isoelectric focusing

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ISOELECTRIC FOCUSING

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生物化学与分子生物学实验室技术　第5卷　第2部分
«等电聚焦»

这一部分详细介绍了等电聚焦在分离和分析蛋白质中的应用技术。内容丰富。可以供生物化学、分子生物学、及医学临床分析等工作者参考。

共五章，目次：①等电聚焦的理论及基本方面，②制备等电聚焦，③分析等电聚焦，④常用实验方法，⑤等电聚焦的应用。

This book is the pocket-edition of Volume 5, Part II, of the series 'Laboratory Techniques in Biochemistry and Molecular Biology'.
Volume 5 of the series contains the following parts:
Part I Techniques of sample preparation for liquid scintillation counting, by Brian W. Fox
Part II Isoelectric focusing, by P.G. Righetti and J.W. Drysdale

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Isoelectric focusing

To Professor H. Rilbe
father of the 'fine art' of isoelectric focusing

LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

Edited by

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Contents

Contents

Theory and fundamental aspects of isoelectric focusing

1.1. Introduction

Electrophoretic mobility has long been used as basis for separating and characterising proteins. The resolving power of conventional electrophoresis is limited, however, and characterisation by mobility is not unique because of its dependence on the composition, pH and ionic strength of the medium. The development of isoelectric focusing (IEF) represents a major advance in the field of electrophoretic separations of proteins and other amphoteric substances. Isoelectric focusing is essentially an equilibrium electrophoretic method for segregating amphoteric macromolecules according to their isoelectric points in stable pH gradients. The method offers unique advantages for both analytical and preparative procedures.

Isoelectric focusing has now been refined to the point where one can display all components whose isoelectric points differ by as little as 0.01 pH unit. Such exquisite resolution is not normally obtainable by other procedures based on charge separations such as electrophoresis or ion exchange chromatography. With these latter methods, specially tailored conditions usually have to be devised for particular separations. By contrast, the built-in resolution of IEF allows the separation in a single experiment of all components with measurably different isoelectric points. IEF is, therefore, a more definitive technique for examining charge heterogeneity and is particularly suitable for differentiating closely related molecules. By the same token, IEF

Subject index p. 387

is also a rigorous test of homogeneity. In addition, because molecules are concentrated during separation by IEF, the technique lends itself to preparative as well as analytical purposes. Finally, IEF defines an important parameter, the isoelectric point (pI), which gives information about the composition of macromolecules and also allows a rational approach to other experimental manipulations.

The principle of IEF is outlined in Fig. 1.1. A stable pH gradient increasing progressively from anode to cathode is established by electrolysis of carrier ampholytes in a suitable anticonvective liquid medium. When introduced into this system, a protein or other amphoteric molecule will migrate according to its surface charge in

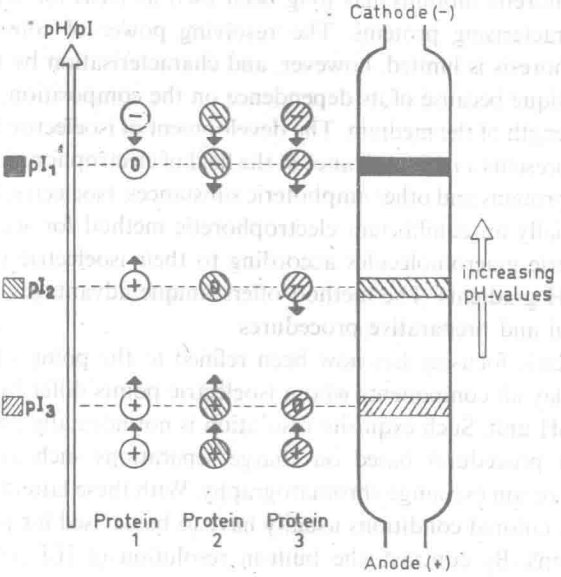

Fig. 1.1 Principle of IEF. Three amphoteric molecules, represented as pI_1, pI_2 and pI_3, migrate to their respective isoelectric points in a stable pH gradient generated by electrolysis of carrier ampholytes in an anticonvective medium. Each species will eventually reach an equilibrium zone where its net electrical charge is zero, i.e. its pI (Courtesy of LKB Produkter AB).

the electric field. Should its initial charge be positive, it will migrate towards the cathode into regions of higher pH. As it does so, the molecule will gradually loose positive charges and gain negative charges, e.g. through deprotonation of carboxyl or amino functions. Eventually, it will reach a zone where its net electrical charge is zero, i.e. its pI. Should the molecule migrate or diffuse away from its pI, it will develop a net charge and be repelled back to its pI. Thus by countering back diffusion with an appropriate electrical field, a protein or other amphoteric macromolecule will reach an equilibrium position where it may be concentrated into an extremely sharp band. As might be expected, the degree of separation of two ampholytes is a function, among other parameters, of the slope of the pH gradient in which they are focused. The shallower the pH gradient, the better is the separation (Fig. 1.2). This principle is discussed more fully later (§1.3.6).

IEF was originally developed as a preparative technique. Fractionations were conducted in sucrose density gradients which served as an anticonvective medium to stabilize the pH gradient and focused protein zones. Experiments were usually performed in column volumes of 110 or 440 ml and usually required 2–4 days to reach equilibrium. These systems were, however, rather expensive in time and required careful standardization. They also suffered from some practical problems arising from isoelectric precipitation and excessive diffusion during elution of the column in the absence of the electrical field. Finally, sample detection was laborious and often difficult. Consequently, these early systems were not convenient for routine analytical procedures. Fortunately, many of the problems inherent in IEF in sucrose density gradients have now been overcome with the use of more suitable anti-convective media such as polyacrylamide gel or Sephadex beds. With these media many of the potential advantages of IEF for high resolution separations of amphoteric substances can be realised. The methodology for IEF in gels has now been fairly well standardized for both analytical and preparative procedures. Lastly, IEF in gels is a remarkably forgiving technique in which a minimum of technical skill and effort is amply rewarded in the quality of the fractionations achieved.

Subject index p. 587