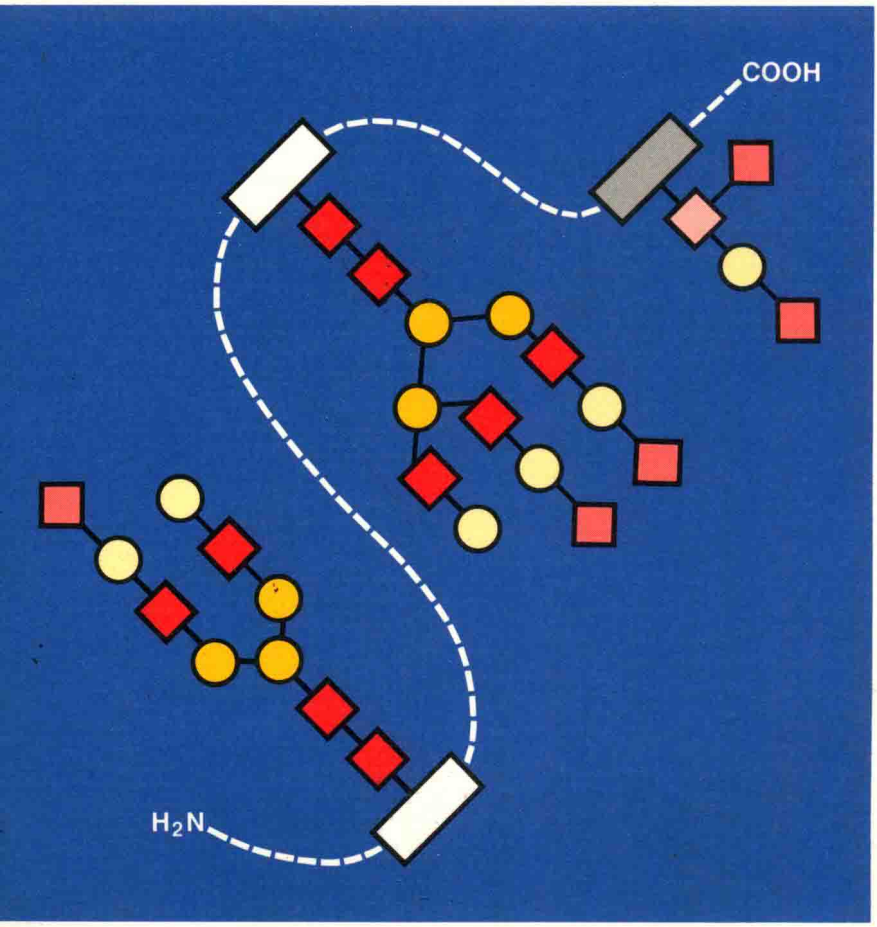
GBF Monographs Volume 15

Protein Glycosylation: Cellular, Biotechnological and Analytical Aspects

Edited by
H.S.Conradt



VCH

GBF Monographs Volume 15

Protein Glycosylation: Cellular, Biotechnological and Analytical Aspects

Edited by
H.S.Conradt

Contributions to the International Workshop on Protein Glycosylation June 28 to 30, 1990 Braunschweig, Germany

VCH

Dr. H. S. Conradt
Gesellschaft für
Biotechnologische Forschung mbH
Mascheroder Weg 1
D-3300 Braunschweig
Federal Republic of Germany

Published jointly by
VCH Verlagsgesellschaft mbH, Weinheim (Federal Republic of Germany)
VCH Publishers, Inc., New York, NY (USA)

Copy Editor: Dr. J.-H. Walsdorff, Gesellschaft für Biotechnologische Forschung, Braunschweig
Responsible for the contents: The contributors

Library of Congress Card No.: 91-24528

British Library Cataloguing-in-Publication Data:
Protein glycosylation: cellular, biotechnological
and analytical aspects. – (GBF monographs)
I. Conradt, H.S. II. Series
547.7
ISBN 3-527-28367-6

Deutsche Bibliothek Cataloguing-in-Publication Data:
Protein glycosylation : cellular, biotechnological and analytical
aspects ; contributions to the International Workshop on
Protein Glycosylation, June 28 to 30, 1990, Braunschweig,
Germany / ed. by H. S. Conradt. – Weinheim ; Basel
(Switzerland) ; Cambridge ; New York, NY : VCH, 1991
(GBF monographs ; Vol. 15)
ISBN 3-527-28367-6 (Weinheim ...)
ISBN 1-56081-184-6 (New York)
NE: Conradt, Harald S. [Hrsg.]; International Workshop on Protein
Glycosylation <1990, Braunschweig>; Gesellschaft für
Biotechnologische Forschung <Braunschweig>: GBF-Monographien

Printing: betz-druck gmbh, D-6100 Darmstadt 12. Bookbinding: Verlagsbuchbinderei Kränkl, D-6148 Heppenheim. Cover design: Graphik-Design Zisowsky & Partner, Braunschweig.
Printed in the Federal Republic of Germany

GBF Gesellschaft für
Biotechnologische Forschung mbH

Distribution:
VCH, P.O. Box 101161, D-6940 Weinheim (Federal Republic of Germany)
Switzerland: VCH, P.O. Box, CH-4020 Basel (Switzerland)
United Kingdom and Ireland: VCH (UK) Ltd., 8 Wellington Court, Cambridge CB1 1HZ (England)
USA and Canada: VCH, Suite 909, 220 East 23rd Street, New York, NY 10010-4606 (USA)

ISBN 3-527-28367-6 (VCH, Weinheim)
ISBN 1-56081-184-6 (VCH, New York)

ISSN 0930-4320

Protein Glycosylation:
Cellular, Biotechnological and Analytical Aspects

Preface

Glycoproteins (i.e. proteins containing covalently bound carbohydrate) are ubiquitous constituents of all living organisms including bacteria. The posttranslational modification of polypeptides with carbohydrate groups is very common for secretory as well as integral membrane proteins of higher organisms which may function as enzymes, antibodies, hormones, structural or carrier proteins and receptors.

Over the past two decades the principal biosynthetic pathways leading to the final carbohydrate structures of glycoproteins have been elucidated. The introduction of improved techniques such as high-resolution NMR and fast atom bombardment mass spectrometry as well as the introduction of novel chromatographic techniques for oligosaccharides over the past decade have expanded our knowledge of the enormous microheterogeneity of oligosaccharide structures that can be present at even a single glycosylation domain. The biological significance of this structural diversity seen in glycoproteins is unclear.

Recombinant DNA technology has permitted the efficient production of many biologically important glycoproteins (membrane receptors as well as their ligands) by expression in heterologous mammalian cell lines. By using defined glycosylation mutant cell lines as hosts as have been derived from CHO and BHK cells (see paper by P. Stanley, this volume) it should be possible to obtain pure glycoproteins of defined carbohydrate structures. The study of the biological functionality of these glycosylation forms will considerably increase our understanding of the role of protein linked oligosaccharides.

Pharmaceutical companies' interest in the production of clinically important human proteins (many of which are glycoproteins) by biotechnological means, will undoubtedly have an impact on the development of glycoprotein biochemistry in the near future. The efforts of the pharmaceutical industry are directed toward human medicine, and many clinically useful glycoproteins (immune-modulators, differentiation factors, glycoprotein hormones and receptors) are now available from recombinant sources. They should be used to develop our understanding of biological phenomena associated with protein linked carbohydrates. However, only a multidisciplinary approach including molecular structure research, computer graphic model building as well as genetic engineering and cell biology is likely to be successful.

The present volume evolved from a workshop held at the GBF in Braunschweig in June 1990 with the aim of bringing together a balanced mixture of people from university settings whose interest runs from basic science to the possible practical application of their research, i.e. including researchers from industrial laboratories with strong biotechnological interest.

I thank the GBF administration, especially Sabine Peters, for help in running the workshop. My special thank goes to all speakers, chairpersons and contributors to the book. The professional help of Dr. J.-H. Walsdorff in editing this volume is gratefully acknowledged.

Braunschweig, January 1991 Harald S. Conradt

Contents

List of Authors

A. Cellular Aspects

Processing and Re-Processing of Asparagine-linked Oligosaccharides

R. C. Hughes

National Institute for Medical Research
Mill Hill, London NW7 1AA

Summary

The assembly of asparagine-linked oligosaccharides in glycoprotein biosynthesis is cell specific, polypeptide specific and glycosylation site specific. Recombinant glycoproteins produced in non-homologous cells are likely to be glycosylated abnormally and the consequences on protein stability, conformation and biological activity need to be considered. Although the major pathways of assembly of asparagine-linked oligosaccharides are identified, their regulation during biosynthesis is not understood. The early events in oligosaccharide processing catalyzed by glucosidases I and II and specific mannosidases are particularly complex. Experiments using various inhibitors of processing glucosidases and mannosidases as well as structural analysis of processing intermediates, show that different processing pathways are selected for assembly of glycans substituted at specific sites in glycoproteins. New mannosidases are being described that participate in these diverse pathways. A novel mannosidase of rat liver is concentrated in endosomes as well as the cis Golgi compartment and may play an additional role in remodelling of glycoproteins that occurs during internalisation and recycling of cell surface glycoproteins.

1. Introduction

The increasing application of recombinant DNA technology for manufacture of therapeutic glycoproteins using large scale cell culture has raised general awareness of the importance of carbohydrate composition on glycoprotein stability, activity and pharmacodynamics. The carbohydrate moieties of glycoproteins form a major part of the total molecular mass (1) and carry distinct functions among which are protection of proteins against proteolytic and immunological attack, induction and maintenance of protein conformation and stability, recognition and association with viruses or bacteria and other pathogens, recognition in intercellular adhesion and recognition in hormone action and growth control. The rapid increase in interest in the last few years has paralleled improvements in analytical techniques for the characterization of large carbohydrate structures, techniques that increasingly are

becoming instrument-led by use of FAB-MS and high resolution NMR, and a better understanding of glycan biosynthesis although as I shall discuss later, many blank areas need to be mapped. Nevertheless, even at this stage it seems likely that the controlled modification of the carbohydrate moieties of glycoproteins will be an exploitive approach for development of improved, secondgeneration recombinant products. Certainly, careful consideration of the consequences of producing recombinant glycoproteins in heterologous cells which may or may not have the same glycosylation potential as the homologous cell type seems sensible.

2. N-Glycosylation of Proteins

Mammalian cells produce glycoproteins with four main types of carbohydrate chains: high mannose, hybrid and complex asparagine-linked N-glycans and serine- or threonine-linked O-glycans. In this paper I shall discuss only the N-glycans.

Protein N-glycosylation is cell specific, polypeptide specific and glycosylation site specific. This means firstly that glycosylation of any protein is determined by the cell in which it is produced. Secondly, it implies that a polypeptide encodes information that direct its own pattern of glycosylation. These controls are by no means rigid and in general there can be considerable heterogeneity in the carbohydrate side chains, particularly with respect to chain terminating groups, many of which are highly antigenic and are targets for physiological uptake systems in the circulation. Nonetheless, controls do exist which need to be considered for the production of recombinant glycoproteins in heterologous cells.

Fig. 1 shows an imaginary polypeptide containing four consensus Asn.X Ser (Thr) sequences for N-glycosylation, three of which are glycosylated. Initiation of N-glycosylation is catalysed by transfer co-translationally of a preformed oligosaccharide from a lipid linked intermediate catalysed by oligosaccharyl transferase within the limen of the endoplasmic reticulum (ER). The transferase is active with synthetic peptides containing the Asn.X Ser(Thr) triplet (2) and it is still unclear why some potential sites for glycosylation are consistently not utilized during glycoprotein biosynthesis. Probably conformational restrictions apply to limit recognition of the triplet sequence by the oligosaccharyl transferase *in vivo*.For example, the triplet could simply be buried in a folded or partially folded nascent polypeptide chain or could be in an inappropriate conformation. By analogy with the latter point, it is known that the triplet sequence Arg.Gly.Asp functions as a common recognition sequence for many receptors interacting with extracellular matrix molecules such as fibronectin, fibrinogen, laminin and various collagens (3). Yet the receptors in general display unique binding specificity for a particular matrix component or subset of components. For example, the $\alpha_5\beta_1$ integrin receptor for fibronectin

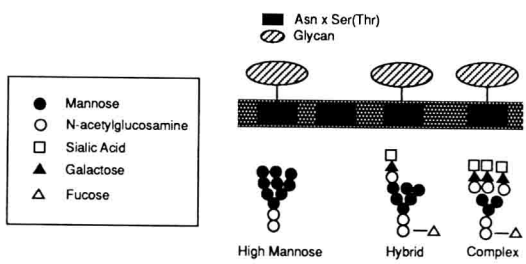


Fig. 1. N-glycosylation of a polypeptide. Four potential sites for N-glycosylation are shown, three of which are substituted by unique classes of oligosaccharide structure.

which binds to an Arg.Gly.Asp containing sequence in fibronectin has no affinity for the same triplet in laminin or collagen. This behaviour has been explained by differences in the conformation and hence presentation of the triplet to the receptor in the folded matrix proteins (3).Conceivably, similar constraints could operate to limit the recognition of potential glycosylation sites by oligosaccharyl transferase *in vivo*, provided glycosylation proceeds after at leastsome folding of the nascent polypeptide chain during biosynthesis.

Similar conformational constraints may be involved as an explanation for another common feature found in multi-glycosylated proteins, illustrated in Fig. 1. Usually it is found that the pattern of glycosylation is site-specific. Although all asparagine-linked oligosaccharides begin as the same defined structure, subsequently during assembly this precursor is processed into the various main classes of N-glycans as discussed in the next section.

Many of these aspects of control of N-glycosylation are nicely shown by a transfection experiment carried out by Sheares and Robbins (4). In this experiment, a cDNA for the chick ovalbumin gene was introduced into heterologous mouse L cells (Fig. 2). Ovalbumin contains two potential glycosylation sites, only one of which is utilized in the homologous chick oviduct cell producing this glycoprotein.

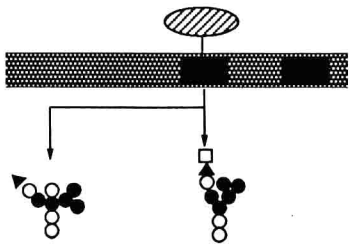


Fig. 2. Glycosylation of ovalbumin in heterologous mouse L-cells. A plasmid containing ovalbumin genomic DNA was introduced into L-cells and the carbohydrate structures of the product determined for comparision with those of the natural glycoprotein.

The same is true for the transfected gene in L-cells. Furthermore, the structures of the N-glycans produced in either chick oviduct or in L-cells are broadly similar, in each case being a hybrid-type oligosaccharide. The exact structures obtained reflect differences in the glycosylation potentials of the two cell types: mouse L-cells lack the enzyme N-acetylglucosaminyl transferase III (GlcNAc T III) and hence the N-glycan lacks the bisecting GlcNAc residue found in ovalbumin produced in chick oviduct which is rich in this enzyme. Conversely, the chick cells lack sialyl transferase and only the N-glycan produced in L-cells contains sialic acid. Despite these differences, the pattern of processing at the defined glycosylation site in the two very different cell types is remarkably similar, strongly supporting a role of the polypeptide itself in directing the assembly of its sugar residue.

3. Processing Reactions

Recent evidence summarized in Fig. 3 has shown that oligosaccharide processing is much more complex than previously thought: *in vivo* it seems increasingly likely that several independent pathways are operating simultaneoulsy to maximize the heterogeneity of oligosaccharide structures found in glycoproteins.