DETERMINATION OF TRANSIENT AND STEADY STATES IN ELECTROPHORESIS

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An apparatus for continuous registration of the electric field within an electrophoretic column was developed. It comprises a rectangular separation trough with an ordered array of 256 electrodes over a length of 10 cm and perpendicular to the current flow. Electrodes are photoetched on a supporting glass plate from a vapor deposited thin layer of SnO_2 semiconductor (width: 60µm, height: 0.1µm, space between: 340µm). The rectangular trough is defined by a gasket between the glass plate and a block of plexiglass. This block contains the sample inlet system and the connections to the electrode compartments. A power supply, appropriate detection electronics and fluid handling devices complete the apparatus. The electrode array is scanned mechanically by two graphite brushes, controlled by a microprocessor. The measured potential gradient between adjacent electrodes is recorded and stored in the computer memory of a Heathkit H8 computer, which handles all control functions, data treatment and printout of results.

Measuring data of each detection channel nearly simultaneously enables us to follow the dynamics of electrophoretic processes. This allows the automation of an isotachophoretic analysis by following the vanishing of mixed zones (asymptotic approach of the steady state). The device is a useful tool for validation studies of computer modeling in electrophoresis. Examples showing the electrophoretic evolution in each of the four classical modes (MBE, ZE, ITP and IEF) as well as in combinations are given. Results are compared with theoretical predictions. Various isotachophoretic applications are presented to demonstrate the usefulness of this new device in instrumental analysis.

Memo:

ISOTACHOPHORESIS AND SEPARATION OF PROTEINS USING CARRIER-FREE ELECTROPHORESIS APPARATUS

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We have attempted analysis of soluble proteins and membrane proteins by isoelectric focusing using carrier-free electrophoresis apparatus without supports such as polyacrylamide gel.

In this report,we describe separation of protein by isotachophoresis using a carrier-free electrophoresis apparatus. This apparatus was combined with a pump-out device, a UV detector and a fraction collector.

CONDITIONS OF ISOTACHOPHORESIS

Separation column : polyethylene tube ϕ 5 mm x 145 mm
Leading : 10 mM HCl pH (Ammediol) 0.04% Hydroxy propyl methyl cellulose (HPMC) 0.05% Agarose
Terminating : 10 mM ε-amino caproic acid 10 mM Ammediol pH 10.8 (NaOH) 0.04% HPMC 0.05% Agarose
Current : 1 mA constant current

Since the polyethylene tube was translucent,the migration process of colored proteins,hemoglobin and ceruloplasmin could be observed. During isotachophoresis,the bands of the two proteins were concentrated into adjacent two thin disks. When asparagine was mixed with the proteins,it formed a mixed zone between the zone of hemoglobin and that of ceruloplasmin.

Human plasma,mixed with amino acids or Ampholine, was subjected to isotachophoresis and the proteins in pump-out subfractions were identified by micro-dimentional electrophoresis.

Memo:

APPLICATION OF A RULE TO ILLUSTRATION OF ELECTROPHORETIC RESULTS AND STANDARIZATION OF ELECTROPHORESIS APPARATUS

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I am propose to the application of a rule to the illustration of photographs and diagrams of the electrophoretic results and standarization of the electrophoresis apparatus.

I. Illustration of photographs and diagrams

In the One-Dimensional Electrophoresis with the exception of IEP, the Anode should be oriented to the down or right-hand of the page, and in the IEP, the Base should be oriented to the right-hand side of the page.

Two-Dimensional Electrophoresis

In the first dimensional electrophoresis with the exception of the IEP, the Anode should be oriented to the right-hand side of the page, and with the IEP, the Base should be oriented to the right-hand side of the page. In the second dimensional electrophoresis, the Anode should be oriented to the down side of the page. In the Crossed immunoelectrophoresis, the Anode should be oriented to the top side of the page.

II. Electrophoresis apparatus

A. Power supply

Ammeter and Voltmeter on the front panel of the power supply should be located from left to right, or from top to down, respectively.

Anodic terminal colored with red should be located to the top, or right-hand side on the front panel.

B. Electrophoresis cell

Anodic electrode colored with red should be located to the right-hand side of the cell for Horizontal type, and the down side of the cell for Vertical type.

Memo:

HIGH-RESOLUTION AUTOMATIC EVALUATION OF ELECTROPHEROGRAMS

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Aiming at a more accurate and precise evaluation of 1D and 2D electropherograms a scanning system has been developed consisting of a scanning microphotometer, a laboratory computer and computer programs for data analysis. The photometric performance of the scanner is optimized, thus allowing accurate evaluation of even highly distorted bands or spots in electropherograms by digital signal processing /1/.

Original gel-electropherograms up to 5 mm thick and 250 mm X 250 mm large can be placed on a mechanical stage and scanned in two dimensions at steps of 100 µm and at a step frequency of 2000 Hz. The sampling grid can be refined along the lines up to 20 µm for the acquisition of high-resolution 1D electropherograms. Under these conditions measurements of 4000 distinct gray levels are obtained covering the absorbance range of 0.0001 to 3.6 optical density units.

Besides the quantification of 2D electropherograms by implicit modelling of the spots /1/ also 1D electropherograms will be evaluated two-dimensionally in order to take into account that some bands may be rather distorted or expanded transversally. For the comparison of 2D electropherograms spots will be identified semi-automatically by specifying a few landmarks which facilitate spot matching by a method of pattern recognition based on interspot distance relations /2/.

References:

/1/ Kronberg, H., Zimmer, H.-G., Neuhoff, V.:
Electrophoresis 1, 27-32 (1980)
/2/ Pardowitz, I. Zimmer, H.-G., Neuhoff, V.:
submitted to Anal. Biochem.

Memo:

ANALYSIS OF CULTURED SKIN FIBROBLASTS FROM PATIENTS WITH TRISOMY 18 USING ELECTROPHORETIC TECHNIQUES.

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In the present study we have employed the gel electrophoresis in the presence of SDS, polyacrylamide isoelectrofocusing and two dimensional high resolution polypeptide mapping to compare the proteins of cultured skin fibroblasts from normal healthy individuals and patients with trisomy 18. The cell cultures were also grown in the presence of ^{35}S-Methionine and the gel patterns were obtained by fluorography. In our gel patterns, apart from visible quantitative differences, certain qualitative spot differenes are indicated.These results would be presented and discussed in view of the data in some other type of trisomies.

Memo:

DIFFERENCE IN ANDROGEN-DEPENDENT CHANGES OF SUBCELLULAR PROTEINS BETWEEN VENTRAL AND DORSOLATERAL LOBES OF RAT PROSTATE AS DETECTED BY POLYACRYLAMIDE GEL ELECTROPHORESES

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Androgen-dependent changes of proteins in the subcellular fractions (cytosol, post-nuclear particulate and nuclear fractions) of the rat prostate were analyzed by SDS-polyacrylamide and two-dimensional gel electrophoreses. By castration, the content of the most abundant species in the cytosol fraction, 16K/5.6 (molecular weight x 10^{-3} · K/pI) for the ventral lobe and 67K/6.3 for the dorsolateral lobe, was decreased in the ventral lobe, but increased in the dorsolateral lobe. Their restoration to normal by replacement of androgen were slower with the ventral lobe than with the dorsolateral lobe. In the post-nuclear particulate fractions, there were similarities between the two lobes in the androgen-dependent changes of the contents of the most abundant species, 20K/8.5 for the ventral lobe and 110K for the dorsolateral lobe. Of nuclear proteins, the content of non-histone proteins per DNA were changed, whereas those of histones were hardly influenced, by castration and replacement of androgen. The content of the most abundant species of non-histone proteins, 20K/11.5 for either lobe, was more markedly decreased by castration with the dorsolateral lobe than with the ventral lobe. Its restoration by replacement of androgen was slower with the dorsolateral lobe than with the ventral lobe. The 20K/11.5 was extractable with 0.35 M NaCl from the nuclei previously washed with 1% Triton X-100. The protein in the dorsolateral prostate was purified into homogeneous state by CM-Sepharose column chromatography. Its amino acid composition revealed that the protein was not caused by the modification of histones, but a novel species of non-histone protein.

Memo:

ELECTROSTAINING ON TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

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Electrostaining is a simple method for the detection of proteins on polyacrylamide density gradient gel electrophoresis. Details of the procedure have been described in ELECTROPHORESIS '81. We applied this method to 2-dimensional electrophoresis composed of isoelectric focusing and polyacrylamide density gradient gel electrophoresis without denaturating agents.

Among the human plasma proteins, IgG and haptoglobin polymers were less sensitive than other proteins, while some protein spots which have not been identified yet, were relatively more sensitive than the case of the conventional staining method.

IgG and haptoglobin polymers became more sensitive when the dye solution containing 0.5% SDS was used. Passing through the gel, SDS may cause conformational changes of proteins, then the dye become to bind to proteins more easily. Excess dye cause partial solubilization and dissociation of protein complexes and disturbed protein map, especially on IgG and α_2-macroglobulin.

This staining method in the slightly alkaline condition may be based on the hydrophobic interactions of a dye and proteins. The results showed that the strengths of the bindings varied according to the characteristics of proteins. These were not by isoelectric points nor molecular weights, but conformations of proteins.

Electrostaining is a simple and useful method for the determination of proteins which can not be specified with the ordinary dipping stain method, by selecting proper dyes.

Memo:

TWO-DIMENSIONAL ELECTROPHORESIS OF BOVINE BRAIN PROTEINS —SOLUBLE AND INSOLUBLE FRACTIONS—

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Total analysis of bovine brain proteins were attempted by the combination of two-dimensional polyacrylamide gel electrophoresis (in macro system (160 x 120 x 3 mm slab gels) and in micro system (38 x 35 x 1 mm slab gels)), ammonium sulfate fractionation, and DEAE-Sephadex chromatography.

For the soluble protein analysis (cytosol fraction), proteins were separated by ammonium sulfate fractionation (0-30%, 30-85%, and 85-100% saturation) and then by DEAE-Sephadex column chromatography. Two electrophoretic buffer system were used for two-dimensional electrophoresis; (1) ISO-GRAD system, without any chaotropic reagent in the first and the second dimension and (2) ISO-SDS system, 1% SDS in the second dimension.

For the insoluble protein analysis (including nuclei, mitochondria, and cell membranes), the sample was prepared by 2M urea solubilization and analysed by ISO(urea)-SDS electrophretic system, with 4M urea in the first dimension and 1% SDS in the second dimension.

About 200 spots were detected in the soluble fraction by ISO-GRAD macro electrophoresis system,and about 400 spots were detected by ISO-SDS system. About 100 spots less than 20,000 dalton were newly appered by the treatment with SDS. Major spots in these maps were located in the protein maps of eight fractions separated by DEAE-Sphadex chromatography.

In the insoluble fraction, GFA, tubulin, actin and other fibrous proteins were located successfully.

Memo:

ANALYSIS OF GENETIC VARIATION IN AMERINDIAN SERA BY 2-D PAGE

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Studies of genetic variation in 2-D PAGE preparations of lymphocytes and fibroblasts have revealed levels of heterozygosity closer to one percent than to the six percent projected from 1-D studies of a variety of enzymes. On the other hand, employing Sammon-stained 2-D PAGE preparations of serum proteins from a population of new-born Caucasians, we have encountered heterozygosity levels of 6.4 percent in a series of randomly selected proteins. The validity of all variants has been confirmed by family studies. We now report a similar study of sera/plasma from Amerindians. Reading the preparations was complicated by the high gamma-globulin levels of the Amerindian samples. Despite this, a sufficient number of protein moieties have been scored for a valid comparison; the precise figures will be presented.

Memo:

TWO-DIMENSIONAL ELECTROPHORESIS OF RAT AND RABBIT LIVER PLASMA MEMBRANES

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We have used the technique of Two-Dimensional Electrophoresis for analysis of rat and rabbit liver plasma membrane proteins and peptides. We have adapted the sample preparation conditions as well as those for electrophoretic separations. The membranes are isolated as described by Neville (1).

The conditions of isolectric focusing and electrophoresis were adapted to plasma membranes. Isoelectric focusing iscarried out in the presence of an ampholine mixture (pH 3,5-10 and 5-7) with 700 V for 15 hours. The second dimension was performed on polyacrylamide gradient gel (11 and 14%).

The technique of solubilisation of membrane proteins mainly determine the quality of electrophoretic separation.

To determine optimal conditions of membrane solubilisation we studied the effect of some chemical agents and detergents: urea, nonidet Np-40, sodium dodecylsufate (SDS), sodium deoxycholate (DOC)... The data obtained confirmed the need for use of urea for membrane solubilisation. However we have assayed SDS and DOC. The use of DOC at a final concentration of 0.5% permitted to considerably increase the number of spots in the electrophoretic pattern.

Thus using silver staining technique we could detect more than 300 proteins and peptides in these membranes. The electrophoretic patterns of plasma membranes of rat and rabbit liver have a large number of peptides in common.

Now, we are trying to identify some of these proteins, after their transfer on nitrocellulose, by specific "antigen-antibody" reaction.

(1) J.D.M. NEVILLE
Isolation of an organ specific protein antigen from cell surface membrane of rat liver
Biochem. Biophys. Acta, 1968, 154, 540-552

Memo:

AN ISO-DALT ELECTROPHORESIS SYSTEM WITH REDUCED BUFFER AND CURRENT REQUIREMENTS

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Stegemann in 1970 first combined isoelectric focusing in one dimension and electrophoresis in the presence of sodium dodecyl sulfate (SDS) in the second, and O'Farrell in 1975 optimized the system to resolve approximately 1200 protein subunit spots. Our interest is to develop the hardware to run many two-dimensional electrophoresis samples in parallel, with the long-term goal of complete automation of the system. The semi-automated system developed at Argonne is termed "ISO-DALT," to indicate a combination of isoelectric focusing in the first dimension (ISO) with a moleuclar weight separation in daltons using SDS in the second dimension (DALT). To use the system effectively in a clinical or research setting, it was necessary to develop methods for running many analyses rapidly at the same time with good reproducibility.

Utilizing the prototype developed at Argonne, the system was made safer and more economical, and the buffer and current requirements of the second dimension DALT tanks were reduced while achieving the same results. This paper discusses the evolution of the system to its present form, and illustrates the advantageous technological spinoff of work at the National Laboratories to industry.

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Memo: