

The Glycan Patterns at the Individual Glycosylation Sites in Orosomucoid from Allergic Reaction Patients

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Key Words

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Summary

Little is known about the alterations that have occurred at the individual glycosylation sites in allergy patients or how these glycosylation patterns may change after anti-allergy treatments. Using reverse-phase HPLC, we have quantitated the glycoforms present at the individual glycosylation sites on orosomucoid isolated from the sera of allergic reaction patients and an allergic reaction patient treated with the antihistamine Terfenadine. The glycan structures isolated from the five glycosylation sites for the individual taking Terfenadine were all within normal ranges. It is suggested that if the changes in glycosylation in OMD in the allergic state are functionally driven, then it should be possible to correlate biological activities with quantitative changes at the individual glycosylation sites, and hence further define the role of OMD in allergy and inflammation.

Introduction

The lipocalin Orosomucoid (OMD; α_1 -acid glycoprotein; AAG), is a positive acute phase reactant containing five *N*-linked complex glycan chains [1]. The glycan structures and the percentage of each at the individual glycosylation sites of OMD isolated from normal sera have been studied extensively [2–4], however, their functional role remains unknown. Recent studies have indicated that the glycan microheterogeneity in OMD varies with the acute phase [5, 6].

Cytokines, products of activated polymorphonuclear (PMN) cells and mediators of hypersensitivity reactions, alter the plasma concentrations of the acute

phase proteins and induce changes in the glycosylation pattern of the OMD produced in hepatocytes [7–9]. During the allergic reaction, a variety of cells are triggered by such mediators as platelet-activating factor (PAF) to release vasoactive amines that contribute to the allergic inflammatory response after initial IgE-mediated mast cell activation. Schmid et al. [10] determined that OMD is capable of suppressing lymphocyte proliferation and a variety of other immune functions. This modulation of activity was shown by Pos et al. [11] to be due to a decrease in the overall biantennary content of the molecule, and it has been suggested [12] that a particular total glycan content on OMD may be responsible for mediating specific biological activities. OMD may control the duration of the allergic response by down-regulating the sensitization stage (lymphocyte activation [10]) or by preventing the mediators PAF [1], histamine [13], and serotonin from causing acute or chronic inflammation. Little, however, is known about the alterations that have occurred at the individual glycosylation sites in allergy patients or how these glycosylation patterns may change after anti-allergy treatments.

In previous work, we have developed chromatographic methods [14] to determine the glycan composition [2] and the percentage of each [2] present at the individual glycosylation sites of OMD isolated from normal sera. These chromatographic procedures have now been applied to the OMD isolated from the sera of allergic reaction patients and an allergic reaction patient treated with the antihistamine Terfenadine (Seldane).

Experimental

Materials and Methods

Chemicals. All reagent grade chemicals and high performance liquid chromatography (HPLC) grade solvents were from Fisher Scientific, Pittsburgh, PA. HPLC grade water was purified in our laboratory.

Affinity chromatography. The immunoaffinity column was made as previously described [15]. One to two mL of sera from allergic reaction patients were loaded onto the affinity column and washed with 300 mL of 0.01 M

phosphate and 0.1 M NaCl. The bound OMD was eluted with 0.01 M phosphate pH 7.4 containing 1 M NaCl. the immunoaffinity purified OMD was reduced and alkylated according to the procedure of Ikenaka et al. [16]. The reaction was stopped by desalting on a Sephadex G-25 (Sigma) column (0.5 × 40 cm) and the product lyophilized.

Endoproteinase Glu-C cleavage. The isolated OMD was cleaved with Endoproteinase Glu-C (V-8, Boehringer Mannheim Chemicals, Indianapolis, IN, USA) in 150 µL of 0.1 M phosphate pH 7.8 at an enzyme to reduced and alkylated protein concentration ratio of 1 : 20 for 24 h at room temperature.

High performance liquid chromatography. Reverse phase HPLC gradients were generated by dual Kratos Spectroflow 400 pumps controlled by a Kratos model 783 and chromatograms were recorded and peak areas integrated using a 7290 SpectraPhysics Integrator. Detection was at 276 nm for the initial V-8 cleavage separation and 214 nm for further purifying the glycopeptides. The purification and identification of the individual glycosylation sites were as previously described [2].

Desialylation Sialidase from *Clostridium perfringens* (25 units insolubilized/gm of agarose; Sigma) was used to desialylate the glycopeptides. The isolated glycopeptides were incubated with 0.250 units of the enzyme in 400 µL of 0.1 M acetate, pH 5.0 for 24 h at 37 °C. The glycopeptides were separated from the beads by

centrifugation in Spin-X centrifuge filter units (Costar, Cambridge, MA, USA), and concentrated from the acetate buffer using C₁₈ Sep Paks (Waters). The isolated glycopeptides were rerun on the C₁₈ column (218-TP-510) to determine changes in the HPLC profiles.

Electrophoresis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed under reducing conditions according to Laemmli [17] using an 11 % slab gel (Hoefer Scientific Instruments, San Francisco, CA, USA).

Results and Discussion

The patients listed in Table I were used for determining the glycan percentages at each of the individual glycosylation sites on OMD from treated and non-treated allergic reaction patients. The OMD was isolated from 1–2 mL of sera from each of these patients. This OMD, isolated by affinity chromatography, was from single donors and not pooled samples. Electrophoresis of the affinity-isolated OMD gave a single band at approximately 41,000 daltons. The percentages of each major glycan type at the five glycosylation sites were determined for this isolated OMD based on the peak areas from the HPLC chromatograms. (In this context, we define the major glycan forms as the classes of bi, tri and tetraantennary.

Table I Percentages of the complex glycan types at each of the five glycosylation sites for OMD isolated from allergic reaction patients. Tet – tetraantennary. Tri – triantennary. Bi – biantennary. The standard deviation calculated for each of the five glycosylation sites for the OMD obtained from 'normal' sera were determined previously [2]. GPI denotes the composition for only gene product I.

Site	'Normal' OMD	Allergic Reaction								
		Percentage of Tetraantennary			Percentage of Triantennary			Percentage of Biantennary		
		Patient			Patient			Patient		
		1	2	3	1	2	3	1	2	3
I	Tet 0.0	0.0	0.0	0.0						
	Tri 91.4 ± 0.3				95.5	94.6	92.5			
	Bi 8.6 ± 0.3							4.5	5.4	7.5
II	Tet 14.6 ± 0.3	20.8	21.8	14.9						
	Tri 24.8 ± 0.6				42.0	28.4	25.9			
	Bi 60.6 ± 0.4							37.2	49.7	59.2
III	Tet 58.9 ± 0.5	69.3	68.5	61.2						
	Tri 32.0 ± 0.3				26.6	29.1	30.4			
	Bi 9.0 ± 0.2							4.1	2.4	8.4
IV	Tet 79.1 ± 0.8	77.1	75.9	78.3						
	Tri 20.9 ± 0.8				22.9	24.1	21.7			
	Bi 0.0							0.0	0.0	0.0
V-GPI	Tet 84.8 ± 0.4	81.3	82.0	82.7						
	Tri 15.2 ± 0.4				18.7	18.0	17.3			
	Bi 0.0							0.0	0.0	0.0

Subclasses of mono or bifucosylated or the addition of a nonbisecting GalGlcNAc are included with the form from which they were derived). The glycan percentages are reported for only the desialylated forms of the glycopeptides generated from the five glycosylation sites of OMD.

Table I gives the percentages found for each of the major glycan forms on OMD from these allergic reaction patients and are reported according to individual glycosylation sites. These are compared to the percentages found previously for "normal" OMD [2].

All of these allergic reaction individuals presented with type I hypersensitivities. Patients 1 and 2 (Table I) were currently having an allergic response, to varying degrees, at the time the blood was drawn. Patient number one, who was hospitalized, (this serum was obtained from the Diagnostic Immunology Laboratory of University Hospital) had a much greater deviation from the normal percentages for the glycans present. However, each individual showed the same direction of change at each site, per glycan, suggesting that these glycan patterns are specific to the allergic response. The severity of the allergic response is reflected in the magnitude of these changes (Table I). This illustrates the importance of looking at the direction of change and not just the magnitude. Thus, standard deviation is less important as a method of measuring variation in the glycan structures at individual sites, since the severity of the response determines the magnitude of change in the allergic group. The increase in severity of the allergic reaction for this group of individuals was 1 > 2 > 3 (Table I). This situation where the severity of the disease determines the magnitude of the change in the glycan structures may also occur in other disease states as well.

Tentative support for the changes in the glycan structures being coupled to the allergic response comes from the results of patient #3 on antihistamine therapy (Seldane, 60 mg b.i.d.). The glycan structures isolated from the five glycosylation sites for this individual were all within normal ranges (Table I). This suggests that the medication inhibits the induction of changes in the OMD glycan profiles resulting from the allergic response.

The number of patients sampled in this preliminary work was too small to be statistically significant, but the

results are intriguing not only for their internal consistency, but also because the increase in the tetra-antennary glycan content (49.7 % for allergic reaction patients versus 47.7 ± 1.1 [2] for normals) agrees with the results of Pos et al. [11]. In addition, the procedure provides information on the glycan structures present at the individual glycosylation sites for individual allergic reaction patients. If changes in glycosylation in OMD in the allergic state are related to its function, then quantitative correlations may be drawn between these biological activities and the compositional changes at the individual glycosylation sites. This will help to define the role of OMD in allergy and inflammation.

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