Methods in Molecular Biology

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Capillary Electrophoresis of Proteins and Peptides

Methods and Protocols

Edited by

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Preface

Peptides and proteins represent an important class of biomolecules which play essential roles in living organisms by regulating or controlling many biological processes. At the same time, they display very diverse functions, acting as hormones, substrates, neurotransmitters, immunomodulators, enzymes, coenzymes, receptor ligands, transporters, and toxins. Proteins and peptides are also very important biopharmaceuticals, and their therapeutic use requires an extensive structural characterization as well as multiple quality controls of these complex drugs.

This book provides a comprehensive survey of recent developments and applications of high performance capillary electrophoresis in the field of protein and peptide analysis. The focus is given to the analysis of intact proteins. (Glyco)proteins can also be analyzed as fragments (peptides, glycopeptides, oligosaccharides, monosaccharides) after their enzymatic or chemical cleavage, and this would represent another set of analytical strategies and topics that have not been considered in this book. This book covers different modes of capillary electrophoresis (CE) useful for protein and peptide analysis, CZE, CIEF, ACE, CGE, and different types of application such as the quality control of therapeutic proteins and monoclonal antibodies, clinical analyses of chemokines in tissues, qualitative and quantitative analysis of vaccine proteins, and determination of binding constants in complexes involving peptides or proteins.

CE is a powerful separation technique; it brings speed, high resolution, automation, and low consumption of samples and buffers. However, the separation of proteins by CE is often complicated by their tendency to adsorb onto the negatively charged surface of fused silica capillaries. This occurs primarily via coulombic interaction but can rapidly get amplified by the unfolding of the adsorbed proteins which can in turn participate, through a cooperative effect, to adsorb other proteins involving at this stage all kind of molecular interactions (hydrophobic, dipolar, hydrogen bounding, etc.). Different strategies, with different levels of success, can be employed to circumvent these issues. They may rely on simply working at extreme pHs (acidic or alkaline) or increasing ionic strengths of the buffers to more drastic solutions that entail dynamic or permanent capillary coatings.

The first part is devoted to detection methods employed in CE for proteins and peptides, a topic as important as the separation itself. Proteins can be easily detected with UV detection in CE; however, to achieve more sensitive detection, laser-induced fluorescence detection may be preferable. Even if proteins have tryptophan residues that possess intrinsic fluorescence properties, most of the sensitive applications require the derivatization of peptides or proteins with fluorophore dyes. Another way to achieve high sensitivity is to use mass spectrometry (MS) as a detector. The number of applications using the coupling of CE to MS has significantly increased these last 10 years. CE-MS indeed combines the high separation efficiency of CE with the possibility of mass detection and analyte characterization through MS-MS. Besides, the interfacing technology has considerably evolved proposing, at present configurations with or without addition of sheath liquid, to maintain the electrical continuity required for the electrophoretic separation. It remains that not all CE separation conditions are compatible with MS, but researchers are progressing, trying to
push the current limits of CE-MS. CIEF combined with MS is possible, while this coupling presents major challenges as the compatibility between the separation medium, which contains anticonvective gel and ampholytes, and MS detector.

The second part provides the readers with the latest breakthroughs and improvements in CE. This part encompasses many contributions showing that CE is an evolving technique which is still very active in providing innovations and new solutions to circumvent protein adsorption, to increase detection sensitivities or specificities. The recent advances have mainly been focused into two directions: the sample pretreatments online to the separation and the integration of electrophoretic processes into microchips. Sample treatment is often required for real biological samples either to eliminate interfering compounds, matrices, or to enrich the sample with the protein present at a too low concentration if body fluid or tissues are studied. Monoliths which can be easily synthetized into capillaries are among the solid supports that rank amongst the most adapted for online sample pretreatments.

Part 3 highlights different recent applications in the field of quality control of therapeutic proteins. This part is fully illustrated by protocols dealing with recombinant proteins such as growth hormone, insulin, plasma-derived proteins such as human serum albumin as well as monoclonal antibodies. Those applications are proposed for formulated pharmaceutical preparations, and this can complicate the analytical development of the CE method due to the presence of specific excipients in those formulations aimed at protecting the active proteins from degradation or ensuring a longer or controlled release of the drug.

Finally, Part 4 illustrates quite specific applications of CE analysis in the field of vaccine proteins, or peptide/alkali metal ion complexes, showing that CE can be applied to very different areas in health and therapeutics and even to give more insight on the way proteins are acting or interacting.

This book is useful for a wide audience, including researchers, technicians, and students; it can also be a reference for experienced researchers as well as for beginners and newcomers in this field. Indeed, besides specific methods fully detailed, several important principles related to protein analyses by CE are briefly noted in several chapters (separation modes, capillary coatings, chemical and physical protein degradation ways, CE modes for intact glycoprotein analysis, methods for coupling solid phase extraction to CE, methods for pre-capillary, in-capillary, or post-capillary derivatizing proteins, CE-MS coupling).

As editor and co-editors of this book, we would like to thank all the chapter contributors who made the editing of this book possible by their excellent work covering quite exhaustively the current and most active topics of CE for peptides and proteins.

*Châtenay-Malabry, France*  
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