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Note

Isoelectric point electrophoresis: a new technique for protein purification

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Purification of a given protein from a complex mixture usually requires a series of purification steps based on different principles of separation. Petrilli *et al.*[1] reported one isoelectric chromatographic method using ion-exchange resins. Preparative electrophoresis techniques often serve as a useful step in protein purification. Electrophoretic methods previously described[2,3] are based on differing rates of protein movement through the gel and then elution of the desired product. Our aim was to demonstrate a new technique based on a unique principle, electrophoresis performed at the isoelectric point (p*I*) of the protein to be purified. Theoretically, this protein should not migrate under these conditions and most of the contaminants should move into the gel, leaving a purified product in the original slot. We will describe a simple test of this principle, showing that it is possible to obtain a considerable purification of cytochrome *c* from a complex mixture in only 25 min.

MATERIALS AND METHODS

The apparatus (Fig. 1), consists of a rectangular plastic box with electrodes at each end. The center area has a block of 5% polyacrylamide (Cyanogum 41, Fisher Scientific, Pittsburgh, Pa., U.S.A.) gel made with the sample buffer and containing a slot for the sample. As shown in Fig. 1, gel separates the sides and bottom of the slot from the container.

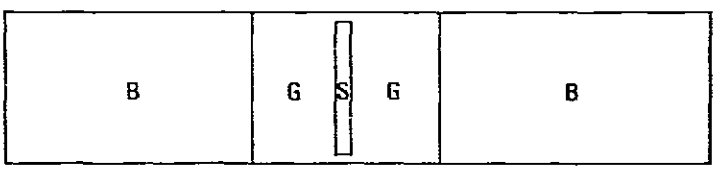

Fig. 1. Electrophoresis apparatus: B is buffer, G is gel, and S is sample slot. Box dimensions are 21.5 cm × 5.5 cm × 5 cm deep. The gel is 5.5 cm × 5.3 cm × 2.1 cm deep. The slot is 4.4 cm × 0.3 cm. The electrodes are placed at each end of the container.

We prepared, as samples, two artificial test mixtures, TM1 and TM2. TM1 contained 2 mg/ml of horse cytochrome *c*, Sigma type III (protein to be purified), and 5.7 mg/ml of horse heart extract previously centrifuged at 105,000 *g* for 2 h (con-

taminants) in 0.02 *M* cyclohexylaminopropane sulfonate (CAPS) buffer, pH 10.00. TM2 contained 0.2 mg/ml of the same cytochrome *c* and 3.2 mg/ml of bovine serum albumin (Calbiochem, Los Angeles, Calif., U.S.A.) in 0.05 *M* NaOH–glycine buffer, pH 10.55.

It is known that the observed p*I* of a protein depends on the temperature and buffer used[4]. We could not use the p*I* values for horse cytochrome *c* (9.0 and 9.4) published in the literature[5] because they were measured in a different buffer system. A specific identification test for the protein to be purified was required to determine its p*I* value in the given buffer. Using the apparatus described above, the direction and rate of migration of cytochrome *c* into the gel was measured at various pH values, and the approximate p*I* was estimated. The final p*I* value was obtained by trial and error as the pH at which the protein did not migrate. We determined the p*I* values for cytochrome *c* in 0.02 *M* CAPS and 0.05 *M* NaOH–glycine buffers as pH 10.00 and 10.55, respectively at 23°.

Electrophoresis was carried out as follows: the appropriate buffer was placed in both the buffer chambers (B) (Fig. 1) and slot (S), and preelectrophoresis was performed at 200 V and 60 mA for 10 min at room temperature. Then the buffer in B was replaced with fresh buffer and that in S was replaced with TM1 or TM2. Electrophoresis was performed at 200V and 50–60 mA for 25 min at room temperature (23°), and the product was removed from S. Product and standards for TM1 were examined by slab gel electrophoresis in 7.5% polyacrylamide gel with 0.05 *M* Tris–HCl buffer, pH 9.0, and for TM2 by spectrophotometry at 280 and 550 nm.

RESULTS AND DISCUSSION

Fig. 2 shows that the product from TM1 (channel 3) no longer contains most of the contaminant proteins which were present in the horse heart extract and move toward the anode (channels 1 and 4). The cytochrome *c* yield was more than 60%.

Sample TM2, purified with the NaOH–glycine buffer was freed of 97% of the contaminant protein with a cytochrome *c* yield of 64%. These results were obtained in spite of the fact that the pH of the NaOH–glycine was at the very limit of its buffering capacity. In similar experiments, cytochrome *c* yields of 70–80% were obtained.

Contaminant proteins with p*I* values close to that of cytochrome *c* as well as very-high-molecular weight substances, which may still remain, can probably be removed by other techniques. Excessively long periods of electrophoresis reduce the yield of the product, presumably because of a pH change. An apparatus with a continuous flow of buffer through the electrode chambers should overcome this problem. The maximum sample volume treated by this technique is correlated with the slot capacity and the current density (A/cm^2) used. Both can be increased in a large apparatus provided with an appropriate cooling system. In theory, this technique should separate proteins similar in p*I* with an apparatus designed to hold the pH and buffer temperature constant, and with a longer electrophoresis run. In practice, it is hard to predict the degree of resolution obtainable, but the results reported here employing a primitive apparatus are indeed promising.

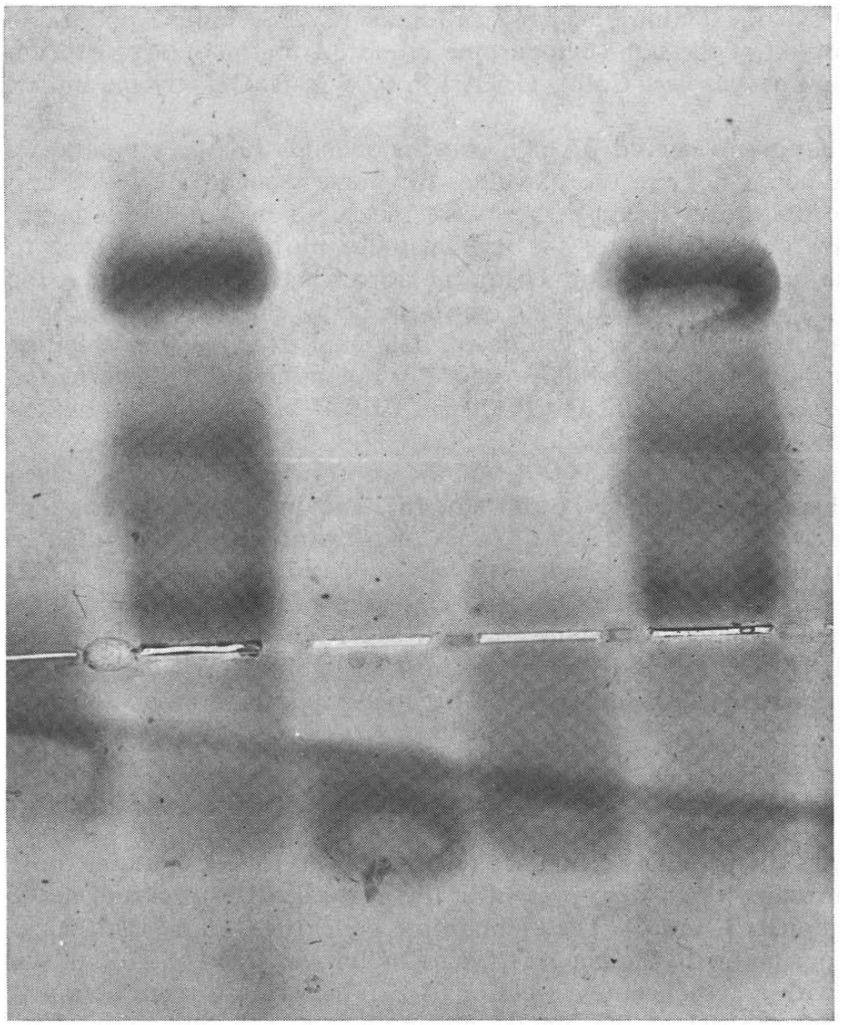

Fig. 2. Analytical polyacrylamide gel electrophoresis of product from TM1 and standards after protein staining. From left to right: channels 1 and 4 are horse heart extract without added cytochrome *c*; channel 2 is horse cytochrome *c*; and channel 3 is the purified product. Electrophoresis was performed for 4 h at 13 V/cm. The anode is at the top of the figure, and samples were placed into the central slots. The diagonal line visible in the bottom section of the gel is an artifact.

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