Analysis for Purity: Crystallization

10.1 Electrophoretic Analysis

Resolution of separate components of a protein mixture is achieved most clearly using an electrophoretic method. As discussed in section 6.2, preparative electrophoresis has major problems despite its theoretical potential, and is consequently not often used. But in an analytical mode, electrophoresis is a most widely used method; indeed, it is almost obligatory to characterize a purified protein preparation by an electrophoretic technique. Analytical electrophoresis in a gel system requires only 5–25 μg protein (or less with sensitive silver-staining techniques); this is rarely a significant proportion of what is available. Before gel systems were developed, electrophoretic analysis was carried out in the Tiselius free-boundary apparatus, requiring tens of mgs of protein. This did not resolve closely similar proteins, and analyzing a single sample required a great deal of effort and attention. Paper and other cellulose-based supports were introduced for zone analytical electrophoresis, which eliminated two of the disadvantages of the Tiselius apparatus; only small amounts of protein were needed, and the technique was easy, requiring only simple equipment. But resolution is scarcely any better even on the superior modern cellulose acetate strips, because separation is based only on a rough charge/size ratio; many proteins move together as a single peak (cf. section 6.2).

When Smithies (178) used starch gel as a support medium, an immediate improvement in resolution was achieved. As a result of the small pore sizes in the gel (compared with paper, agar gels, and other materials that had been used up till then), large molecules were retarded. This is a result of the apparent increase of viscosity; effective viscosity becomes dependent on molecular size. Moreover, diffusion is lessened, so very sharp zones could be
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Figure 10.1. Disk gel electrophoresis system. Each protein component resolves as a disc in the tube of polyacrylamide gel.

found even after overnight electrophoresis. A few years later, Ornstein (179) introduced a synthetic gel medium, cross-linked polyacrylamide, which is more controllable than starch and has some other desirable advantages. Polyacrylamide gel electrophoresis was originally associated with “disk gel electrophoresis,” because the system was promoted in which samples are run in individual tubes of gel, resolving into “disk” of protein zones (Figure 10.1). Although still widely used, disk gel electrophoresis has largely been replaced by slab gels, on which several samples can be run simultaneously with direct comparison of mobility. Thin-slab gel electrophoresis, using polyacrylamide, has been developed extensively over the past few years, and the technical problems associated with pouring and with sample application have been resolved, so that the system is as simple as, but more powerful than, disk gels. A variety of commercial apparatuses are available, using gel thicknesses down to less than 1 mm (Figure 10.2). One advantage of a thin gel is that less heat is produced per square centimeter of gel surface for a given applied voltage. Also, during staining and destaining of the protein bands diffusion of dye is more rapid into the thin gel slab.

There are at least five distinct electrophoretic procedures using polyacrylamide gel as the medium:

Simple Electrophoresis

This involves running the sample in a buffer at a pH where the proteins remain stable and in their native form. This method was the original procedure, making use both of differences in charges between proteins and their different sizes. The buffer chosen depends somewhat on the nature of the proteins, but generally it is slightly alkaline, in the pH range 8–9, where most proteins are negatively charged and so move toward the anode. The anode is normally at