〔Short Communication〕

An Approach to Quantitative Analysis of Two-dimensional Electrophoretogram*

By

Tosifusa Toda, Toshiko Fujita and Mochihiko Ohashi**

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Introduction

Two-dimensional electrophoresis[1,2] has been a powerful tool in various fields of life science. The high resolution of two-dimensional electrophoresis provides the capability of detecting minor protein changes which appear with aging, carcinogenesis, disease states and therapies. We developed a new type of two-dimensional electrophoresis[3], which was performed on the layers of cellulose acetate membrane. This method had many remarkable properties : (1) it required only a little time to perform ; (2) membrane was an easy supporting medium to handle ; (3) enzyme activities were detectable after electrophoresis ; (4) several replicas of protein map were available for various detection methods, and so forth. However, there was difficulty in using the two-dimensional method to determine quantitative differences in protein spots. Although several groups had reported their methods of two-dimensional densitometry[4~6] using a minicomputer system, the minicomputer was less accessible than a microcomputer for us. Thus, we devised a microcomputer-aided system for two-dimensional densitometry. In this paper, the method of quantitative analysis of two-dimensional electrophoretogram is discribed and discussed.

Materials and Methods

Two-dimensional electrophoresis of human serum proteins

Two-dimensional cellulose acetate electrophoresis was performed as described previously[3]. Aliquots of 0.5, 1, 1.5 and 2 μl of serum from a healthy male human were applied to strips of cellulose acetate membrane (Titan III, 75×10 mm) for the first-dimensional concentrating electrophoresis. 1 μl of the standard protein solution (10 μg/μl acetylated cytochrome c, 5 μg/μl *R. rubrum* cytochrome c_2, 12.5 μg/μl horse heart metmyoglobin) was dropped on the strip near the anodic end. Then the strip was laid on a sheet of cellulose acetate membrane (Separax EF, 110×60 mm) impregnate with Ampholine (pH 3.5-10) solution. Isoelectric focusing was carried out on the membrane. Protein spots on the membrane were visualized by staining with Coomassie Blue G-250 as described previously[3].

Apparatus for two-dimensional densitometry

Optical density (OD) of the stained membrane was measured by a Joyce Loebl Microdensitometer 6 (Gateshead, UK) equipped with a NOVA/4 minicomputer (Westboro, USA). The minicomputer controlled the scanning of a sample stage of the densitometer to read optical densities of an electro-

* 2次元電気泳動像の定量分析法.

** 戸田年総，藤田敬子，大橋望彦，(財)東京都老人総合研究所生化学部基礎第2研究室.

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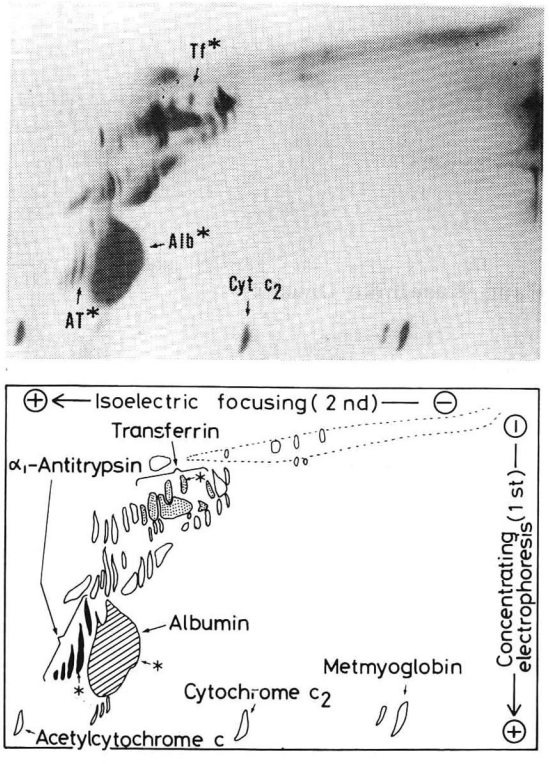

Fig. 1. Two-dimensional cellulose acetate electrophoretogram of human serum proteins.

An aliquot of 2 μl of human serum was applied to a strip of cellulose acetate membrane (Titan III, 75×10 mm) for the first-dimensional concentrating electrophoresis. 1 μl of the standard protein solution was dropped on the membrane strip after the first-dimensional electrophoresis. The strip was laid on a sheet of cellulose acetate membrane (Separax EF, 110×60 mm) impregnate with Ampholine (pH 3.5-10) solution. Isoelectric focusing was carried out on the membrane for 3 h. Proteins on the membrane were stained with Coomassie Blue G-250. All electrophoresis procedures and Coomassie staining were performed as described in our previous paper[3]. The shaded, dotted and striped spots were assigned to α_1-antitrypsin, transferrin and albumin, respectively, by immunochemical technique. The protein spots, pointed by an arrow with an asterisk, were quantified by two-dimensional densitometry for the linearity test.

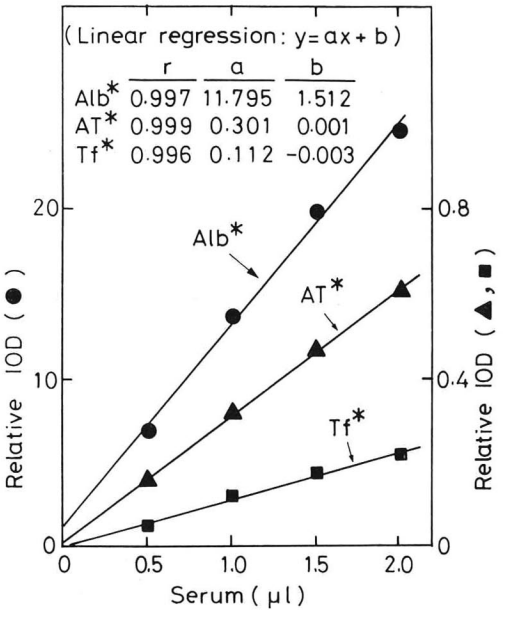

Fig. 2. Linearity of relative IOD.

Linearity of relative IOD, measured in the ratio to an internal standard spot, was examined using a human serum sample. Albumin (●-●). a subfraction of α_1-antitrypsin (▲-▲) and a subfraction of transferrin (■-■) were quantified. One unit of relative IOD was defined as an integrated pixel value for the Cyt-c_2 spot. The inclinations and the intersecting points of the regression lines are shown in the figure.

phoretogram. The pixel data were, then, transferred to a SORD microcomputer M 223 mark V (Tokyo, Japan) through 2 ports of serial interfaces (at 9,600 bits/sec). Image processing for quantitative analysis was performed by the SORD microcomputer equipped with a color CRT monitor screen, a video plotter, a line printer, and 2 disk drives for 8-inch floppy diskette.

Program and procedure of two-dimensional densitometry

A set of programs for two-dimensional densitometry (TWODIM/TIG) was written in Z-80 assembly language. The program package included ACCEPT, PACK, WHOLE, and LOCAL. The measured OD values, transferred from the NOVA /4 minicomputer, were accepted under "ACCEPT", and stored in a floppy diskette. Then the pixel data in 12-bit binary were packed into 8-bit binary under "PACK". The whole view of the electrophoretogram was displayed on the color CRT monitor

screen under "WHOLE" to determine the background level and the X, Y-positions of a given protein spot. The local area, including the protein spot, was displayed on the screen under "LOCAL" to quantify the size and the integrated optical density (IOD) of the spot. The obtained results were then typed down on a chart by a printer.

Results

IOD linearity of Coomassie-stained protein spots

Linearity of the measured IOD values of serum protein spots was analyzed. Because the absolute value of IOD on the two-dimensional electrophoretogram might have been changed by the experimental conditions, relative IOD value to a standard protein spot was determined as the quantity of a given protein spot. Fig. 1 shows a two-dimensional electrophoretogram of human serum proteins. Protein spots were identified by both immunofixation and electrosyneresis. Details of the spot assignment will be reported in a separate paper. Acetylcytochrome c (Ac-cyt c), cytochrome c_2 (Cyt c_2), and metmyoglobin (MetMb) were standard proteins applied after the first-dimensional electrophoresis. Albumin (Alb), a subfraction of α_1-antitrypsin (AT*) and a subfraction of transferrin (Tf*) were quantified relative to cytochrome c_2. The result is shown in Fig. 2. The relative IOD values show high linearity over the range of applied volumes.

Conclusion

Two-dimensional cellulose acetate electrophoresis was followed by quantitative analysis using a simple two-dimensional densitometer system. The densitometer was constructed with a microcomputer system in combination with a scanning densitometer. A set of programs for quantitative analysis (TWODIM/TIG) was written in Z-80 assembly language.

The relative IOD values of Coomassie-stained protein spots, measured in the ratio to cytochrome c_2, showed a high linearity. Similarly high linearities were also observed when other marker proteins (Ac-cyt c and MetMb) were used as the internal standard. The results supported the usefulness of our two-dimensional technique for the quantitative studies on protein changes.

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