Capillary Electrophoresis of Proteins

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Preface

This book evolved from a review of capillary electrophoresis (CE) of proteins that was published in *Advances in Chromatography* in 1996. We recognized that although a literature review was timely and needed in this growing field, there was no comprehensive practical guide for researchers interested in applying CE to protein analysis. Our own experience had shown us that simply reading published research papers was necessary but not sufficient to achieve successful results in the laboratory. To fill this gap in the published information on capillary electrophoresis and to aid practitioners with limited expertise in CE of proteins, we undertook preparation of this work.

Capillary Electrophoresis of Proteins is designed to be both a reference and a handbook. It is organized by separation mode (zone electrophoresis, isoelectric focusing, and sieving) with discussions on separation principles, method development, and optimization. Practical details of buffer preparation, capillary selection and handling, and troubleshooting problem separations are emphasized. For readers who are new to the area of capillary electrophoresis, introductory chapters on the basic principles of capillary electrophoresis and CE instrumentation are included. Where appropriate, step-by-step methods are provided. The literature review we published in 1996 has been updated in this book to provide a current overview of published applications with 386 references. The book is intended to serve as an introduction to protein CE and as a useful reference for scientists applying CE to protein analysis on a regular basis.

Much of the practical information in this work originates from our experience in the capillary electrophoresis group at Bio-Rad Labora-

tories over the last decade. In addition to the development of CE instrumentation and separation methods, we worked continually with scientists in the field to apply CE to real-life bioanalytical problems. This book represents a distillation of our experience, which we hope will be beneficial to practicing scientists in both academic and industry environments.

Tim Wehr
Roberto Rodríguez-Díaz
Mingde Zhu

Capillary Electrophoresis of Proteins

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Introduction

Separation technology has been central to the elucidation of protein structure and function. Both chromatography and electrophoresis have been used for decades to isolate and characterize proteins and their components. Open column and low pressure chromatography were essential for the purification of sufficient amounts for protein for structural analysis, and the preparation of high-resolution ion exchange resins enabled development of the amino acid analyzer which paved the way for analysis of protein composition. The introduction of protein-compatible ion exchange and size exclusion supports allowed high-performance liquid chromatography to be used for obtaining highly pure proteins, while high-efficiency reversed phase columns proved invaluable for high-resolution peptide mapping and purification, and for identification of the PTH amino acids generated by automated Edman sequence analysis. Gel electrophoresis evolved in parallel with chromatography, providing an inexpensive method for separating complex protein mixtures. The development of the Laemmli system for separating SDS-protein complexes on polyacrylamide gels proved to be such a powerful technique that it is used on a daily basis in virtually every protein chemistry laboratory in the world. Isoelectric focusing provided an alternative separation technique for separation of proteins based on their isoelectric points, and the combination of IEF and SDS-PAGE by O'Farrell resulted in a two-dimensional separation technique which has the power to resolve thousands of proteins on a single 2-D gel. The development of blotting techniques to transfer separated proteins from the gel to a

suitable support for other analyses such as immunoassay or sequencing greatly simplified many experiments in protein chemistry.

As powerful as these protein separation techniques are, they are not without limitations. Chromatographic separations are based on interaction of an analyte with the surface of the stationary phase. However, proteins are by nature very surface-active molecules. They also possess low diffusion constants and display poor mass transfer kinetics during the chromatographic process. As a consequence, resolution is often less than desired, protein recovery may be low, and native proteins may be denatured during the separation. Gel electrophoresis, on the other hand, is a laborious and time-consuming technique requiring preparation of the gel, separation of the sample, staining and destaining, and gel-drying. The gel must be treated with a dye or stain to visualize the separated proteins and because the uptake of stain may occur in a nonlinear fashion, the intensity of the stained bands may be poorly correlated with amount of protein. For this reason, gel electrophoresis is, at best, a semiquantitative technique.

Capillary electrophoresis (CE) is a relatively new separation technology which combines aspects of both gel electrophoresis and HPLC. Like gel electrophoresis, the separation depends upon differential migration in an electrical fied. Since its first description in the late1960s, capillary electrophoretic techniques analogous to most conventional electrophoretic techniques have been demonstrated: zone electrophoresis, displacement electrophoresis, isoelectric focusing, and sieving separations. Unlike conventional electrophoresis, however, the separations are performed in free solution without the requirement for a casting a gel. As in HPLC, detection is accomplished as the separation progresses, with resolved zones producing an electronic signal as they migrate past the monitor point of a concentration-sensitive (e.g., UV absorbance or fluorescence) detector. Therefore the need for staining and destaining is eliminated. Data presentation and interpretation is therefore also similar to HPLC; the output (peaks on a baseline) can be displayed as an electropherogram and integrated to produce quantitative information in the form of peak area or height. In CE and HPLC, a single sample is injected at the inlet of the capillary and multiple samples are analyzed in serial fashion. This contrasts to conventional electrophoresis in which multiple samples are

frequently run in parallel as lanes on the same gel. This limitation of CE in sample throughput is compensated by the ability to process samples automatically using an autosampler. Compared to its elder cousins, CE is characterized by high resolving power, sometimes higher than electrophoresis or HPLC. The use of narrow-bore capillaries with excellent heat dissipation properties enable the use of very high field strengths (sometimes in excess of 1000 V/cm), which decreases analysis time and minimizes band diffusion. When separations are performed in the presence of electroosmotic flow (EOF), the plug-flow characteristics of EOF also contribute to high effciency. In contrast, the laminar flow properties of liquid chromatography increase resistance to mass transfer, reducing separation efficiency.

Because of its many advantages, CE shows great promise as an analytical tool in protein chemistry. In some cases it may replace HPLC and electrophoresis, but more often it is used in conjunction with existing techniques, providing a different separation selectivity, improved quantitation, or automated analysis. An anticipated benefit of performing protein separations in open-tubular capillaries was the reduced potential for surface interactions. In fact, this proved not to be the case; the high surface-to-volume ratio of the capillaries and the high surface activity of the fused silica capillary wall has proven to be a major problem in applying CE to protein separations. Much of the research in separation chemistries and capillary wall modifications has been directed toward improving CE performance in protein separations.

This book is designed to provide guidelines for separating proteins using CE. It has been compiled from a review of the published literature and from experience gained in the authors' labs over the last several years. It is written with an eye to the practical aspects of protein CE, and covers each of the major separation modes. Specific applications are included to illustrate the various methods as applied to different classes of proteins and different separation problems. A discussion of the basic principles of capillary electrophoresis is provided in Chapter 2 for readers less familiar with the field.