Protein Purification by Elution Convection Electrophoresis

Abstract. *Purified protein components can be recovered from high-resolution gel-electrophoresis patterns by elution-convection electrophoresis. The protein components are all simultaneously eluted from the gel pattern by horizontal electrophoresis and are then simultaneously concentrated by electroconvection to recover the proteins in nearly the original sample concentration. Thirty individual protein fractions can be recovered in 3 hours from a sample containing 200 milligrams of protein.*

A number of attempts have been made to apply the high resolving power of gel electrophoresis to the preparation of purified protein components (*1*). The published techniques are based on some form of column electrophoresis in which the sample is applied as a single specimen to a column of gel and is carried through the column by electrophoresis. A mixture is resolved in the column, and successive individual fractions are carried off the end of the column by electrophoresis. The emerging components are swept off and collected in a stream of buffer solution flowing across the end of the column. These column techniques suffer from two major disadvantages: (i) a relatively long time is required to move the entire protein sample off the column, particularly when very slow-moving components such as γ-globulin are present; (ii) a relatively large volume of buffer solution is required to sweep up the protein emerging from the end of the column; individual fractions are therefore recovered in dilute solution and must be dialyzed and concentrated to recover them in a concentration approaching the initial value.

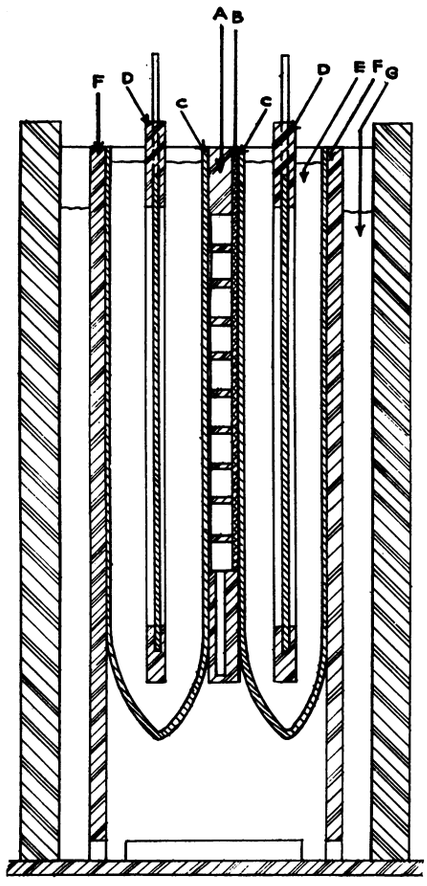

Fig. 1. Elution-convection cell, diagrammatic cross section. *A*, separating grid; *B*, gel slab carrying electrophoretic pattern; *C*, dialysis bags; *D*, electrodes; *E*, buffer level inside bags; *F*, cell supports; *G*, buffer level outside bags.

These difficulties can be overcome by the use of the apparatus and technique to be described. The technique comprises two successive steps.

1) A fully resolved pattern is developed in the usual way by electrophoresis on a gel matrix. An acrylamide gel is preferred (*2*) because it is technically easier to handle in the laboratory than starch gel, it has high resolving power, and it can carry a greater quantity of protein per unit volume. A vertical flat slab is used in preference to a cylindrical column because the flat slab, with a larger ratio of surface area to gel volume, affords closer control of temperature and also permits the excision of a segment from the pattern for staining before elution of the separated zones.

2) The fully developed gel-slab pattern is removed from the electrophoresis cell and replaced in the elution convection cell for elution and recovery of the separated protein components. The pattern is rotated 90 degrees before being placed in the cell so that the zones are aligned with vertical channels in the face of the separating grid. The spacing of the vertical channels is purely arbitrary, as it is impracticable to make a grid with spacings exactly corresponding to a specific protein pattern. Since the pattern must be eluted while the proteins are in the undenatured state it is normally not possible to see the zones. The recovered fractions are to some extent arbitrary, but they are spaced sufficiently close (3 mm per zone) so that no significant loss of resolution in the pattern is to be expected.

The protein zones are eluted by electrophoresis through the face of the gel slab. Each protein zone, therefore, has to travel only a short distance (less than 3 mm) before it emerges completely from the gel matrix. The emerging proteins are trapped in the vertical channels, which are sealed on one side by contact with the face of the gel slab and on the other side by contact with a sheet of dialyzing membrane which effectively bars the passage of the proteins. Within the vertical channel thus enclosed, a vertical convection current is set up by the mechanism of electroconvection (*3*). The elution cell, in effect, consists of a large number of microsized electroconvention cells (*4*) arranged in parallel on one face of the gel slab. In these electroconvection microcells the protein components are carried downward to the reservoirs at the bottom of the channels where they accumulate as concentrated protein solutions. The entire process of elution requires approximately 1 hour for proteins with the normal range of mobility of serum proteins at *p*H 8.0 to 9.0.

The elution convection cell comprises a vertical sandwich array of first electrode, first dialyzing membrane, gel matrix, separating grid with vertical channels, second dialyzing membrane, and second electrode. This array is positioned vertically in a plexiglas chamber filled with buffer solution. The buffer is circulated from one electrode to the other by means of an external

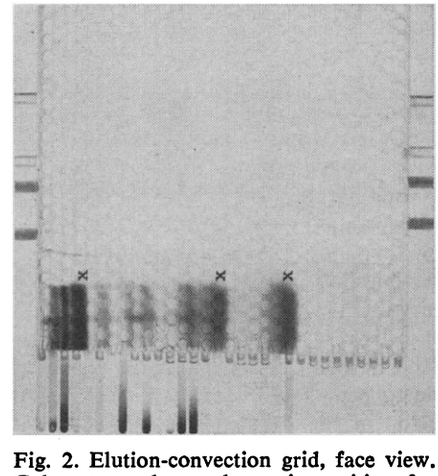

Fig. 2. Elution-convection grid, face view. Gel pattern above, shown in position for elution; collecting tubules below, containing components eluted from another strip of the gel pattern. The crosses mark the position of diffusable low-molecular-weight dye components.

pump which mixes the buffer solutions surrounding the two electrodes, thereby neutralizing changes in the *p*H or concentration of the buffer as a result of electrode reactions (*4*). The heat developed in the cell (*5*) by passage of the electric current is removed by cooling plates in the walls of the buffer chamber. The two electrodes are made of stainless steel, and their large area (10 × 10 cm) provides a reasonably uniform electric field through the face of the gel slab. The electrodes are placed in close proximity to the gel matrix and to the separating grid in order to decrease the heat load generated within the apparatus by the passage of the electric current. A relatively low voltage and high current are required (25 v at 200 to 500 ma) (Fig. 1).

There are certain necessary conditions and limitations for the apparatus to function effectively. First, the proteins must all migrate in the same direction through the gel and through the buffer solution. This, in effect, precludes the use of starch gel as electrophoresis matrix since some slow moving components, such as γ-globulin, which migrate toward the negative electrode in the starch gel matrix, migrate toward the positive electrode in the buffer solution. In such a case, the protein could never set up within the vertical channel the electroconvection currents necessary to concentrate the protein in the collecting reservoir at the bottom. Presumably a cell could be designed with electroconvection channels on both faces of the gel matrix to accommodate both forward and retrograde migrating proteins, but the additional complications in the design of such an elution cell seem to be greater than the useful applications such a cell would warrant.

Second, the individual proteins comprising the eluting zones must have a positive density increment in the buffer used. In other words, the protein-buffer solution must have a higher density than the protein-free buffer. If this condition is not met the eluted protein will tend to float to the top of the cell instead of descending by convection to the bottom. Again, it might be possible to provide collecting reservoirs at the top of the elution cell to meet this difficulty, although the area of usefulness of such a design would be extremely limited.

Finally, an important requirement is that the protein must be large enough to be nondialyzable, that is, it must not pass through the dialysis membrane used to form the base of the electroconvection channel. The usual Visking dialysis tubing effectively bars the passage of proteins whose molecular weight is 20,000 or more, so that this is not a serious limitation in the applications of the cell.

An example illustrating the use of this apparatus is presented in Fig. 2. The sample consisted of an artificial mixture of five colored proteins and three dialyzable dyes. A section of the gel pattern before elution is shown placed on the separating grid in position for elution. The protein components, collected from another section of the original gel pattern, are visible in the collecting tubules, while the dye components, having dialyzed through the barrier membrane, were not concentrated in the tubules corresponding to these bands. The original photograph clearly shows in two colors the separation of the two components on the left (closest to the origin) in the gel pattern.

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References and Notes

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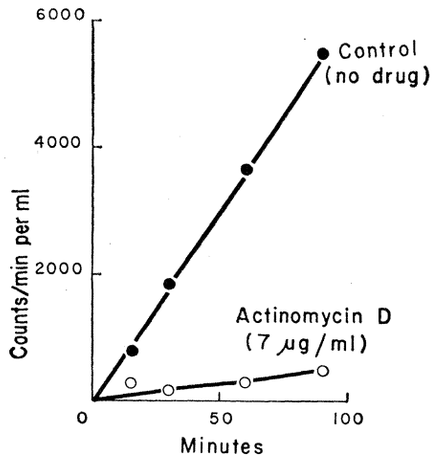
19 August 1964

Actinomycin Resistance in Bacillus subtilis

Abstract. *The incorporation of uracil-C^{14}, a precursor of cellular RNA, into whole cells of the sensitive parent strain* Bacillus subtilis *No. 6051 is completely inhibited by actinomycin D, whereas only partial inhibition of uracil-C^{14} incorporation occurs with actinomycin-resistant intact cells of* B. subtilis *strain II 15. Incorporation of uracil-C^{14} into protoplasts of the resistant strain of* B. subtilis *is completely inhibited. The sensitivity of resistant-cell protoplasts to drug effect clearly supports the conclusion that actinomycin penetration is altered in resistant intact whole cells. Since other experiments have shown that the antibiotic is not broken down in the presence of resistant cells, resistance is considered to result from a change in cell permeability.*

Actinomycin D has proved a useful tool in blocking RNA synthesis in many mammalian and microbial cell systems (*1*). It binds reversibly with the guanine moiety of DNA, the result being inhibition of DNA-dependent RNA-polymerase (*2*).

Little is known concerning the chemical and biological reactions occurring in the emergence of resistance to actinomycin D in any biological system susceptible to this antibiotic. Goldstein *et al.* (*3*) and Journey and Goldstein (*4*) characterized a HeLa line resist-



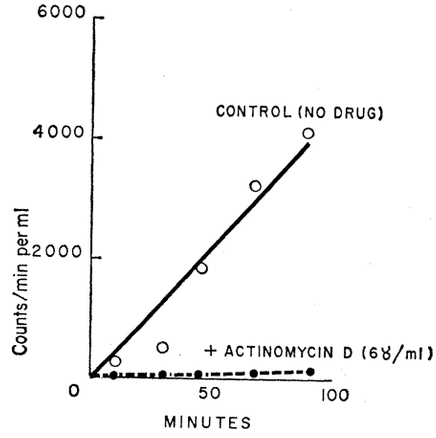


Fig. 1 (left). Uracil-2-C^{14} incorporation into *B. subtilis* strain 6015/S in the presence and absence of actinomycin D. Fig. 2 (right). Uracil-2-C^{14} incorporation into protoplasts of *B. subtilis* strain 6051/S in the presence and absence of actinomycin D.