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A logarithmic sample slot former for sodium dodecyl sulfate electrophoresis

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) is a useful tool for the estimation of the molecular weights of polypeptide chains[1-6]. Recently, the method was applied satisfactorily to polypeptides labeled with 1-dimethyl-amino-naphthalene-5-sulfonyl (DNS) chloride[7-9]. The fluorescent label permits direct visual observation of the protein zones in the gel and increases the sensitivity of detection.

This paper illustrates the use of a newly designed "logarithmic sample slot former" to simplify the approximation of protein molecular weights by their relative migration rate in SDS-polyacrylamide gel slabs.

Experimental

Cleavage of the disulfide bonds, alkylation of sulfhydryl groups and dansylation of the test proteins were performed by procedures described previously[1,2,7].

SDS-gel electrophoresis was performed in the E-C Vertical Gel Electrophoresis apparatus[10] (EC 474). Gel formulations and buffers were prepared mainly as described by WEBER AND OSBORN[4]. The gel buffer, diluted 1:1 with water, was used for both the upper and lower compartment of the apparatus. The protein sample was mixed with a small volume of glycerol and bromophenol blue tracking dye for analysis (usually 10 to 20 μl total volume).

Polymerization, prerun, sample application and electrophoretic run were performed as described in the "Operating Instructions" manual[11]. Electrophoresis was carried out for 5.5 h at 150 mA using a Shandon power supply. After electrophoresis, the gel slab was removed from the apparatus and it was photographed in a Chromato-Vue box (Ultraviolet Products, Inc.) using long wave ultraviolet light and polaroid black and white 4 × 5 Land film positive/negative (55 P/N). An exposure of 30 sec at f:8 was adequate to produce a negative of good contrast. A sheet of semi-logarithmic graph paper was also photographed without changing the lens to subject distance. The semi-logarithmic paper was identical to that used for designing the logarithmic sample slot former described below. The two negatives (gel and semi-log paper) were appropriately superimposed and printed on a Fotorite (Agfa-Gevaert) FP1 (contrast No. 4) paper using a photographic enlarger. A rapid print processor (Fotorite) produced a photograph in a few seconds.

For recording the migration patterns of non-labelled proteins, the gels after the staining–destaining procedures[3] were immersed in the original electrophoresis buffer for 1 h to diminish the effect of gel swelling[12]. The gels were then photographed with fluorescent light.

The sample slot former was designed to accomodate five unknown samples and five marker proteins of known molecular weight. The slots of the marker proteins were positioned at a specified distance from each other related to the logarithm of their molecular weight. This was accomplished as follows: Five marker proteins were chosen with molecular weights of 66,000 (bovine serum albumin); 46,000 (ovalbumin); 34,400 (carboxypeptidase A); 25,740 (chymotrypsinogen A); and 14,400 (lysozyme). The position of these numbers (M.W.) was marked on the logarithmic axis of a No.

340-1210 Dietzgen semi-logarithmic graph paper and served to indicate the center of the slots assigned to the marker proteins. The positions of the slots of the unknown proteins was marked at a convenient location. Each slot was assigned a length of 0.3 cm. The actual distance in centimeters between sample slots was measured with a vernier caliper. The sample slot former was subsequently custom made by E-C Apparatus Corporation to our specifications. A photograph of this device is shown in Fig. 1. Starting from the left side, slot formers No. 2, 4, 5, 7, and 10 accomodate the marker proteins whereas the rest form the sample slots of the unknown proteins.

Since the construction of the slot former is very inexpensive, several of these can be available in the laboratory designed for different marker proteins.

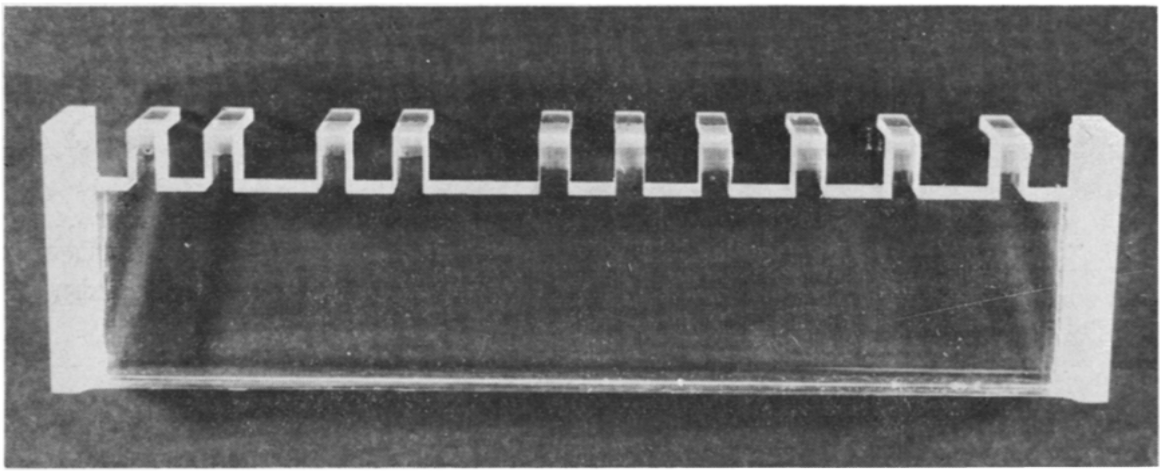

Fig. 1. Custom made sample slot former. Starting from the left, slot formers No. 2, 4, 5, 7, and 10 are positioned at a distance related to the logarithm of the molecular weight of five marker proteins. Slot formers No. 1, 3, 6, 8, and 9 form the slots for the unknown protein samples.

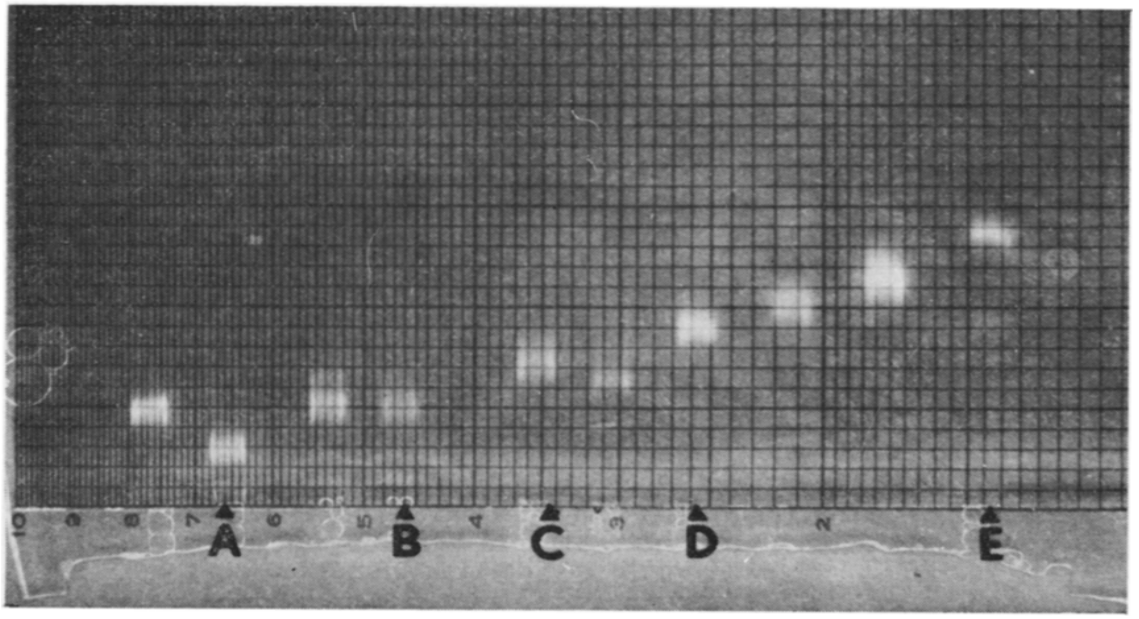

Fig. 2. SDS-polyacrylamide gel electrophoresis pattern of the DNS derivatives of the following proteins (starting from left): alcohol dehydrogenase, bovine serum albumin, aldolase, ovalbumin, carboxypeptidase A, pepsin, chymotrypsinogen A, trypsin, β-lactoglobulin, and lysozyme. A, B, C, D, and E show the positions of the marker proteins.

Results and discussion

A composite photograph of the SDS-gel electrophoresis pattern of the DNS derivatives of five "marker" and five "unknown" proteins projected through a semi-logarithmic screen is shown in Fig. 2. The pattern offers direct visualization of the relationship between electrophoretic migration distance and the logarithm of the molecular weight of the marker proteins. For estimation of the molecular weight of the "unknown" proteins, the mid-point of each band is located and the corresponding mobility in the standard curve is used to read directly the apparent molecular weight of this protein on the logarithmic scale.

Some of the advantages of this technique are that all the samples are subjected to electrophoresis under identical conditions, the unknown proteins are compared directly to a standard curve obtained directly with marker proteins, and calculations are greatly facilitated.

The recent description of a method that permits visual observation and photography of gels during electrophoresis[13], lends additional flexibility to the use of the logarithmic slot former.

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