ANALYSIS OF C^{14}-LABELED PROTEINS BY DISC ELECTROPHORESIS[1]

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Ornstein and Davis (1964) have introduced a method, called "disc electrophoresis", which yields extremely high resolution of proteins electrophoresed in cylindrical columns of polyacrylamide gel. By means of disc electrophoresis, a large number of protein components in a complex mixture can be separated and detected in a single operation. Bands of C^{14}-labeled proteins in disc electropherograms can be detected using techniques described by Heideman (1964); and Jovin, Chrambach, and Naughton (1964). This report presents an alternative procedure involving autoradiography of dried longitudinal gel slices. The method is relatively uncomplicated and can be used to develop the entire pattern of radioactivity in a gel without significant sacrifice of resolution.

Methods. The procedure described here was developed for application to gels containing 7.5% (w/v) acrylamide polymerized in 8 mm o.d. tubing. Disc electrophoresis was carried out as described by Ornstein and Davis (1964)[4] with minor modifications.

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4 Davis suggests numerous modifications of the method originally described in a preprint distributed by Distillation Products Industries, Div. Eastman Kodak Co., Rochester, N. Y. In the experiments described in this report, disc electrophoresis was carried out using the earlier procedure.

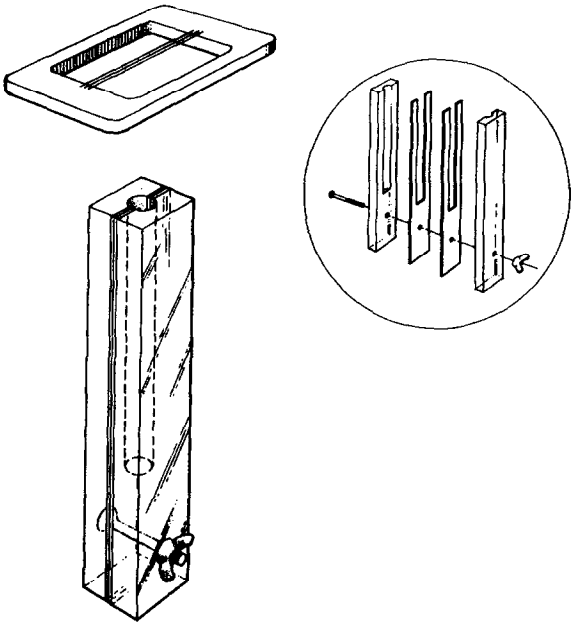

Figure 1: Apparatus for longitudinal slicing of cylindrical polyacrylamide gels. Over-all dimensions of plexiglass gel holder, 1 x 1 x 6 in.; cavity length, 4 in.; cavity diameter, 9/32 in. Wire separation and spacer thickness, 1/16 in.

After staining and destaining, gels are placed in the cavity of the plexiglass holder shown in Fig. 1. Each gel is sliced with a cutting device consisting of three 5 mil stainless steel wires stretched taut in a brass jig. When the wires are drawn through a gel column while it is firmly supported by the holder, four longitudinal slices are produced.

The two inner slices, 1/16 in. thick with two flat faces, are then dried onto filter paper. The bottom plate of an apparatus for suction filtration (Millipore Filter Corp. test filter holder; cat. no. NY 22 142 00) supports the filter paper and provides a means for supplying vacuum below. Gel slices to be dried are arranged on the wet filter and the entire array is covered with Saran wrap. A soft rubber gasket smeared with silicone lubricant makes a vacuum seal with the plastic film at the periphery of the filter support screen. When wrinkles have been smoothed in the covering wrap, vacuum is

applied to the filter apparatus and the wrap applies uniform pressure to the slices and filter paper. The slices thus receive mechanical support which, together with their adherence to the filter paper, prevents them from shrinking in length or width during drying. If the apparatus is heated by an infrared lamp from a distance of about one foot, drying takes one to two hours. The slices are reduced to thin, hard, smooth strips which adhere to the filter paper. (Many other drying procedures were tried, but all others caused gross distortions of the band patterns).

A home-made filtration apparatus has also been used successfully for drying gels. It has a rectangular filter stage cut from a sheet of porous, hydrophilic linear polyethylene (Bel-Art Products, Pequannock, N. J.; cat. no. F-1255). The bottom plate was machined from 1 in. thick linen-backed Micarta. A drying apparatus of this type is relatively inexpensive and has the additional advantage of the more convenient rectangular surface.

The pattern of radioactivity in the gels is developed by autoradiography of the dried slices. Kodak No-Screen x-ray film is used for this purpose because of its high sensitivity. Slices and films are clamped together between masonite plates secured with spring clips, and the assembly is wrapped in foil or stored in a light-tight box for the duration of the exposure. Exposed film is processed using Kodak x-ray developer and fixer as directed by the manufacturer.

Quantitative estimation of the intensity of bands in stained gels or autoradiographs has been carried out using a double beam recording microdensitometer. (Joyce, Loebl and Co., Ltd., Model E12 MK III).

Examples and Discussion. A number of experiments have been performed to investigate quantitative aspects of the autoradiographic technique. In one experiment, a series of gels was prepared containing various concentrations of uniformly dispersed C^{14}-labeled protein. In preparing these samples, the labeled suspensions were mixed with the acrylamide solution before polymerization -- no electrophoresis was performed. After the gels had been stained, sliced and dried, segments 10 mm long were cut from each dried slice and counted in a gas flow counter. The dried segments were then autoradiographed for various

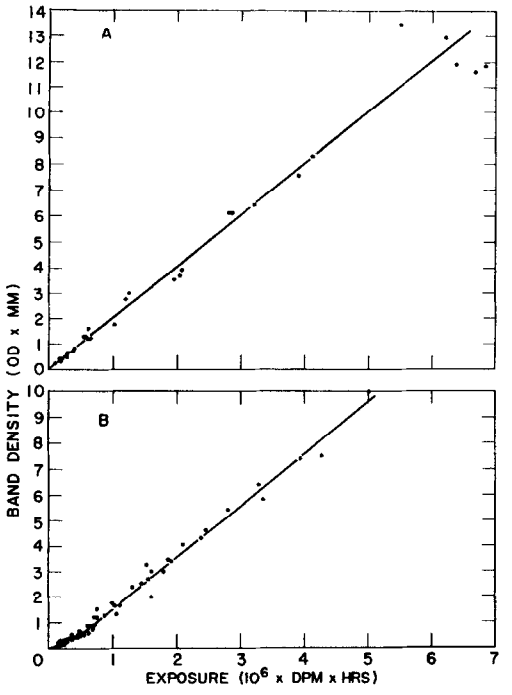

Figure 2: Calibration curves for autoradiographic measurement of band radioactivity in disc electropherograms. Exposure is calculated in (duration in hours) x (estimated dis/min in whole gel). (A) Film response to dried slices from 10 mm gel segments containing uniformly dispersed C^{14}-labeled protein not subjected to electrophoresis. Linear band density is (10 mm) x (measured optical density). (B) Response to bands of C^{14}-labeled hemoglobin. Linear band density in (mm) x (optical density units) is area under microdensitometer trace.

length of time, and the resultant film blackening was measured with the microdensitometer. Fig. 2A displays the results of this experiment, which, because it did not involve electrophoresis, constituted a test of the linearity and sensitivity of the film.

The data plotted in Fig. 2B are results of an investigation of the over-all efficiency and accuracy obtainable in making quantitative determinations on actual electropherograms. Measured amounts of purified C^{14}-labeled hemoglobin were electrophoresed, and a series of autoradiographs prepared with each dried slice. Band densities in the film were determined by measuring areas under microdensitometer tracings through the center of the bands. Ten dried slices in this series were counted in a gas flow counter: the number of counts which could be

detected at the face of a dried slice averaged 7.1% of the total acid-precipitable counts electrophoresed into the gel. The percentage for individual slices varied, however, from 4.7 to 8.8%. This considerable variability is not reflected in Fig. 2B, since exposures in terms of total counts in gels were estimated from counts in slices using the average value of 7.1% as the efficiency of detection. Differences in the residual water content of the dried slices is probably a major cause of

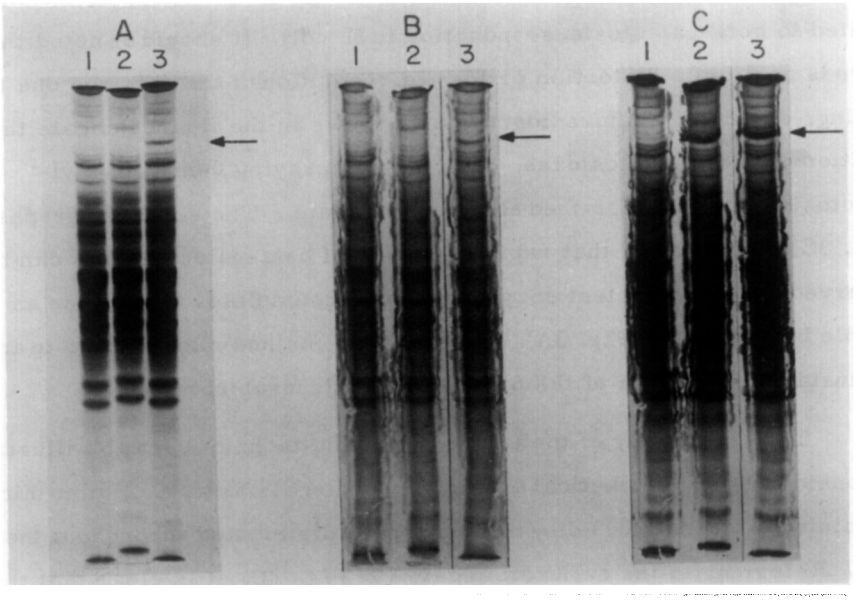

Figure 3: Disc electrophoresis of soluble proteins of E. coli.
(A) Stained gels, unsliced. (B) Dried slices from stained gels. (C) Autoradiographs of dried gel slices -- exposure 81 hours.

E. coli K 10 was grown on lactate at 37 °C to optical density (540 mμ) about 1.0; 90 ml of cell suspension was then transfered to each of three flasks. Each of the three cultures received a 4 min pulse consisting of 20 μC C^{14}-leucine of specific activity 160 mC/mM; 80 % was incorporated into acid-precipitable material during the pulse. Cultures 2 and 3 were induced for β-galactosidase by addition of isopropyl-β-D-thiogalactoside to 5×10^{-4} M. Culture 1 was an uninduced control. Onset of the pulse was 2 min after induction in Culture 2 and 25 min after induction in Culture 3.

Soluble protein extracts were obtained as supernatants after high speed centrifugation of sonicates of cells resuspended in 2 ml Tris-chloride buffer at pH 8 (10^{-2} M Tris, 10^{-3} M $MgSO_4$). To each gel was applied 0.1 ml of sample consisting of one part 2M sucrose in three parts extract containing 5×10^5 dis/min of acid-precipitable C^{14}.

Gels were stained with 1 % amido black in 7.5 % acetic acid. After destaining in 7.5 % acetic acid, the length of the small-pore gel was 98 mm. The direction of migration was downwards, toward the anode.

this variability. Variations in slice thickness also occur, but have been observed to have negligible effect. The latter observation is consistent with simple calculations which predict that a dried gel slice has an area density near infinite thickness for C^{14} beta radiation. Fortunately, one is usually satisfied with an estimate of the relative radioactivity in different proteins. This information can be obtained by scanning a single autoradiograph and is independent of differences between slices.

Fig. 3 presents a demonstration of the autoradiographic technique applied to beta-galactosidase induction in E. coli. It should be noted that there is negligible distortion or loss of resolution in the patterns due to slicing, drying, or autoradiography. Arrows in the figure indicate the position of beta-galactosidase, detected by spraying O-nitrophenyl-galactoside onto an unstained electropherogram. The autoradiographs (Fig. 3C) demonstrate that rapid synthesis of beta-galactosidase can be observed before sufficient enzyme protein accumulates to produce an easily visible band of stain (Fig. 3A). Clearly, the method may be used to analyze the instantaneous state of the protein synthetic machinery.

The senstitivity of the autoradiographic technique may be illustrated by considering an hypothetical example -- a discoid band, 0.25 mm thick, containing a total of 1000 dis/min uniformly distributed throughout the disc. Referring to the calibration curve in Fig. 2B, it is clear that the band would be visible, exhibiting an optical density of about 0.1, in an autoradiograph developed after exposure to a dried slice for a single day. This sensitivity is sufficiently high to make routine the detection and measurement of protein components which are synthesized relatively rapidly but which, due to turnover or transiency of synthesis, do not accumulate in sufficient quantity to be visualized by staining. Reports in preparation will describe application of the method in a study of the differentiation of chick lens tissue, and in experiments on bacteriophage maturation, effects of metabolic inhibitors, and the relative functional stability of different bacterial messengers.

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