Structure and function of laminin 1 glycans; glycan profiling

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Instrumental advances are progressing at a rapid pace and nowhere are the effects more apparent than in carbohydrate mass spectrometry (MS). Problems with sample gasification, for decades a major obstacle, have now been overcome with the introduction of electrospray (ES) ionization (Fenn et al., 1989). This has provided for the first time molecular ion profiles in the absence of fragmentation and fidelity in glycoform distributions with the potential for quantification in a single analysis. These techniques are being matched with developments in MS analysers where ion trap technology brings unprecedented gains in sensitivity and resolution.

Capturing this technology to unravel biopolymer problems is not always straightforward and frequently demands modifications in both instrumental operation and analyte preparation. Such is the case for carbohydrate samples which electrospray better from methanol solutions when prepared as metal adducted lipophilic analytes. Molecular weight profiling by these techniques provides a measure of composition to assess glycotypes (complex, hybrid, high mannose) and their concentration (glycoform distribution). Further detailed studies may proceed on any selected glycan by periodate oxidation, reduction and methylation (ORM), collision induced dissociation (CID), and tandem mass spectrometry (MS/MS). As we proceed to study glycoconjugates of greater complexity, comprising epitopes of diverse function and vanishing concentration, molecular profiling by these techniques will be a great asset.

As an example, consider the glycan complexity of laminins (LN). These glycoproteins constitute a family of basement membrane molecules (~1 000 000 Da) (Timpl and Brown, 1994), that in quaternary structure exist in three heterotrimeric chains which were previously classified as A, B1, and B2, and are now referred to as α, β, and γ, respectively (Burgeson et al., 1994). Engelbreth-Holm-Swarm (EHS) tumour was the first source of laminin, now referred to as laminin 1, and it remains the prototypical molecule for studies in development and maintenance of tissue architecture, as well as in tumour biology. To date, six additional laminin molecules have been isolated from animal cells and all chains share structural similarities with distinct domains containing globular regions, cysteine-rich repeats and helical structures. These structures are involved in selected aspects of morphogenesis, cell growth, and differentiation, neuritogenesis and cell migration (Engel, 1993).

Functional activity appears to be associated with different domains of the molecule and is mediated upon binding to cellular receptors which have been identified as integrins (Hynes, 1992), $\beta(1\rightarrow4)$ galactosyltransferase (Shur, 1993), galectins (Hughes, 1994), and dystroglycan (Ervasti and Campbell, 1993; Gee et al., 1993). The structural bases of these interactions vary within the models studied; for example, $\beta1$ integrins interact with peptide domains, while $\beta(1\rightarrow4)$ galactosyltransferase and galectins interact with carbohydrate residues. Interestingly, in a role reversal, dystroglycans–laminin 1 interactions appear to involve carbohydrate residues from dystroglycan (since interactions are abolished with periodate treatment of the receptor). This is analogous to the interactions between laminin 1 and gp120/140, a glycoconjugate immunochemically related to $\alpha6\beta1$ integrins from melanoma cells (Chammas et al., 1993).

The glycans of laminin 1 comprise almost one-fifth of the molecular weight, all of which are N-linked. Structural studies (Arumugham et al., 1986; Fujiwara et al., 1988; Knibbs et al., 1989) have revealed unique patterns of glycosylation such as: (i) the presence of terminal galactosyl residues; (ii) expression of polylactosamines (necessary for galectin interactions); and (iii) terminal N-acetylglucosaminyl residues (ligands for $\beta(1\rightarrow4)$ galactosyltransferase recognition). This last feature was only reported in the first structural study of laminin 1 glycans (Arumugham et al., 1986).

In an effort to correlate the structural variations with function, among different laminin 1 preparations, we initiated a profile analysis of laminin glycans using ES-MS. The glycans were released from the protein enzymatically or with hydrazine, methylated, and the extract directly infused into the MS (Reinhold et al., 1994). Further structural details were obtained by ORM (Linsley et al., 1994). Glycan tables are assembled on the premise that glycotypes follow known motifs with monomers and linkage patterns as previously established. Application of this technology to laminin 1 identified over 30 different glycan structures (Table I). All major glycotypes were identified with the high-mannose structures comprising ~19%, hybrids 1.2%, and complex ~80%, dispersed between ~30 biantennary structures. The majority of antennae terminate with N-acetylglucosamine (~50% of all the glycans), with ~3% displaying lactosamine repeating units. These structures indicate the summed presence of terminal α-galactosyl residues at ~59%. The profile suggests a form of incomplete processing, which may be due to the tumoral origin of the laminin 1.

In a general sense, these profiles support the role of galectins and $\beta(1\rightarrow4)$ galactosytransferase in laminin 1 mediated functions. The detection of abundant high-mannose structures also upholds the recently described finding of oligomannoside-dependent spreading of melanoma cells on laminin (Chandrasekaran et al., 1994). Although these analyses suggest glycan topology in a general sense, the question remains whether these glycans are present in non-tumoral laminins, how such interactions play a role in vivo, and what are their site specificity and detailed structural relationships to other family members. ES-MS provides an opportunity to rapidly profile indigenous structures at considerable sensitivity which may be followed by peptide mapping for site specificity relationships and tandem mass spectrometry for a detailed linkage and branching analysis.

Acknowledgements

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Table I. Glycan structures of laminin 1

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R1\
   \Man\                    R5
R2/     \                   |
R3\      Man—GlcNAc—GlcNAc
   \Man /
R4/
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R1	R2	R3	R4	R5	%
M	M				4.9
M	M	M			3.9
M-M	M	M	–	–	3.1
M-M	M-M	M	–	–	2.9
M-M	M-M	M-M	–	–	3.9
GN	–	GN	–	–	5.6
GN	GN	–	–	–	2.8
Gal-GN	–	G N	–	–	7.6
Gal-GN	GN	–	–	–	2.5
Gal-GN	–	Gal-GN	–	–	6.8
Gal-Gal-GN	–	GN	–	–	2.7
Gal-GN	Gal-GN		–	–	2.5
Gal-GN	Gal-GN	Gal-GN	–	–	1.0
(Gal-GN)^2	–	Gal-GN	–	–	1.1
GN	GN	GN	–	Fuc	2.0
Gal-GN	GN	GN	–	Fuc	1.3
Gal-GN	Gal-GN	GN	–	Fuc	1.6
Gal-GN	Gal-GN	Gal-GN	–	Fuc	1.6
(Gal-GN)^2	–	Gal-GN	–	Fuc	0.5
Gal-(Gal-GN)^2	–	Gal-GN	–	–	1.8
Gal-(Gal-GN)^2	–	Gal-Gal-GN		–	0.9
Gal-(Gal-GN)^2	–	Gal-GN	–	Fuc	0.8
Gal-(Gal-GN)^2	–	Gal-Gal-GN	–	Fuc	0.9
Gal-Gal-GN	–	Gal-GN	–	–	4.5
Gal-Gal-GN	–	Gal-Gal-GN	–	–	3.1
GN	–	GN	–	Fuc	5.3
Gal-GN	–	GN	–	Fuc	6.2
Gal-GN	–	Gal-GN	–	Fuc	3.9
Gal-Gal-GN	–	GN	–	Fuc	3.3
Gal-Gal-GN	–	Gal-GN		Fuc	1.1
Gal-Gal-GN	–	Gal-Gal-GN	–	Fuc	1.8
GN	GN	GN	–	–	2.7
Gal-GN	GN	GN	–	–	2.8
Gal-GN	Gal-GN	GN	–	–	2.9
Gal-GN	Gal-GN	Gal-GN	GN		0.6
Gal-GN	Gal-GN	Gal-GN	Gal-GN	–	0.7
Gal-GN	Gal-GN	Gal-GN	GN	Fuc	0.6
NA-Gal-GN	–	Gal-GN	–	–	0.7
NA-Gal-GN	–	Gal-GN	–	Fuc	0.2
NA-Gal-GN	Gal-GN	Gal-GN	–	–	0.4
Gal-GN	Gal-GN	GN	GN	–	0.7
Gal-GN	GN	GN	GN	–	0.7
GN	GN	GN	GN		2.0
Gal-GN	GN	GN	GN	Fuc	0.6
Gal-GN	Gal-GN	GN	GN	Fuc	0.6

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Meeting Announcements

First EuroConference on Carbohydrate Mimics

Strasbourg, France
May 15–18, 1995

The prominent role of carbohydrates in many biological processes is now well recognized. This has promoted numerous studies in the new field of analogues, often so-called 'mimics' of biologically significant carbohydrates. The purpose of this new series of two Euroconferences on Carbohydrate Mimics is to summarize the different aspects of this new expanding field of glycobiology. The first of these meetings will be held in May 1995 in France and will deal with the following topics: chemical synthesis, biological evaluation, conformational aspects of carbohydrate analogues, including mono- and oligosaccharides, cyclitols, nucleosides and nucleotides.

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Two day meeting on 'Mucins'; information from

Dr J.Sheehan,
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University of Manchester.

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September 13, 1995

One day meeting on 'Carbohydrate Recognition Proteins'; information from

Dr M.Taylor,
Glycobiology Institute,
Department of Biochemistry,
University of Oxford.