TWO-DIMENSIONAL ELECTROPHORESIS FOR "PROTEIN MAP" AND FOR "POLYPEPTIDE MAP"

T. Okuyama and T. Manabe

There are two types of 2-D electrophoresis method, depending on "with" or "without" chaotropic reagent. The former method relying on the random coil structure of protein is called as "Polypeptide Map" method and the latter method relying on the natural structure or the higher dimensional structure of protein should be called as "Protein Map" method. The differences between the two methods were tested with the addition of 8M urea, mercaptoethanol, sodium dodecyl sulfate or/and nonionic detergent.

The protein map method was useful for the analysis of protein complexes, such as lipoproteins or haptoglobin-hemoglobin complexes and also protein polymers such as myeloma immunoglobulin polymers or haptoglobin polymers and further useful for the analysis of various isoenzymes. Thus the method is more useful for the physiological or clinical purposes and the conventional "Polypeptide Map" is useful for genetic studies.

Memo:

ELECTROPHORETIC METHODS IN HORIZONTAL ULTRATHINLAYER POLYACRYLAMIDE GELS. - A VERSATILE TIME AND COST SAVING SYSTEM

R. Westermeier, W. Postel, and A. Görg
Lehrstuhl für Allgemeine Lebensmitteltechnologie der
Technischen Universität München, D-8o5o Freising-Weihenstephan

Since the introduction of ultrathin-layer isoelectric focusing (Görg, A., Postel, W. and Westermeier, R., Anal. Biochem. 89, 6o-7o (1978)) there is a growing trend towards the use of thinner gels in analytical isoelectric focusing. The many advantages of ultrathin-layer gels polymerized on plastic supports, which are easy handling, better heat dissipation, higher resolution, shorter separation and staining times, higher sensitivity, are also valid for other electrophoretic methods. Because electrodecantation and vertical thermal gradients are eliminated in ultrathin gel slabs, the inherent advantages of the horizontal system can be realized also for zone PAGE (polyacrylamide gel electrophoresis), disc-PAGE, pore gradient PAGE, SDS-PAGE and 2D-electrophoresis. Under nondenaturing conditions anodally as well as cathodally migrating protein fractions are separated in a single gel matrix.

Performing 2D-electrophoresis in a horizontal system is more simple than in the vertical system. Since the ultrathin focusing gel is cast on a plastic membrane, the handling and transfer of the 1D-gel strip to the 2D-electrophoresis gel is facilitated. Due to the optimal flat to flat contact, the agarose overlay step is eliminated, and there is a complete removal of the proteins from the IEF gel strip into the gel of the second dimension.

Memo:

MICROCOMPUTER-AIDED TWO-DIMENSIONAL DENSITOMETRY

T. Toda, T. Fujita, and M. Ohashi
Dept. of Biochemistry, Tokyo Metrop. Inst. Gerontol.,
Tokyo, Japan

Two-dimensional electrophoresis provides the capability of analysing many proteins at once. Especially 2-D electrophoresis in polyacrylamide gel with SDS such as ISODALT system provides the excellent separation of polypeptides. In the method, however, the SDS-denaturation of protein structure may spoil their biological activities. We have developed a new type of 2-D electrophoresis on the layers of cellulose acetate membrane for the analysis of native proteins. The time-saving and easy-handling properties of the membrane method are advantageous for both clinical and laboratory use. We have been applying this method to the analysis of protein disorder which appears in aging process of mammalian tissues. In this study, it is necessary that the corresponding spots on two electrophoretograms are compared quantitatively. Therefore, we have made a tiny program for quantification of spots on 2-D electrophoretograms which runs on a commercially available microcomputer (SORD M223) interfaced with an appropriate optical reader. In the first case, Joyce-Loebl microdensitometer was connected. The X,Y-scanning densitometer showed the high resolution, high sensitivity and high reliability in the measured OD values. The system with the densitometer is useful for our studies. However, the densitometer is not suitable for routine work because it consumes hours. Then, in the second step, we connected a CCD-TV camera unit to the microcomputer system. The TV camera unit provides the high-speed reading of 2-D electrophoretogram and shows the enough reliability in the quantified values for our studies. In this paper, The method and performance of the microcomputer-aided 2-D densitometry system will be presented and discussed.

Memo:

MICRO TWO-DIMENSIONAL ELECTROPHORESIS ON SOLUBLE PROTEINS OF ALBUMEN GLAND (LAND SNAIL, EUHADRA)

Y. Suda, M. Fujishiro and T. Inoue
Dept. of Biology, Tokyo Gakugei Univ., Tokyo, Japan.

The albumen gland in Pulmonates is a secretory organ to serve the egg as the nutritive substances.

In the feeding, the secretory cells in the albumen gland of Helix pomatia were filled with the secretory substances consisted of galactogen, proteins, glycoproteins, amino acid and calcium.

The biochemical properties of galactogen have been studied by many authors, whereas the protein constitution of the albumen gland is little known.

Recently, we reported that the soluble proteins in the albumen gland and the egg of the Japanese land snails, Euhadra peliomphala, Euhadra hickonis and Euhadra quaesita were analyzed by the disc electrophoresis and the immunoelectrophoresis.

In this paper, we report that the transfer of the soluble proteins in the albumen gland (E. peliomphala) to the egg and the seasonal variation of the protein constitution in the albumen gland.

The micro two-dimensional electrophoresis which was recently developed by our laboratory and O'Farrell's two-dimensional electrophoresis were used in all experiments. The methods of silver staining and periodic acid-Schiff silver staining were also used for micro detection of proteins and glycoproteins.

Memo:

HIGH PERFORMANCE HORIZONTAL TWO-DIMENSIONAL ELECTROPHORESIS OF SERUM PROTEINS

M. Yoshida and M. Itoh
Central Research Laboratory, Hitachi, Ltd., Tokyo, Japan

A new two-dimensional electrophoretic technique suitable for routine clinical analysis has been developed. Separation in both directions was performed on a horizontal polyacrylamide gel (open gel) bound to a silanized glass. An isofocusing gel (0.5 mm thick) for the 1st dimension was bound to a narrow glass plate of 4 mm width, and 90 mm length. 3µl of serum were applied to the strip of gel that contains 2 % Ampholite (pH3.5-9.5, LKB.). After forcusing at 1500 V x h, the gel strip was placed in a groove of a gradient gel (4.2-18 %, size, 100 x 100 x 0.5 mm). They were then attached to each other with a low melting temperature agarose, and were subjected to a 2nd electrophoresis at 1000 V x h.

Proteins thus separated on a gradient gel over a total electrophoresis time of 6 h were stained by a new, rapid and sensitive method whereby they were converted into an artificial peroxidase. The gel was treated with hemin chloride(1×10^{-4} M) in a phosphate buffer (0.07 M, pH7.5) containing 50 % methanol for 10 min. The gel was then rinsed with the phosphate buffer for 8 min., to remove background hemin. It was then treated with a mixture of 1 mM 3.3'-diaminobenzidine, 2 mM xylenol and the buffer for 3 min. Finally H_2O_2 (0.1 M) was added to the mixture, and blue spots appeared within 10 min. The pattern thus obtained by means of this 30 min. process was similar to that obtained by staining and destaining with Coomassie Blue for 15 h.

Memo: