STUDY ON THE COLORIMETRIC ANALYSIS OF TWO DIMENSIONAL P.A.G. ELECTROPHORESIS BY SILVER STAINING METHODS

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There are various approaches to the analysis of Two Dimensional Electrophoresis images. Recently, the study of the color analysis on protain map by silver staining method has become a center of attention.

The silver stain process is described by Adams and Sammons with net charge, molecular weight and color. In addition to a characteristic position (orthogonal coordinates) on a gel and an intensity, the spot staining is a characteristic hue as well, usually of various shades of yellow, red, green and blue. This multi dimentional analysis will permit greater resolution, accuracy and reliability of either automatic or manual analysis, which is attainable via existing monochrome approches.

However, usual method of color analysis is often unreliable and uncertain. In order to remove this defect, a new colorimetric instrument for electrophoresis use is designed, which displays the digital color information. Based on advanced color science, direct digital reading of tristimulus value XYZ (red, green and blue), CIE chromaticity and color difference delta E* by CIELAB system (Recommendation of Commision Internationale de l'Eclairage) can be measured immediately. Mutual relationship between color difference and molecular weight was sufficiently estimated from the result of measurement.

Memo:

AGE-RELATED CHANGES OF TWO-DIMENSIONAL ELECTROPHORETIC PROTEIN PATTERN OF RAT LIVER

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We have developed a high-performance and easy-handling procedure of 2-D electrophoresis using cellulose acetate membrane. The first-dimensional electrophoresis was performed on a cellulose acetate strip posterior to the concentrating electrophoresis on it. Isoelectric focusing in the second dimension was done on several layers of cellulose acetate membrane. Thus, several 2-D replicas of a sample were obtained at once. Those replicas were stained separately with Coomassie blue, silver and Shiff's reagents. The quantitative analysis of protein spots was performed by a Joyce-Loebl microdensitometer connected to the SORD M223 microcomputer system. This newly developed method was applied to analysis of the age-related changes of proteins in rat tissues.

Young (1.5M), adult (6, 12M) and old (24, 30M) rats of Wistar/Slc and Fischer 344/DuCrj strains were sacrificed and tissues were removed. Tissues were homogenized with 9 vol. of distilled water. Their 10,000 xg supernatants were subjected to the 2-D electrophoretic analysis.

Approximately 80 protein spots were detected on the 2-D pattern of rat liver. Six spots in them were markedly changed with aging. There was, however, sexual difference in the quantitative trends. From these results, we determined an alteration of the amount of protein by a castration and by a treatment of testosterone. There was also tissue specificity on the six proteins. Three protein spots were found only in the liver extract. Therefore, these three proteins might be liver specific proteins, and others were distributed widely such as in kidney, muscle and brain so far as studied.

Memo:

SLAB GEL ELECTROPHORESIS AT ANY THICKNESS WITH EFFECTIVE SAMPLE MOVEMENT & GRADIENT FLATTENING OF SDS AND IEF GELS

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Using techniques of our multibarreled microelectrophoresis, new apparatuses are developed for SDS slab gel, IEF gel and gradient flattening. The new SDS one makes good effective protein movement from first-dimension gel into second-dimension gel, makes running time short, is convenient for the simultaneous processing of many polyacrylamide gel slabs and ultra thin(100μm) gels, is adaptable, easy and inexpensive, and has many other advantages. The new IEF one reduces anodic and cathodic drifts in vertical gel rod IEF, can be used for many gels, can change length, diameter and shape of the gel arbitrarily, and can use high concentrations of detergent to dissolve protein samples. The third one accomplishes MW gradient flattening of SDS slabs and pH gradient flattening of IEF gels at any gel segment, so any crowded areas in the gel can be easily widened and any dispersed areas in the gel can be easily narrowed. These methods does not need any special expensive apparatuses. From the gradient maker to the gel dryer, all the necessary apparatuses are simple, home-made and inexpensive. Many gels are simply piled up between two ordinary glass pans for electrophoresis. Glass plates do not need notches and plate-sealing layers are straight and narrow. Size and shape of the slab gel can be easily changed. Acrylamide polymerization can be adjusted by ice. With the simple and convenient gradient flattening for SDS and IEF gels, every gel segment can be used effectively and meaningfully.

Memo:

FURTHER CHARACTERIZATION OF ERYTHROCYTE MEMBRANE PROTEINS BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

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Studies of the erythrocyte membrane in our laboratory using two-dimensional gel electrophoresis (2D PAGE) showed a complex pattern including over 600 spots (Clin. Chem. 28:925, 1982). Major cytoskeletal proteins (spectrin and actin) were not present in these patterns due to selective solubilization. In the present studies, we have modified the solubilization procedure to include spectrin and actin in 2D PAGE patterns. In addition we have further characterized the multiple polypeptides seen in the original 2D PAGE pattern. Membranes were solubilized in various solutions all containing 2% Nonidet P-40, 2% β-mercaptoethanol, 2% pH 3.5 Ampholines, but varied in the concentration of urea and the presence of ionic detergents. Membranes solubilized with 4 M urea for 2D PAGE resulted in the pattern previously observed. The same membranes when solubilized with 9 M urea containing ionic detergents resulted in three major Coomassie blue staining polypeptides, corresponding in molecular weight to the two spectrin bands and actin. Further staining with an ultra-sensitive silver stain showed additional polypeptides, however, these did not correspond to the polypeptide seen in the 4 M urea pattern. Membrane polypeptides separated by 2D PAGE were transferred for the detection of glycoproteins. The membrane pattern obtained by solubilization with 4 M urea showed that many of these polypeptides were positive for carbohydrate. These studies show that erythrocyte membrane proteins can be selectively solubilized to reveal a 2D pattern that corresponds to either cytoskeletal or non-cytoskeletal protiens.

Memo:

THE EFFECT OF RADIOACTIVE AMINO ACIDS USED FOR LABELING CELL PROTEINS ON TWO-DIMENSIONAL PROTEIN PATTERNS

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Two-dimensional electrophoresis (2DE) of proteins followed by autoradiographic (fluorographic) visualization of the protein patterns leads to a high resolution of complex protein solutions. Patterns showing several thousands of protein spots can be obtained from one type of cells. However, to reach such a high resolution, a sufficient amount of radioactive amnio acids must be incorporated into the proteins of the cells. We investigated, whether the radiation of the radioactive compounds affects the viability of the cells and therefore alters the normal protein pattern.

Human cells (Hep- and Hela-cells) and chinese hamster cells (CHO-cells) were cultured with 20 µCi ^{14}C amino acid mixture per ml medium for 5, 8 and 20 h and with 200 µCi for 0.5 h. Thereafter, the number of cells which survived were determined by cell cloning (plating efficiency). Furthermore, the cells were investigated for chromosome mutations. Finally, the proteins were extracted from the cells and separated by 2DE. The protein patterns were analysed for quantitative and qualitative changes.

The results showed a considerable decrease in the plating efficiency of the cells and a considerable increase in the number of chromosome breaks when the cells were increasingly exposed to the radioactive amino acids. In contrast, obvious changes in the 2DE patterns of proteins could not be observed. Some explanations for this discrepancy will be discussed.

Memo:

TWO-DIMENSIONAL ELECTROPHORESIS OF CEREBROSPINAL FLUID PROTEINS IN THE ABSENCE OF DENATURING AGENTS

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Previously, we described a "normalized map" of human plasma proteins, which illustrated the standard distribution pattern of the proteins two-dimensionally separated in the absence of denaturing agents. The present report describes two-dimensional electrophoretic analysis of cerebrospinal fluid proteins without denaturant.

Forty specimen of human cerebrospinal fluid were subjected to macro-(employing 16 x 12 x 0.3 cm slab gels) or micro-(simultaneous run of 8-16 slab gels of 3.8 x 3.5 x 0.1 cm in size) two-dimensional electrophoresis.

2-D patterns of human cerebrospinal fluid proteins resembled with those of human serum proteins, except the following features were observed. (1) High-molecular weight proteins, such as IgM, α_2 macroglobulin, and LDL were relatively less in amount. (2) Several protein spots characteristic to cerebrospinal fluid were detected. (3) Haptoglobin polymers were not detected in most of the patterns,which suggested the selective filtration of serum proteins.

A "normalized map" of cerebrospinal fluid proteins was prepared by comparing the patterns obtained from "healthy" individuals. When the patterns obtained from multiple sclerosis patients were compared with the normalized map, increase of proteins at haptoglobin region was observed.

Memo:

PROTEIN ALTERATIONS ASSOCIATED WITH RADIATION INDUCED CHROMOSOMAL DELETIONS

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The study of genetic effects of radiation in the mouse has led to the identification of lethal albino deletion mutations. In previous studies, deletions of the albino locus (c) in chromosome 7 were selected for mutational analysis because homozygotes are nonviable and thus are easily detected. In heterozygotes, the radiation induced mutations interact with the albino allele causing albinism.

We have detected protein alterations associated with radiation induced deletions using two-dimensional electrophoresis. Liver proteins obtained from homozygous ($\underline{c}^{14CoS}/\underline{c}^{14CoS}$, $\underline{c}^{3H}/\underline{c}^{3H}$) and heterozygous ($\underline{c}^{ch}/\underline{c}^{14CoS}$, $\underline{c}^{ch}/\underline{c}^{3H}$) mutant mice were analyzed. Both homozygous and heterozygous deletions altered the protein pattern. Several polypeptide spots were absent in patterns corresponding to mutant mice, whereas other spots that were undetectable in the normal were quite obvious in the mutant group. Agreement with the normal protein pattern was much closer for heterozygotes than for homozygotes. In homozygotes, the extent to which protein alterations were observed between mutant and normal mice was proportional to the length of the deletion. The longer deletions expressed substantially more protein differences than the shorter deletions. We conclude that two-dimensional electrophoresis allows the identification of protein products of deleted structural loci as well as the identification of secondary effects related to the deletion of regulatory loci.

Memo: