficacy is necessary. A biomarker database structure has to be developed and decision rules have to be identified for the organisation of various types of data to support a chemical risk assessment [1] While looking clinically, proteomics can be considered as a promising new potential approach that can fasten the discovery and validation of biomarkers that associate with disease and permit the assessment of therapeutic treatments. Development and optimized enhancement of quick and cost effective quantitative mass spectroscopic assay for biomarker validation is required. MS- based assays can greatly strive with antibody- based methods in areas of cost, specificity and accuracy as they can analyse those protein for which no antibody has been recognised. High Performance Liquid Chromatography (HPLC), Electron Spray Ionisation (ESI), Matrix Assisted Laser Desorption Ionisation (MALDI), can prove to be valuable tools for analysis, validation and successful diagnostic. This will impact on novel discovery of biomarker as well. Multiple Reaction Monitoring (MRM) technology can help in determination of changes in particular protein samples and help in approaches for establishment of validity [13]

Potential approaches to evaluate and validate therapeutically relevant biomarkers include developing a classifier by selecting a prediction model, splitting sample data into training and test sets, performing and then estimating the prediction accuracy with test set. Appropriate method validation experiments should include steps of sample collection, relation precision and accuracy, selectivity, specificity, parallelism, ‘fit’ for purpose and stability factor so as to meet the necessities for advanced specified research [10]

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