Electrofocusing and protein detection and isolation

S. LEWIN

M.Sc., Ph.D., F.R.I.C.

Physical Biochemistry Laboratory,

Waltham Forest Technical College, London, E.17

PROTEINS are generally least hydrated and least soluble at their respective isoelectric points. In this connection the process of electrofocusing of proteins (Svenson, 1962; Vesterberg & Svenson, 1966) is relevant. In this method several proteins are electrophoretically propelled—through an electrophoretically established pH gradient—until they reach the particular pH regions which equal their respective isoelectric points; there they remain stationary as their individual electrophoretic propulsive potentials disappear. In their electrofocused isoelectric pH regions, the individual proteins will be at their respectively least soluble conditions; and provided that the individual concentrations exceed their respective isoelectric point solubilities, precipitation should take place. (It should be noted here that the particular solubilities may differ somewhat depending upon the type of medium employed, e.g. ordinary aqueous solution or high density aqueous sucrose medium, or highly capillary medium such as acrylamide gel, where both buoyance and protein hydration may affect the solubility values. Also, the solubility, or suspendability, of a colloid should be affected when it is aligned in an electric field.)

Protein separations and identification have been carried out using vertical columns of aqueous media in which the pH gradient is supported by a sucrose gradient; the presence of a protein can then be detected by using a fraction collector and appropriate tests, e.g. spectrophotometric. However, in this method, precipitation is undesirable because it interferes with separation and detection. Acrylamide gels have also been used for electrofocusing (Dale & Latner, 1968; Leaback & Rutter, 1968). However, such gel electrofocusing is normally followed by fixation of the protein on treatment with trichloroacetic acid solutions followed by suitable staining. These procedures are necessary because the quantities of the proteins used, e.g. serum, are usually too low to result in the respective solubilities being exceeded at the particular isoelectric points. So far as the requirements for purification by precipitation are concerned, concentrations higher than those

normally used would be necessary, when the proteins should appear as white precipitated bands at their respective isoelectric points. It must, however, be emphasized that the use of excessive quantities of proteins could well result in increase in respective band widths to an extent in which band fusion would result thereby making clean separation impracticable.

FIG. 1. The application of the thesis to acrylamide gel electrofocusing of several preparations of insulin. (A) Purified pork insulin (ten times recrystallized); (B) purified beef insulin (ten times recrystallized); (C) insulin B.P.; (D) Insulin Zinc Protamine; (E) Insulin Zinc Semilente; and (F) Insulin Neutral Actrapid.

We have applied the principle to various protein mixtures. Fig. 1 shows the application of this thesis to acrylamide gel electrofocusing of several preparations of insulin. Seven per cent acrylamide gels were

prepared by photopolymerization in tubes of *ca.* 0.4 cm internal diameter in a similar manner to that proposed by Dale & Latner (1968). The Ampholine (pH gradient 3–10; LKB, Sweden) concentration was *ca.* 0.8% and 5×10^{-2} mg of insulin were used in each tube. Purified beef insulin (ten times recrystallized) appeared as one band (though microscopic examination suggested the possibility of two merged bands) while purified pork insulin (ten times recrystallized) gave two clearly separated bands. Several commercial insulin preparations precipitated themselves in the form of two or more white bands already after the first 3 hr. In most cases the bands sharpened effectively and were fully stabilized prior to the standard 16-hr run. The photographs show the respective bands obtained after 16 hr, without any staining, when viewed against a dark background.

The significance of the existence of more than one insulin band as well as relevant experimental results using other proteins will be considered elsewhere.

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