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Review

Glycoaffinity chromatography and biological recognition

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Abstract

The potential of bioaffinity chromatography as a tool for study of biological recognition mechanisms is gaining increasing recognition. Biochromatographic methods allow the separation of proteins according to both the structure of their polypeptidic chain and their post-translational modifications. Among the various post-translational modifications which proteins undergo, glycosylation has conducted to the development of original methods (glycotechnologies). This review discusses the applications of glycotechnologies in bioaffinity chromatography, and particularly the use of biochromatography to elucidate mechanisms involved in glycobiology.

Keywords: Reviews; Biological recognition; Glycoaffinity chromatography; Proteins

Contents

1. Introduction

The interaction between proteins capable of binding glycoconjugates and carbohydrates is quite stimulating as sugars are the postulated mediators for the transmission of biological information by forming specific recognition codes [1–3]. Owing to the progress in understanding the importance of glycoconjugates in many biological interactions, the development of carbohydrate-based technologies (or

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Table 1
Broad spectrum applications of glycotechnology

Applications
1. Glycoaffinity chromatography
2. Blotting technology (affinoblotting)
3. Solid-phase affinoassays
4. Bioaffinity sensors
5. Affinity cytochemistry (cytological glycoprobes)
6. Glycohistochemistry
7. Flow cytometry
8. Production and characterization of recombinant glycoproteins
9. Production and characterization of monoclonal antibodies to tumor oligosaccharides
10. Diagnosis (isoenzymes, use of glycoprobes, etc.)
11. Carbohydrate-mediated targeting (glycodrugs delivery)
12. Selective retrieval or elimination of cell populations
13. Prevention of interactions between bacterial lectins and cell receptors
14. Basic studies of cellular functions (adhesion, development, apoptosis, etc.)

glycotechnologies) is being intensively pursued. Table 1 summarizes the diverse fields of application of these glycotechnologies. Among this broad spectrum of technologies glycoaffinity chromatography allows the purification and the study of two interacting components: a carbohydrate-binding protein (lectin, antibody, etc.) and a glycoligand. The interaction of these two components is due to the aptitude of a particular protein domain (carbohydrate recognition domain, CRD) to bind specifically to a certain sugar sequence in oligosaccharides and glycoconjugates. The abundance of lectins in plants and the solubility of these proteins have helped to make plant lectins immobilized on various supports the most widely used category of tools for the purification of glycoproteins, glycopeptides and oligosaccharides to date [4–6]. Moreover, affinity adsorbents derived from these lectins are useful not only for preparative chromatography but also for other purposes such as analysis and diagnosis. In addition, glycosylated polymers are used as models of glycoproteins and glycolipids, and as affinity supports to purify and identify complementary proteins from tissue extracts [7–9]. In this paper, biospecific adsorbents obtained by immobilization of carbohydrates or lectins are considered as models that may be used advantageously for studies of mechanisms involved in glycobiology. Since this short review attempts to give a general view of the applications of glycotechnologies in affinity chromatography, the readers are encouraged to refer to recent reviews, if necessary [10–12].

2. Use of soluble glycosylated polymers

Since glycoconjugates and glycans have been shown to interact with many biological systems both in vivo and in vitro, a great deal of attention has been focused on the development of polymer- or microsphere-bound glycosidic ligands [13–17]. In principle, a glycosidic ligand should retain its selective interaction after attachment to a polymeric material, despite some limiting factors arising from the heterogeneity introduced by the polymer. Therefore, a large variety of glycopolymers have been designed and their behavior toward different carbohydrate-binding proteins have been investigated [14,15,18–20].

2.1. Synthesis of chemically defined multivalent polymers

The conjugation of a carbohydrate to a polymer and its subsequent presentation is of fundamental importance. Ligand presentation in glycobiology is very important as both the number and the proximity of carbohydrate structures can significantly modulate binding affinity. The linkage type can also affect the interaction of glycosylated polymers with carbohy-

drate-binding proteins in solution as well as in solid-phase [9,21].

Many functional polymers have been prepared from polyacrylamide because of its ability to yield soluble as well as solid derivatives and its chemical stability. Numerous investigations on the reactivity of these materials with plant lectins [18,20,22] have employed water-soluble copolymers prepared by copolymerization of acrylamide, allylamine and an allyl glycoside [16] (Fig. 1A). The sugar content of the polymer can be varied by changing the amount of allyl glycoside used in the copolymerization reaction. These polymers were designated as poly(glycosyloxyallylaminoallylacrylamide) copolymers, or poly(*O*-glycosylacrylamide) copolymers (COPs). The efficiency of the linked sugars in comparison with that of the corresponding free sugars, tested for their ability to inhibit hemagglutination, is markedly increased (500-times more efficient for mannose derivatives [22]). The increased inhibitory potencies of linked sugars are consistent with their enhanced affinities being due to the presence of multiple binding residues in individual molecules which statistically increases their probability of binding and their sterical arrangement (cluster effect). Moreover, modification of the free amino groups of COP with biotin- or with fluorescein-isothiocyanate (FITC) yielded soluble copolymers very useful for the detection of lectins or saccharide-binding proteins.

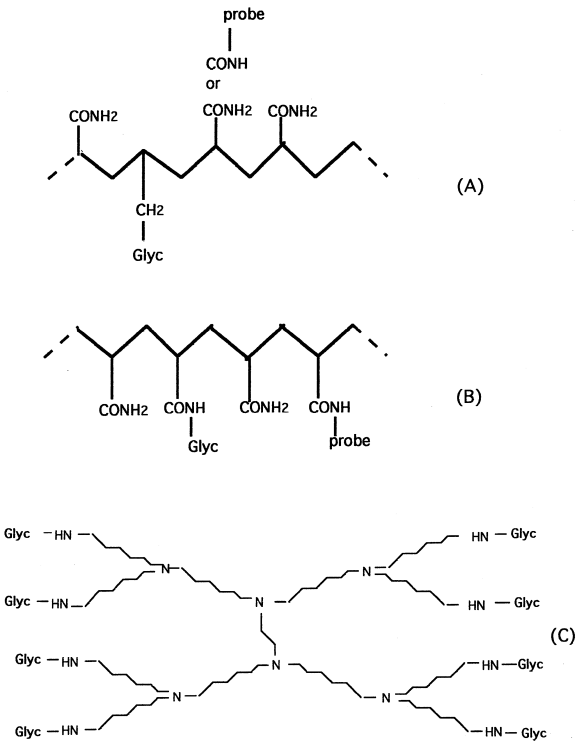

Fig. 1. Schematic representations of glycopolymers prepared from acrylamide. Sugar–polyacrylamide copolymers synthesized according to Refs. [16] (A) and [23] (B). (C) Glycodendrimers [17]. Glyc represents the glycosylated ligand.

A second group of sugar–polyacrylamide conjugates has been developed by Bovin [23]. These conjugates were synthesized by the condensation of aminoalkyl glycosides with poly(4-nitrophenyl acrylate) (Fig. 1B). A complete substitution of the nitrophenoxy group by aminoalkyl glycosidic groups does not take place. Therefore, after this substitution, the polymer matrix can be modified by a label (biotin, FITC, etc.), the unused activated groups being deactivated by the action of ethanolamine [24]. This method permits the obtention of pseudo-glycoproteins, pseudomucins and other complex constructions.

A third category of glycosylated polymers has been given the name of ''glycodendrimers'' [17,25] by virtue of their resemblance to naturally occurring multiantennary glycoproteins (Fig. 1C). Synthetic multiantennary dendritic mannosides have been prepared from polyamidoamine dendrimers onto which peracetylated mannopyranosyl isothiocyanate was directly attached [17]. Other dendrimers with sialoside and lactoside analogs have also been prepared [26,27]. Multivalent 3′-sulfo-Lewis^x-(Glc) clusters showed relative binding potencies 250–625-fold higher than the monomer for L-selectin [28], suggesting the necessity of arrays of clustered-ligands for strong carbohydrate-binding interactions.

2.2. Applications of glycopolymers

Glycopolymers have been used to prepare soluble gels for affinity electrophoresis [29–32] and affinity precipitation procedures [33]. Affinity electrophoresis on polyacrylamide gels containing COP was used for the determination of dissociation constants of lectin–sugar complexes [34], in the investigation of the interaction of modified lectins with sac-

charides [32], in following the pH dependence of lectin interactions with sugars [20], and in studying the effect of ionic strength on the interaction of lectins with saccharides [35]. The possibility to perform this method in acidic as well as in alkaline buffers was used to study the role of Mn^{2+} in the activity of the soybean lectin. Affinity electrophoresis on polyacrylamide gel containing immobilized galactosyl residues showed that the interaction of the demetallized lectin does not differ from that of the native lectin in alkaline media but is decreased in acidic media [36].

The interaction of glycopolymers with biotinylated carbohydrate–proteins allowed to develop simple enzyme affinoassays for detection and estimation of carbohydrate-binding activities. These affinoassays employ the polymer dotted on nitrocellulose (affinodotting assay) and the polymer coated on microtiter plates as well as detection of bound biotinylated carbohydrate-binding proteins by streptavidin/horseradish peroxidase reagent [22].

The ability of polyacrylamide conjugates to bind with nitrocellulose made it possible to use these conjugates in affinodotting and affinoblotting techniques. The sensitivity and carbohydrate specificity of dotting assay was tested against biotinylated-plant lectins and human galectin 1 (GAL1). Peanut lectin and GAL1 detected up to 0.2 ng of lactosylated polymer while the same amount of mannosylated polymer was detected by *Lathyrus ochrus* lectin. Affinodotting assays provide reproducible binding, inhibitable by specific sugars.

Microtiter plate competitive enzyme-lectin assay (CELA) is well suited to sensitive detection of carbohydrate-binding activities in tissue extracts and biological fluids [22,37]. It consists of competing proteins to be tested with a biotinylated lectin for binding to a glycopolymer coated in the microtiter plate. This assay was used for the characterization of oligoclonal β-galactoside-binding immunoglobulins in human serum and cerebrospinal fluid [37]. A microtiter plate assay was also used for studying influenza virus hemagglutinin specificity [23]. The viruses were adsorbed into the wells of the plate coated with fetuin, and the binding of polyacrylic derivatives of *N*-acetylneuraminic acid by solid-phase attached virus was quantified by their competition with fetuin–horseradish peroxidase conjugate. This study confirmed that the increase in polymer substitution by a carbohydrate not always lead to an enhanced interaction with a complementary protein, as a sharp activity maximum was observed for 10–12% substitution, whereas the conjugates with 5 and 20% content of neuraminic acid were considerably less active [38].

2.3. Applications of glycoprobes

Glycopolymers can be prepared with fluorescein or biotin probes (Glycoprobes). Modification of the free amino groups of COPs with FITC yielded soluble copolymers having covalently-bound fluorescein and glycosyl groups [16]. Affinity electrophoresis showed that the presence of FITC-modified aminogroups does not influence the ability of sugar residues to interact with lectins. A biotin derivative (6-aminohexylamide) and a fluorescein derivative (2-aminoethylamide) were also introduced in the sugar–polyacrylamide derivatives developed by Bovin et al. [39] without interfering with the polymers solubility and hydrophobicity.

The study of the specificity of the lectin from *Butea frondosa* is an example of microtiter plate assay with a soluble biotinylated glycoprobe. The lectin was applied onto the plastic and then the glycoprobe and the inhibitors were added, permitting the evaluation of the fine specificity of the lectin [40]. More generally, glycoprobes can be adapted to most of the methods using biotin or fluorescein derivatives of antibodies. They are most widely used for revealing and studying the endogeneous cell lectins in histochemical and cytochemical experiments [41].

3. Glycoaffinity chromatography and carbohydrate-dependent recognitions

Lectins are frequently used to purify and to characterize glycoproteins, cell-derived oligosaccharides and glycolipids from alien source by affinity chromatography [42]. Another field of application for immobilized lectins is in immobilization of glycoenzymes [43]. Nevertheless, the interactions between lectins and these biological structures never appear in nature.

3.1. Study of the biological ligands of carbohydrate-binding proteins

In view of the biological role of lectins, which is poorly known, any interaction found between a lectin and a constituent of the same origin (plant, animal or human tissue, etc.) deserves attention. This type of interaction was first described for the interaction of Leguminosae lectins with proteins of the same plant (lectin binders) [44]. The study of the two lectins from *Vicia cracca* by affinity chromatography demonstrated that they react with each other [45]. If one of both lectins is attached covalently to CH-Sepharose, it is able to retard the other one from a solution. This interaction is dependent on the environmental conditions, and particularly on the sugar concentration in the eluent, suggesting that they bound together by carbohydrate–lectin interactions. Therefore, both proteins, which not only occur in the same plant but also in the same cell compartment, may be associated inside the cell.

Affinity adsorbents are also useful for the study of carbohydrate-dependent mechanisms involving carbohydrate-binding proteins found in animal cells (endogeneous lectins). A general scheme for the purification of endogeneous glycoligands is represented in Fig. 2. A variation of these scheme was used to isolate biological ligands of human brain GAL1. A loss of affinity was previously described for calf GAL1 coupled to CNBr-activated Sepharose [46]. However, immobilization of alkylated GAL1 on divinylsulfone-activated agarose, in the presence of haptenic sugar, resulted in almost complete maintenance of glycoprotein-binding ability [47]. This affinity support allowed the purification of GAL1-binding ligand from soluble human brain proteins extracts [48]. A GAL1-free protein fraction was obtained by solid-phase adsorption on lactose–divinylsulfone–agarose [49]. The purification of the soluble ligand of GAL1 was then performed by sequential affinity chromatographies on immobilized *Erythrina cristagalli* lectin (EcL), selecting

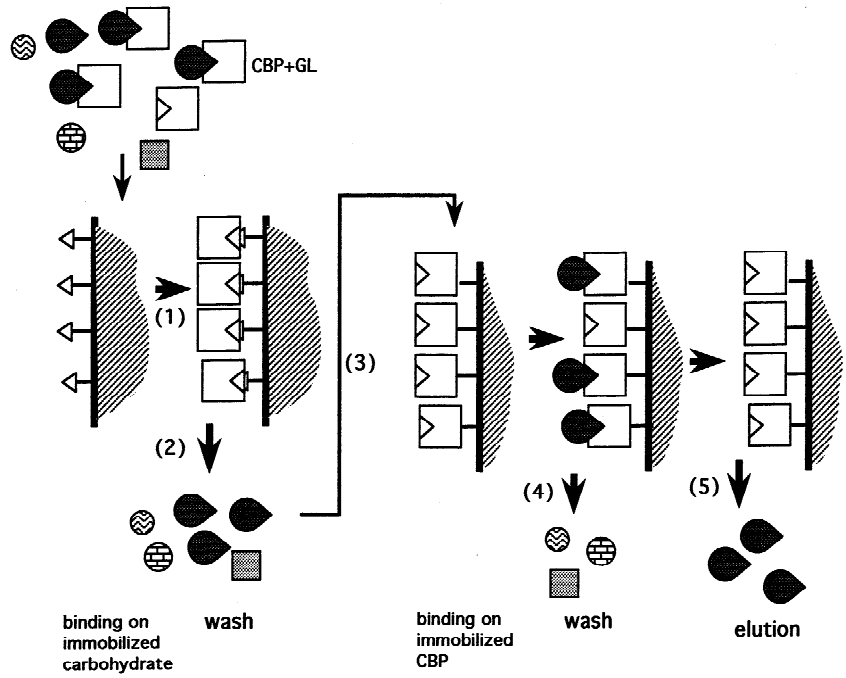

Fig. 2. The purification of the biological glycoligands (GLs) of carbohydrate-binding proteins (CBPs) consists of (1) adsorption on an immobilized sugar specific of CBP of a crude extract containing GL–CBP complexes (CBP+GL), (2) washing of host proteins including free GL, (3) adsorption of the flow through on an CBP column, (4) washing of the unbound proteins and (5) specific elution of GL by a competitive sugar.

glycoproteins containing lactosamines [50] (the carbohydrate specificity of GAL1), and on immobilized GAL1.

3.2. Study of protein–glycolipid interactions

Surface glycolipids, like membrane glycoproteins, are believed to play a major role in cell recognition and cell to cell interactions. They can serve as selective receptor sites for several bioeffectors such as neurotransmitters, lectins and toxins. The detection of glycolipids ligands by direct binding of carbohydrate-binding proteins to thin-layer chromatograms was originally described for the detection of monoganglioside 1 (GM1) using ^{125}I-labeled cholera toxin [51]. This method was subsequently adapted to the detection of glycolipids by antibodies directed against carbohydrate antigens [52,53] and by lectins [54,55]. The interaction of plant lectins with glycolipid-derived oligosaccharides was also studied by affinity chromatography on the immobilized lectins. The blood group A specific lectin from *Helix pomatia* has been used for the detection of glycolipids with terminal GalNac residues on thin-layer chromatography, as well as for the affinity purification of the corresponding glycolipid-derived oligosaccharides [55]. However, protein–ganglioside interactions can be complex, involving contributions of oligosaccharide and ceramide portions of the ganglioside. Therefore, the results of the studies of the interactions between gangliosides and carbohydrate-binding proteins clearly depend on the assay system used and on the experimental conditions.

The feasibility of studying protein–ganglioside recognition by high-performance affinity chromatography (HPLAC) on immobilized glycolipids has been established [56]. The affinity of porous silica beads coated with DEAE dextran, and coupled to lysoGM1 was demonstrated both for toxins [56] and lectins [57]. The saccharidic part of the ganglioside being present at the end of a spacer arm on this affinity matrix, it may rotate freely, adopting a configuration and orientation the proteins can recognize. This support was used to detect a possible recognition phenomenon between two molecules that co-exist in brain tissue, GAL1 and GM1 [57]. GAL1 showed no binding in neutral citrate buffer, although a binding was observed in reducing conditions. These results confirmed a hypothesis about the regulation of the lectin activity by its environment. Bound GAL1 could be eluted either by 0.1 *M* lactose, or by an acidic pH (citrate pH 2.8). Cholera toxin adsorbed on the same support was eluted by an acidic pH whereas no elution of the toxin was obtained with lactose. This most likely results from the different carbohydrate-binding specificities of both proteins.

These examples give an idea of the interest of using glycoaffinity chromatography in the study of carbohydrate-binding proteins and glycoligands. Moreover, our understanding of glycobiological mechanisms has been greatly enlarged by experimental work with endogeneous lectins revealing that certain members of this carbohydrate-binding protein family can actually recognize peptide structures in addition to glycoligands [49,58].

4. Non-carbohydrate binding recognitions in glycobiology

The idea that carbohydrates could have specific functions, for example in cellular adhesion and development, suggests the existence of carbohydrate-binding molecules which in turn could mediate cellular functions. Moreover, there is an increasing evidence that these carbohydrate-dependent interactions are not limited to the over-simple key and lock representation, and involve complex molecular arrangements maintained both by peptide–oligosaccharide and peptide–peptide interactions. Recent data indicate that several endogeneous carbohydrate-binding proteins are capable of bifunctionality or even multifunctionality [58–60]. The existence of others domains, in addition to the CRD has been described for: (i) galectin 3 (GAL3), which possesses both a domain homologous to certain regions of proteins of the heterogeneous nuclear ribonucleoprotein complex and a CRD [61], (ii) a galactoside-binding lectin of 67 000, which has a hydrophobic binding site that associates with a domain on elastin [62], (iii) various soluble mannose-binding lectins displaying collagen-like domains able to promote interaction with hydrophobic species [63], (iv) a nuclear glucose-binding lectin of 70 000, CBP 70 [64], which interacts with GAL3 by a protein–pro-

tein interaction which is modulated by lactose, (v) and several cytokines and growth factors [60,65].

A fruitful hypothesis, initially based on affinity chromatography results, is that the activity of these multifunctional proteins can be modulated by their interaction both with saccharidic and peptidic complementary structures [58].

4.1. The elastin–laminin receptor

The demonstration that the sugar-binding site of a bifunctional lectin could modify its peptide-binding site was initially based on the affinity chromatography of the 67 000 elastin receptor on immobilized elastin [62]. This receptor, which also binds laminin [66–68], was found to be a galactoside-binding lectin based on the facts that it can be eluted from elastin affinity columns by lactose and that it cross-immunoreacts with an antibody directed against GAL1 [69]. The finding that it can be eluted by lactose was unexpected since elastin is not a glycoprotein. In addition, its interaction with elastin occurs through the recognition of a hydrophobic hexapeptide sequence VGVAPG [62] which is represented several times in elastin from various origin [68]. Consequently, the 67 000 elastin receptor bound clearly to immobilized elastin by a protein–protein interaction, even if its elution is efficiently achieved using a lactose solution as eluent. It seems likely that the association between elastin and its receptor is by a tight interaction which is allosterically inhibited by binding lactose to the CRD of the receptor [69].

4.2. Galectin 3

GAL3 is a β-galactoside-binding animal lectin that is localized, depending on cell types and proliferative states, in the cytoplasm, in the nucleus, or on the cell surface. It has two distinct domains. GAL3 CRD is in the carboxy-terminal half of the molecule, and GAL3 amino-terminal part consists of a proline- and glycine-rich repetitive domain.

In the nucleus of HL60 cells, GAL3 and the glucose-specific CBP 70 are complexed with one another. During the affinity chromatography of HL60 nuclear extracts, both lectins are retained on immobilized glucose and eluted by an excess of the same sugar in solution. They are also retained on immobilized galactose, while only GAL3 is retained on immobilized lactose. The co-isolation of CBP 70 and GAL3 on the glucose-column, despite the fact that GAL3 does not bind to this column by itself, suggests that both lectins are complexed together by a protein–protein interaction in binding to immobilized glucose. The observation that only GAL3 was found in the eluate of the lactose-column suggests that binding of lactose by GAL3 dissociated the complex. Moreover, when nuclear extract of HL60 cells was fractionated over a column containing immobilized glucose, the bound CBP 70/GAL3 complex was dissociated upon lactose addition, GAL3 being eluted. Subsequent elution of the same column with glucose released CBP 70. These data demonstrate that the binding of lactose to GAL3 disrupts or prevents its association with CBP 70. Galactose, which interacts with GAL3 with a lower affinity, fails to yield the same effect. The disruption of GAL3/CBP 70 interaction by lactose was verified in isolated nuclei, in a way similar to that observed during affinity chromatography [70].

In human leukemia T cells transfected with GAL3 cDNA, GAL3 overexpression was found to confer resistance to apoptosis induced by anti-Fas antibody [71]. Sequence homology between this protein and the anti-apoptotic protein Bcl-2 suggested the possibility that they may associate with each other. Indeed, affinity chromatographies of leukemia T cell extracts on GAL3-Sepharose shares that GAL3 interacts with Bcl-2 in a lactose inhibitable manner. This inhibition was an intriguing finding, however, since Bcl-2 is not a glycoprotein. This observation suggests that the interaction which occurs between the two proteins is a protein–protein bond, and that lactose binding to GAL3 induces a conformational change in the critical region of this protein that participates in the interaction with Bcl-2, or that this region is located within the CRD of GAL3.

These examples show the increasing importance of affinity chromatography to study biological interactions of various proteins. This technique can be a supplementary powerful tool to understand the organisation of supramolecular complexes in the cell, in addition to immunoprecipitation and two-hybrid system techniques. Moreover, the fact that a protein-protein interaction can be disrupted by a sugar like

lactose strongly suggest that a glycoprotein–lectin interaction could modify a protein–protein binding in vivo. This open a new area for the affinity chromatography method.

5. Conclusions and perspectives

Owing to the increasing relevance of the study of the mechanisms involved in glycobiology, and the development of glycoproteins as human therapeutics, it has been necessary to develop expression systems and purification schemes which allow the production of biologically active glycoproteins and carbohydrate-binding proteins. For exemple, a major contribution to our understanding of the role of carbohydrates would be the ability to produce and purify glycoproteins with defined oligosaccharide structure. Among the purification schemes, affinity chromatography on either immobilized saccharides or immobilized carbohydrate-binding proteins (glycoaffinity chromatography) has proved to be very powerful. Numerous sorbents of this type have appeared in the literature and on the market for the purification or separation of lectins, enzymes, anti-carbohydrate antibodies and glycoproteins. The potential of the method has clearly been demonstrated by the applications on various sorbents reported in this review, and, undoubtedly, the chromatographic approach will show important applications in the future; these include: (i) the use of glycoaffinity chromatography for diagnosis purpose, for example to separate isoenzymes [72–74], (ii) the study of the interactions of endogeneous lectins or other carbohydrate-binding proteins (inhibitors of bacterial toxins, etc.) in order to design strategies for inhibition of microbial and parasite infection, of mammalian cell interactions [75], (iii) the evaluation of recombinant glycoproteins [76], (iv) and, last but not least, more basic purposes such as the understanding of mechanisms involved in cellular adhesion and development, apoptosis, infection and host defence, etc.

References

[1] H.J. Gabius, S. Gabius (Eds.), Lectins and Glycobiology, Springer-Verlag, Berlin, 1993.
[2] E. Van Driessche, P. Rougé, S. Beekmans, T.C. Bog-Hansen (Eds.), Lectins, Vol. 11, Textop, Hellerup, 1996.
[3] S. Gabius, H.J. Gabius (Eds.), Glycosciences, Status and Perspectives, Chapman and Hall, Weinheim, 1997.
[4] C. Doinel, M. Caron, Biosciences 6 (1986) 125.
[5] R.K. Merkle, R.D. Cummings, Methods Enzymol. 138 (1987) 232.
[6] C. Gerard, Methods Enzymol. 182 (1990) 529.
[7] H. Lis, N. Sharon, J. Chromatogr. 215 (1981) 361.
[8] P.J. Hogg, D.J. Winsor, Anal. Biochem. 163 (1987) 331.
[9] H.J. Gabius, Anal. Biochem. 189 (1990) 91.
[10] K. Yamamoto, T. Tsuji, T. Osawa, Mol. Biotechnol. 3 (1995) 25.
[11] T. Endo, J. Chromatogr. A 720 (1996) 251.
[12] R.D. Cummings, in: S. Gabius, H.J. Gabius (Eds.), Glycosciences, Status and Perspectives, Chapman and Hall, Weinheim, 1997, pp. 191–199.
[13] R.T. Lee, Y.C. Lee, Biochemistry 19 (1980) 156.
[14] K. Ek, E. Gianazza, P.G. Righetti, Biochim. Biophys. Acta 626 (1980) 356.
[15] B.K. Brandley, T.S. Ross, R.L. Schnaar, J. Cell Biol. 105 (1987) 991.
[16] M. Ticha, J. Kocourek, Carbohydr. Res. 213 (1991) 339.
[17] R. Roy, Curr. Opin. Struct. Biol. 6 (1996) 692.
[18] V. Horejsi, J. Kocourek, Methods Enzymol. 34 (1974) 361.
[19] V. Horejsi, J. Kocourek, Biochim. Biophys. Acta 538 (1978) 299.
[20] K. Hauzer, M. Ticha, V. Horejsi, J. Kocourek, Biochim. Biophys. Acta 583 (1979) 103.
[21] R.T. Lee, R.W. Myers, Y.C. Lee, Biochemistry 21 (1982) 6292.
[22] A. Chadli, M. Caron, M. Ticha, R. Joubert, D. Bladier, J. Kocourek, Anal. Biochem. 204 (1992) 198.
[23] N.V. Bovin, in H.J. Gabius, S. Gabius (Eds.), Lectins and Glycobiology, Springer-Verlag, Berlin, 1993, pp. 23–28.
[24] N.V. Bovin, S.D. Shiyan, E.V. Mikhalchik, Glycoconjugate J. 12 (1995) 427.
[25] R. Roy, Polymers News 21 (1996) 226.
[26] R. Roy, D. Zanini, S.J. Meunier, A. Romanowska, ACS Symp. Ser. 560 (1994) 104.
[27] R. Roy, W.K.C. Park, Q. Wu, S.N. Wang, Tetrahedron Lett. 36 (1995) 4377.
[28] R. Roy, W.K.C. Park, O.P. Srivastava, C. Foxall, Bioorg. Med. Chem. Lett. 6 (1996) 1399.
[29] V. Horejsi, Anal. Biochem. 112 (1981) 1.
[30] V. Horejsi, M. Ticha, Anal. Biochem. 116 (1981) 22.
[31] V. Horejsi, M. Ticha, J. Chromatogr. 376 (1986) 49.
[32] V. Horejsi, M. Ticha, J. Kocourek, Biochim. Biophys. Acta 499 (1977) 301.
[33] V. Horejsi, P. Smolek, J. Kocourek, Biochim. Biophys. Acta 538 (1978) 293.
[34] V. Horejsi, M. Ticha, J. Kocourek, Biochim. Biophys. Acta 499 (1977) 290.
[35] R. Turkova, M. Ticha, J. Kocourek, J. Chromatogr. 192 (1980) 408.
[36] J. Novakova, M. Ticha, J. Kocourek, Biochim. Biophys. Acta 670 (1981) 401.

[37] R. Joubert-Caron, M. Caron, P. Bochet, A. Chadli, P. Delaporte, E. Schuller, D. Bladier, Int. J. Biochem. 26 (1994) 813.
[38] M.N. Matrosovich, L.V. Mochalova, V.P. Marinina, N.E. Byramova, N.V. Bovin, FEBS Lett. 272 (1990) 209.
[39] N.V. Bovin, E.Y. Korchagina, T.V. Zemlyanukhina, N.E. Byramova, O.E. Galanina, A.E. Zemlyakov, A.E. Ivanov, V.P. Zubov, L.V. Mochalova, Glycoconjugate J. 10 (1993) 142.
[40] O.E. Galanina, S. Padmanabkhan, E.Y. Korchagina, T.V. Zemlyanukhina, V.V. Demin, N.V. Bovin, Bioorg. Khim. 18 (1992) 346.
[41] H.J. Gabius, S. Gabius, T.V. Zemlyanukhina, N.V. Bovin, U. Brinck, A. Danguy, S.S. Joshi, K. Kayser, J. Schottelius, F. Sinowatz, L.F. Tietze, F. Vidal-Vanaclocha, J.P. Zanetta, Histol. Histopathol. 8 (1993) 369.
[42] R.D. Cummings, Methods Enzymol. 230 (1994) 66–86.
[43] M. Saleemuddin, H. Qayyum, Enzyme Microb. Technol. 13 (1991) 290.
[44] R. Gansera, H. Schurz, H. Rüdiger, Hoppe-Seyler's Zeitschr. Physiol. Chem. 360 (1979) 1579.
[45] C.M. Baumann, H. Rüdiger, FEBS Lett. 136 (1981) 279.
[46] R.K. Merkle, R.D. Cummings, J. Biol. Chem. 263 (1988) 16143.
[47] J.D. Cornillot, M. Caron, R. Joubert-Caron, D. Bladier, Int. J. Biochem. 24 (1992) 1585.
[48] A. Chadli, J.P. LeCaer, D. Bladier, R. Joubert-Caron, M. Caron, J. Neurochem. 68 (1997) 1640.
[49] R. Joubert, M. Caron, V. Avellana-Adalid, D. Mornet, D. Bladier, J. Neurochem. 58 (1992) 200.
[50] H. Debray, J. Montreuil, H. Lis, N. Sharon, Carbohydr. Res. 151 (1986) 359.
[51] J.L. Magnani, D.F. Smith, V. Ginsburg, Anal. Biochem. 109 (1980) 399.
[52] J.L. Magnani, M. Brockhaus, D.F. Smith, V. Ginsburg, M. Blasczyk, K.F. Mitchell, Z. Steplewski, H. Koprowski, Science 212 (1981) 55.
[53] J.L. Magnani, B. Nilsson, M. Brockhaus, D. Zopf, Z. Steplewski, H. Koprowski, V. Ginsburg, J. Biol. Chem. 257 (1982) 14365.
[54] D.F. Smith, Biochem. Biophys. Res. Commun. 115 (1983) 360.
[55] B.V. Torres, D.K. McCrumb, D.F. Smith, Arch. Biochem. Biophys. 262 (1988) 1.
[56] J.L. Tayot, J. Holmgren, L. Svennerholm, M. Lunblad, M. Tardy, Eur. J. Biochem. 113 (1981) 249.
[57] M. Caron, R. Joubert-Caron, J.R. Cartier, A. Chadli, D. Bladier, J. Chromatogr. 646 (1993) 327.
[58] S.H. Barondes, TIBS 13 (1988) 480.
[59] H.J. Gabius, Int. J. Biochem. 26 (1994) 469.
[60] J.P. Zanetta, T. Scior, J. Wantyghem, C. Wermuth, M. Aubery, G. Strecker, J.C. Michelski, Histol. Histopathol. 11 (1996) 1101.
[61] S. Jia, J.L. Wang, J. Biol. Chem. 263 (1988) 6009.
[62] R.P. Mecham, A. Hinek, R. Entwistle, D.S. Wrenn, G.L. Griffin, R.M. Senior, Biochemistry 28 (1989) 3716.
[63] S. Thiel, K.B.M. Reid, FEBS Lett. 250 (1989) 78.
[64] A.P. Sève, M. Felin, M.A. Doyennette-Moyne, T. Sahraoui, M. Aubery, J. Hubert, Glycobiology 3 (1993) 23.
[65] A.P. Sherblom, N. Sathyamoorthy, J.M. Decker, A.V. Muchmore, J. Immunol. 143 (1989) 939.
[66] V. Groult, W. Hornebeck, P. Ferrari, J.M. Tixier, L. Robert, M.P. Jacob, Cell Biochem. Funct. 9 (1991) 171.
[67] R.P. Mecham, FASEB J. 5 (1991) 2538.
[68] L. Robert, W. Hornebeck (Eds.), Elastin and Elastases, CRC Press, Boca Raton, FL, 1989.
[69] A. Hinek, D.S. Wrenn, R.P. Mecham, S.H. Barondes, Science 239 (1988) 1539.
[70] A.P. Sève, Y. Hadj-Sahraoui, M. Felin, M.A. Doyennette-Moyne, M. Aubery, J. Hubert, Exp. Cell Res. 213 (1994) 191.
[71] R.Y. Yang, D.K. Hsu, F.T. Liu, Proc. Natl. Acad. Sci. USA 93 (1996) 6737.
[72] K. Arai, K.I. Yoshida, T. Komoda, N. Kobayashi, Y. Sakagishi, Clin. Chim. Acta 210 (1992) 35.
[73] K.I. Yoshida, S. Sumi, M. Honda, Y. Hosoya, M. Yano, K. Arai, Y. Ueada, J. Chromatogr. B 672 (1995) 45.
[74] K.I. Yoshida, M. Honda, K. Arai, Y. Hosoya, H. Morigushi, S. Sumi, Y. Ueada, S. Kitahara, J. Chromatogr. B 695 (1997) 439.
[75] E.F. Hounsell, Carbohydr. Res. 300 (1997) 47.
[76] K. Seamon, Glycoconjugate J. 8 (1991) 3.