Two 2-dimensional electrophoreses: A combination of crossed immunoelectrophoresis and dodecyl sulfate polyacrylamide gel electrophoresis for protein analysis

T. Yamamoto[1] and Y. Tanada[2]

Division of Entomology and Parasitology, University of California, Berkeley (California 94720, USA), 1 October 1979

Summary. An improved method of two 2-dimensional electrophoreses that combined crossed immunoelectrophoresis and 2-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis was developed with a horizontal gel-electrophoretic apparatus.

2 properties of proteins, electrical charge and size, have been applied to separate antigens in 2-dimensional (crossed)immunoelectrophoresis[3-5]. Laurell[3] described a method based on the resolution of the 1st dimensional zone electrophoresis in agarose gel that resolved even proteins of human serum[6]. In contrast with zone electrophoresis, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a more efficient method to resolve proteins, and has been used as the 1st dimension in crossed immunoelectrophoresis[5]. This method however, has some problems, e.g., possible alteration in antigenicity of proteins. To overcome this problem, we have developed an improved electrophoretic method, a combination of crossed immunoelectrophoresis and SDS-PAGE.

Experimental procedure. In the 2-dimensional immunoelectrophoresis[3], one of the gel strips from the 1st dimension was transferred to the serum containing plate for crossed immunoelectrophoresis and a 2nd strip was saved for SDS-PAGE (figure 1).

For the 2-dimensional SDS-PAGE, polyacrylamide gel, 10 × 12 cm with 1 mm thickness, containing 50 mM Tris-succinate, pH 8.2, and 0.1% SDS, was cast on a 12 × 14 cm frosted glass plate. The glass plate, a U-shaped spacer, and a plexiglas cover were clamped together and held vertically until the gel solidified, and then the cover and spacer were removed. The agarose gel strip from the 1st dimensional electrophoresis was dipped in 10 ml of SDS buffer composed of 100 mM Tris-succinate, pH 8.2, 2% SDS, and 2% β-mercaptoethanol, and it was placed on the bare glass plate, along the polyacrylamide gel. On one end of the agarose gel strip, a hole can be punched for reference proteins whose molecular weights are known. The space between the 2 gels was filled with 1.2% agarose gel containing SDS and buffer (figure 1). Both ends of the gel were in contact with 8 layers of gauze saturated with the electrode buffer, 100 mM Tris-succinate, pH 8.2, containing 0.1% SDS. Electrophoresis was conducted at constant 100 V until the tracking dye, bromphenol blue, reached the opposite end. The gel was stained with Coomassie blue R-250.

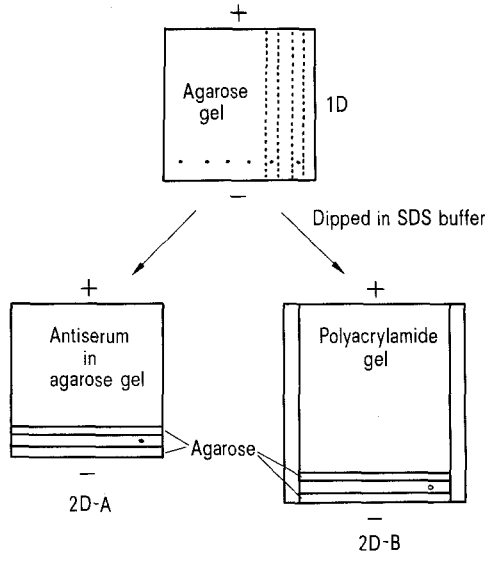

Fig. 1. Diagram of the procedure of the improved two 2-dimensional electrophoreses. After the 1st dimensional electrophoresis in agarose gel, the gel is cut into strips (1D). One of the strips is transferred to serum-containing agarose gel for immunoelectrophoresis (2D-A). Another strip is treated with SDS buffer and transferred to SDS-polyacrylamide gel for two dimensional SDS-PAGE (2D-B). Agarose gel is used to connect the strips and the gels (2D-A, 2D-B).

The apparatus for 2-D SDS-PAGE was made of plexiglas as shown in figure 2. 2 edges of the glass plate, gel side up, were in contact with 2 electrode buffer wells and the plate was covered with a 2-layered lid which had a 1.3-W (6 V, AC) heater between the layers. There was a 2 mm space between the gel and the lid. All electrophoreses, including immunoelectrophoresis, were conducted at 4 °C.

In two 2-dimensional electrophoreses made with reference proteins, each spot on SDS-PAGE was nearly the same size as that of the 1st dimension (figure 3, A). Linear correlation between mol.wts and migration distances was obtained between mol.wts 13,700 and 68,000 with 10% gel, and 30,000 and 93,000 with 7.5% gel (figure 4). With insect blood, the 2-D SDS-PAGE apparently resolved all of the proteins which appeared on the immunoelectrophoretic pattern as precipitin arcs (figure 3, B). The spots on SDS-PAGE, however, appeared broader than those of the reference proteins, probably because of the spots streaking in the 1st dimensional gel.

The size and optical density of the spots were affected by the protein sample size and the incubation time of agarose gel in SDS buffer. Protein amounts exceeding 4 µg increased the spot sizes in both dimensions, and a minimum amount of 100 ng was needed for distinct visibility. Incubation for 5–10 min in SDS buffer produced spots with high

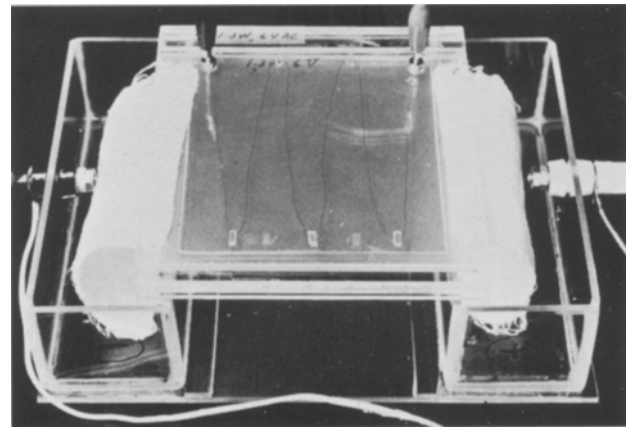

Fig. 2. Photograph of the improved horizontal apparatus for 2-dimensional SDS-PAGE. 2 edges of the gel plate are placed on 2 electrode buffer wells. 2 pieces of 8-layered gauze connect the gel to the electrode buffer. A lid, provided with anti-condensation heater, covers the gel plate.

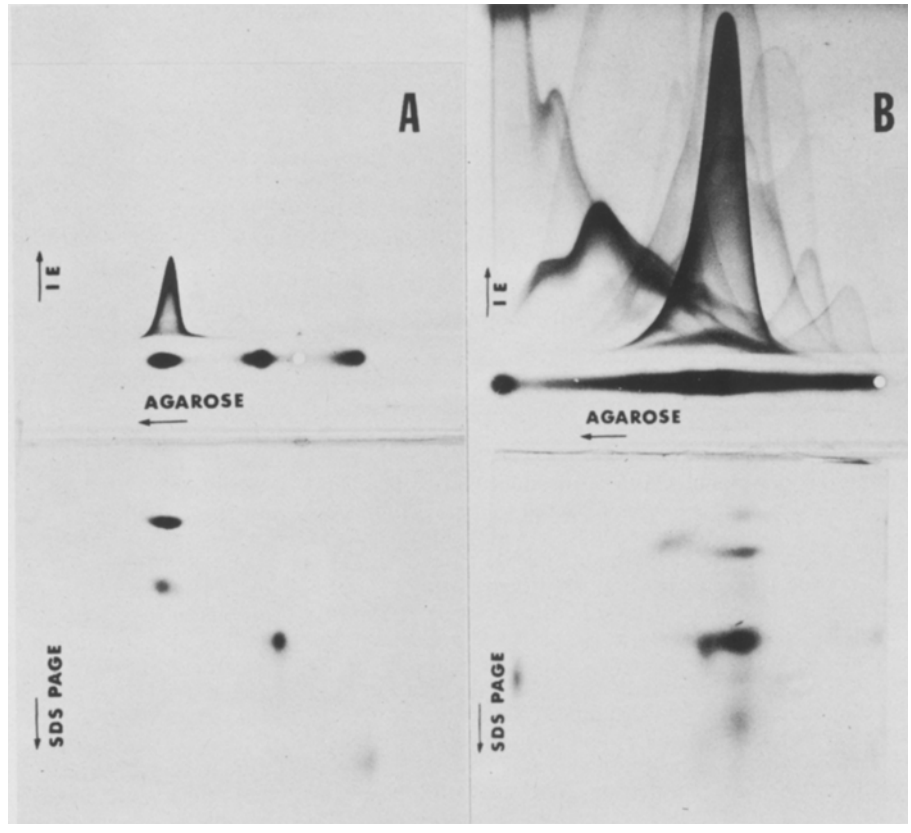

Fig. 3. Two 2-dimensional electrophoretic patterns obtained with water soluble proteins. *A* A mixture of reference proteins (2 µg in 4 µl buffer of each of the following proteins: bovine serum albumin, egg albumin, carbonic anhydrase, and ribonuclease) was electrophoresed in agarose gel as the 1st dimensional electrophoresis. The 2nd dimensional electrophoresis was made with the anti-serum to bovine serum albumin (2.5 µl/cm^2), and with 10% SDS-polyacrylamide gel. *B* Analysis of insect plasma (4 µl) containing 40 µg protein. Two 2nd-dimensional electrophoreses were conducted with the anti-serum to insect plasma (5 µl/cm^2) and with 7.5% SDS-polyacrylamide gel. Arrows point in the direction of the positive electrode.

optical densities, but prolonged incubation reduced the densities of low-molecular-weight proteins which might have escaped from the agarose gel.

Tris-HCl or Tris-acetate, when used as a buffer for SDS-PAGE, produced distorted patterns for proteins with mol.wts above 50,000. The proteins appeared to migrate more than expected from their molecular weights, possibly because of a change in gel pH to one higher than pH 8.2. Tris-succinate buffer eliminated this distortion for proteins of up to 68,000 daltons in 10% gel, and 93,000 in 7.5% gel. When 50 mM buffer was used throughout the system, poor electrical connection developed between the agarose and polyacrylamide gels because of differences in endosmotic pressures resulting from the loss of water in the connecting agarose gel during electrophoresis. This problem was corrected by increasing the buffer concentration in the connecting gel to 100 mM. The difference in electrical conductance between the agarose and the polyacrylamide gels cancelled the difference in endosmotic pressures.

This method is limited by the resolution power of crossed immunoelectrophoresis on the number of proteins in the sample. The method, however, is highly useful for identifying specific antigens. Up to 5 different antisera could be analyzed simultaneously because 5 of 6 gel strips are available from the 1st dimensional electrophoresis. One antiserum would be against a specific antigen which is to be differentiated from a mixture of proteins. At the same time, one of the gel strips would be analyzed by 2-D SDS-PAGE to determine the molecular weight of the specific antigen.

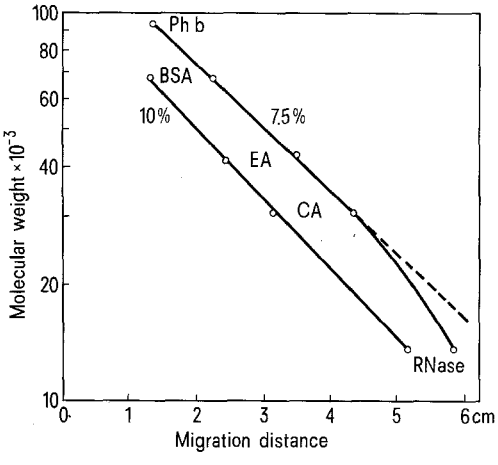

Fig. 4. Estimation of molecular weights on 2-dimensional SDS-PAGE. 2-dimensional SDS-PAGE was conducted with reference proteins, used in figure 3, A, at 2 µg in 4 µl buffer. In 7.5% gel, phosphorylase b was added to the mixture of reference proteins. Migration distances were measured on the chart by tracing with a densitometer.

1 Present address: U.S.D.A./S.E.A./A.R., Cotton Insects Research, P.O. Box 1033, Brownsville, Texas 78520.

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3 C.-B. Laurell, Analyt. Biochem. *10*, 358 (1965).
4 J. Söderholm and C.J. Smyth, in: Progress in Isoelectric Focusing and Isotachophoresis, p.99. Ed. P.G. Righetti. ASP Biol. Med. Press B.V., Amsterdam 1975.
5 C.A. Converse and D.S. Papermaster, Science *189*, 469 (1975).
6 W. Giebel and H. Saechtling, Hoppe-Seylers Z. physiol. Chem. *354*, 673 (1973).