

Book review

Methods on glycoconjugates. A laboratory manual, Edited by André Verbert, Harwood Academic Publishers, Switzerland 1995. ISBN 3-7186-5514-4 hardbound, ISBN 3-7186-5617-5 paperback, 215 pp.

This book dedicated to Professor Bernard Fournet is 'a guide for the first steps in the field of glycoconjugates'. It deals with the general techniques essential for the determination of *N*-glycan and *O*-glycan structure as well as for the metabolism of glycoproteins. However, 'Methods for glycoproteins' would have been a more appropriate title as other glycoconjugates are hardly addressed.

The book starts with a well illustrated overview of the structure and conformation of glycoproteins which underlines the importance of the primary structure of glycans for the study of the functions and metabolism of glycoproteins. It mainly reviews protein *N*-glycan and *O*-glycan structures and stresses their tremendous heterogeneity. The book is divided into 8 sections covering monosaccharide analysis, release of glycans, separation of glycans, structural analysis by chemical methods, use of lectins, structure determination by physico-chemical and biochemical methods, immunochemical probes and metabolism. Each section contains two or three chapters.

This first section explains colorimetric methods for the quantification of neutral monosaccharides, uronic acids, hexosamines or sialic acids and analysis by gas liquid chromatography of trifluoroacetylated monosaccharides, trimethylsilylated monosaccharides or alditols acetates. It also covers different HPLC techniques used in monosaccharide analysis. The techniques for obtaining and derivatization of monosaccharides are generally well explained, but information for the interpretation of chromatograms is missing, notably the retention times of the main monosaccharide derivatives and correspondence of the different columns that can be used. The preparation of methanolic HCl requires specialised material and therefore the author could have mentioned that it can also be purchased.

Three chapters constitute the section 'Release of glycan moieties'. Different methods for obtaining and purifying glycopeptides from a glycoprotein are first described. However, there is no advice on choice of proteolytic enzyme, separation method or type of detection. Techniques are not always clear. For instance, in the UV-determination of peptide liberation, the wavelength at which the products are detected is not stipulated. Moreover, protocols start with 100 mg to 1 g of protein, a quantity not often available. In the following chapter, however, mechanisms of hydrazinolysis and alkaline hydroly-

sis for the release of oligosaccharides from glycoprotein or glycopeptides and the degradations that occur during these reactions are explained in very clear schemes. The enzymatic cleavage of *N*- and *O*-glycosidic linkages is also well documented, with the specificity of the main enzymes (summarised in a table) and their susceptibility to detergents, glycerol, etc.

The third section presents the separation of fluorescently labelled glycans by Biogel P4 chromatography and native glycans by thin layer chromatography. It then covers the separation of native, acidic and neutral oligosaccharides, fluorescently labelled derivatives, benzoylated oligosaccharides or *N*-alkyl-para-amino benzoate derivatives by various HPLC techniques. The set of well chosen and very useful techniques include normal phase chromatography, anion exchange chromatography, reverse phase chromatography and high pH anion exchange chromatography. Finally, the last chapter introduces a less common technique, that of supercritical fluid chromatography of oligosaccharides.

Periodate oxidation, Smith degradation, nitrous acid deamination and methylation are briefly explained in the fourth section. These chapters are far too succinct. The nitrous acid deamination, for example, is often used when studying glycosyl-phosphatidylinositol (GPI) anchors and this would deserve at least a paragraph. In addition, in a book dedicated to Prof Fournet, the Paz Parente procedure for permethylation should be mentioned, despite being more complicated and less frequently used.

The next section is in effect a mini-review on lectins. Lectin specificity, use of immobilised lectins for glycoproteins, glycopeptides or oligosaccharides fractionation and use of radioiodinated lectins, peroxidase- or digoxigenin-labelled lectins for the detection of glycoproteins are explained in detail. With 30 pages and more than 100 references, this is the most developed chapter of the book.

Nuclear magnetic resonance spectroscopy (NMR), mass spectrometry or exoenzymes are indispensable tools for the study of glycan structure. The first chapter tries to explain briefly the basis of carbohydrate NMR and gives a few examples that can be used as references. The chapter on mass spectrometry is particularly interesting. One disappointment, however, is the complete absence of electro-spray ionisation (ESI).

Finally, a study of biological processes is also included in this book. Neoglycoconjugates can be immobilized on an insoluble matrix and used to study interactions between carbohydrates and lectins, receptors or antibodies. A section is devoted to the preparation of such 'Immunological probes' and their use in binding assays. Principles of coupling of carbohydrate to lipid or to protein are depicted with nicely introduced protocols. In the section 'Metabolism', the authors first indicate the biological phenomena (sugar interconversion, degradation of the precursor, turnover of glycoconjugates, etc.) to take into account when performing metabolic labelling of cell culture or glycosyltransferase assays. They then detail the glycosyltransferases of the phosphodolichol pathways and those involved in the processing of *N*-glycans. In this pedagogic section, difficulty gradually increases, allowing the reader to follow easily.

In conclusion, this is an erratic book in which detailed chapters appear alongside brief ones; splendid illustrations appear alongside unreadable figures without legends. It is regrettable that there are unintentional mistakes (eg. elution of compounds from a CarboPac column with methanol, page 44) and numerous mistranslations which sometimes make the text incomprehensible. An abbreviation list should also been included.

However, the book has the merit of describing various experimental pitfalls and tricks to succeed. This original feature is the result of many years of testing by experienced researchers. I particularly appreciate the schemes that describe the chemical mechanisms of the presented methods and facilitate their understanding. The hard cover ring binder helps access whilst working at the bench.

Despite everything, the book is a worthy acquisition which after a few improvements (further editions will follow) will become indispensable to anyone who wants to enter the fascinating field of glycoconjugates, and also to scientists who need to be aware of the recent advances in analytical techniques.

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