
Preface

Glycoconjugates such as glycoproteins and glycolipids play important roles in cell–cell interaction events, including development, differentiation, morphogenesis, fertilization, inflammation, and metastasis. A number of reports have documented the association of unique oligosaccharide sequences to protein targeting and folding, and in mechanisms of infection, inflammation, and immunity. For glycoproteins, these glycan appendages are the result of extensive co- or post-translational modifications of the nascent proteins in the endoplasmic reticulum and in the Golgi apparatus. Although nucleic acids and proteins are copied from a template in a repeated series of identical steps using the same enzymes, complex carbohydrates are formed by the sequential actions of cellular glycosyltransferases that specifically recognize unique substrates. The molecular biology of these transferases and other carbohydrate-modifying enzymes is providing important insights on oligosaccharide recognition events. While it is acknowledged that the definition of the protein complement of cells and tissues (the so-called proteome) remains an enormous task in this postgenomic era, the characterization of all glycans produced by individual organisms (referred to as the glycome) presents an equally important challenge. This task is further complicated by the fact that oligosaccharides cannot presently be cloned.

These complex carbohydrates exist in a staggering diversity of structures, linkages, and branching, thus providing an exquisite molecular repertoire for cellular interactions. In view of the challenges facing the carbohydrate chemist, the further understanding of the structure–function relationship of these glycoconjugates begins with the availability of analytical tools enabling their identification and quantitation. Obviously, progress in this area has been impeded by the structural resemblance existing between isomeric carbohydrate residues and closely related variants conferring on them similar physical and chemical properties. The recent developments in high-resolution separation techniques based on capillary-scale chromatography and electrophoresis have played a pivotal role in deciphering the structural intricacies of these complex biomolecules. Currently, capillary electrophoresis (CE) is one of the most efficient methods for the separation of complex carbohydrates, and excellent procedures exist for the analysis of free and conjugated mono- and oligosaccharides. This field of research has matured significantly over the past two

decades, and it is thus timely that a volume describing protocols for their analysis by CE be presented in this series of *Methods for Molecular Biology*.

All contributors to *Capillary Electrophoresis of Carbohydrates* are well-experienced scientists working in the field of glycoanalysis, and the volume is designed to be a practical companion not only to well-trained glycobiologists, but also to beginners in this field. This volume is separated into five parts with an introductory chapter describing the structural and functional diversity of glycoconjugates. In Part II, protocols for sample preparation prior to CE separation are described in Chapters 2 to 4. Cell membranes are typically composed of glycoproteins and glycolipids, two types of complex carbohydrates in which sugars are covalently bound to proteins and fatty acids, respectively. The use of endoglycopeptidases (Chapter 2) offers a convenient approach to the release of glycans from their corresponding glycoproteins, while preserving their structural integrity. These enzymatic products can be subsequently derivatized with reagents to introduce a charge and a chromophore on neutral oligosaccharides in order to facilitate their CE separation and detection with visible or fluorescent detectors. A list of common derivatization reagents together with reliable procedures are presented in Chapter 3. Proteoglycans, which are important extracellular matrix components and chemical-signaling molecules of animal cells, are composed of glycoaminoglycans (GAG), an unbranched polysaccharide chain comprising repeating units of disaccharide residues. Although the release of these polysaccharides is often difficult to obtain in high yields, some eliminases cleave specific linkages of GAG residues, resulting in unsaturated oligosaccharides that provide valuable structural information of the original glycoconjugate. Chapter 4 describes a series of procedures for the preparation of such oligosaccharides.

The third part of *Capillary Electrophoresis of Carbohydrates* summarizes the separation of mono- and oligosaccharide by CE. Different separation formats are available in CE including capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), capillary isotachopheresis (CITP), capillary gel electrophoresis (CGE), and micellar electrokinetic chromatography (MEKC). CZE is one of the most common separation formats used for the analysis of carbohydrate derivatives, and several applications of this technique are presented in Chapters 5 and 7. The analysis of carbohydrates as borate complexes using CZE and MEKC separation modes are given in Chapter 6. Chapter 8 demonstrates the practical use of affinity electrophoresis using lectins for the separation of oligosaccharides. The analysis of the unsaturated anionic oligosaccharides derived from GAG of proteoglycans is presented in Chapter 9.

The analysis of glycoconjugates in their native state or following minimal chemical or enzymatic treatment is described in Part IV of this volume. Most glycoproteins show microheterogeneity in the N- or O-linked glycan chains appended to the peptide backbone. This glycoform distribution can be monitored using high-resolution separation techniques such as CIEF and CZE with buffer modifiers. The analysis of biologically relevant glycoproteins using these separation formats is described in Chapters 10 to 12. The on-line coupling of CE to mass spectrometry (CE-MS) has also played an important role in the separation and characterization of glycoconjugates. Chapter 13 presents the application of this technique for the monitoring of intact protein glycoforms and for probing the site of glycan attachment in tryptic glycopeptides using specific mass spectral scanning functions. In Chapter 14, a unique application of CE-MS is demonstrated for the separation of closely related glycoform and isoform families in bacterial glycolipids based on their unique molecular conformation and ionic charge distribution.

CE can also be applied to other challenging analytical problems such as the characterization of enzymatic activities of glycosyltransferases as presented in Chapter 15. Another example of application of CE is its use for the determination of association constants (Chapter 16). Since diverse biological functions of carbohydrates can be ascribed to the specific binding of these ligands to proteins, accurate measurements of association constants provide insights toward the further understanding of their structure-function relationships. The last chapter of this volume is dedicated to this important topic. Finally, the appendix describes the structures of the most commonly encountered carbohydrate residues and oligosaccharides from mammalian and bacterial origins.

Capillary Electrophoresis of Carbohydrates is intended to be a practical guide for the analyst contemplating the separation of complex carbohydrates by CE. As such, it is not intended to be a comprehensive survey of analytical tools for the characterization of glycoconjugates. Other reference documents, such as vol. 14, *Glycoprotein Analysis in Biomedicine*, and vol. 76, *Glycoanalysis Protocols*, 2nd edition, both edited by Elizabeth F. Hounsell and published by Humana Press, can provide valuable information to the carbohydrate analyst. Selected examples of applications were chosen by our contributors to illustrate the analytical merits of different electrophoretic techniques. Special attention was given to details on reagent, apparatus, and procedures to provide the reader with all information required to initiate similar investigations. The editors are most thankful to all contributors for their patience and their thoughtful consideration. Also, we owe a special debt of gratitude to Dr. Walker for careful edito-

rial comments and suggestions, and to our publisher for continued support throughout the evolution of this project. We hope that our readers will find in this modest contribution all relevant experimental details to set forth on a fascinating, analytical journey into glycobiology.

Pierre Thibault
Susumu Honda