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Structural analysis of dansyl glyco-asparagines from quail ovalbumin

Johanna H.G.M. Mutsaers [a], Herman van Halbeek [a], Johannes F.G. Vliegenthart [a], Hitoo Iwase [b], Yukinobu Kato [b] and Kyoko Hotta [b]

[a] *Department of Bioorganic Chemistry, University of Utrecht, Croesestraat 79, NL-3522 AD Utrecht (The Netherlands), and* [b] *Department of Biochemistry, School of Medicine, Kitasato University, Sagamihara, 228 (Japan)*

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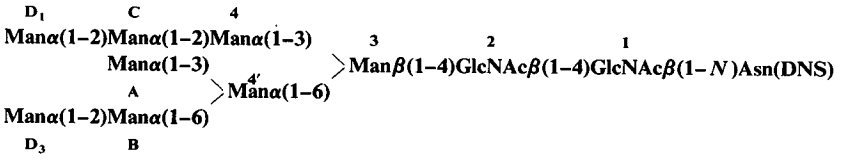
The carbohydrate moiety of quail ovalbumin was isolated in the form of dansyl glyco-asparagines. The various components were separated by high-performance liquid chromatography, yielding two major fractions (90% of the total carbohydrate material). By 500-MHz ^{1}H-NMR spectroscopy they were found to be of the oligomannoside type, containing eight and seven D-mannose residues. The largest one possesses the following structure:



while the other one is its analogue missing mannose D_1. Dansyl glyco-asparagines turned out to be suitable derivatives for ^{1}H-NMR spectroscopic analysis; in combination with HPLC, heterogeneity of sugar chains on glycoproteins can be elegantly characterized.

Introduction

In a previous study [1] the behaviour of ovalbumins from various avian species on Con A/Sepharose was reported. Quail ovalbumin bound more tightly to Con A/Sepharose than did ovalbumins from various chicken subspecies and from turkey. To gain insight into the structural basis of this phenomenon, we determined the primary structure of the carbohydrate chains of quail ovalbumin. The carbohydrate moiety of this glycoprotein is known [2] to be *N*-glycosidically linked to Asn, containing Man and GlcNAc as constituent monosaccharides. For detailed analysis of these chains, we combined the reversed-phase HPLC procedure for fractionation of dansyl derivatives of glyco-asparagines recently developed [2,3] and ^{1}H-NMR spectroscopy, which has proved to be a suitable method for structure determination of *N*-type glycopeptides [4].

Abbreviations: Man, D-mannose; GlcNAc, *N*-acetyl-D-glucosamine; DNS, dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; ODS, octadecylsilyl.

Materials and Methods

Ovalbumin was isolated from quail eggs (*Coturnix coturnix*) purchased from a Japanese supermarket. Glyco-asparagines were prepared from ovalbumin by repeated pronase digestion as described [5]. Dansylation of the glycopeptides was carried out according to Ref. 6.

The HPLC apparatus consists of a TRI ROTAR SR2 solvent delivery system (Japan Spectroscopic Co., Ltd.), an Altex Ultra-sphere ODS-reversed-phase column (5 μm; 0.46 × 25 cm, Mitsubishi Kasei Co., Ltd.) protected by a Brownlee Labs MPLC™ guard column (0.46 × 3.0 cm) packed with ODS-pack (10 μm). The column was eluted

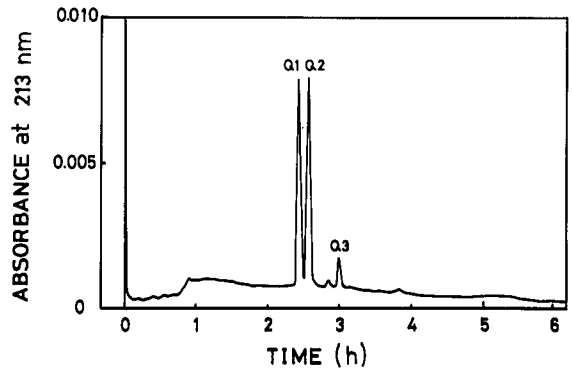


Fig. 1. HPLC elution profile of dansyl glyco-asparagines obtained from quail ovalbumin, on an Altex Ultra-sphere ODS-reversed-phase column.

TABLE I

^{1}H CHEMICAL SHIFTS OF STRUCTURAL-REPORTER GROUPS OF CONSTITUENT MONOSACCHARIDES FOR DANSYL GLYCO-ASPARAGINES Q1 AND Q2 OBTAINED FROM QUAIL OVALBUMIN

Chemical shifts were acquired at 500 MHz for ^2H_2O solutions at 27°C; they are expressed in ppm down-field from internal DSS.

Reporter group	Residue	Chemical shifts in	
		Q2 C–4 A ⟍ ⟩3–2–1–Asn(DNS) 4′ D_3–B ⟋	Q1 D_1–C–4 A ⟍ ⟩3–2–1–Asn(DNS) 4′ D_3–B ⟋
H-1	GlcNAc-1	4.786	4.79 [a]
	GlcNAc-2	4.591	4.592
	Man-3	4.78 [a]	4.78 [a]
	Man-4	5.348	5.339
	Man-4′	4.871	4.871
	Man-A	5.093	5.092
	Man-B	5.147	5.146
	Man-C	5.054	5.304
	Man-D_1	–	5.043
	Man-D_3	5.042	5.043
H-2	Man-3	4.236	4.234
	Man-4	4.116	4.106
	Man-4′	4.150	4.149
	Man-A	4.070 [b]	4.069
	Man-B	4.025	4.027
	Man-C	4.070 [b]	4.089
	Man-D_1	–	4.069
	Man-D_3	4.073 [b]	4.069
NAc	GlcNAc-1	2.029	2.029
	GlcNAc-2	2.068	2.068

[a] Partly obscured by HO^{2}H-line at 27°C (δ 4.77).
[b] Assignments may have to be interchanged.

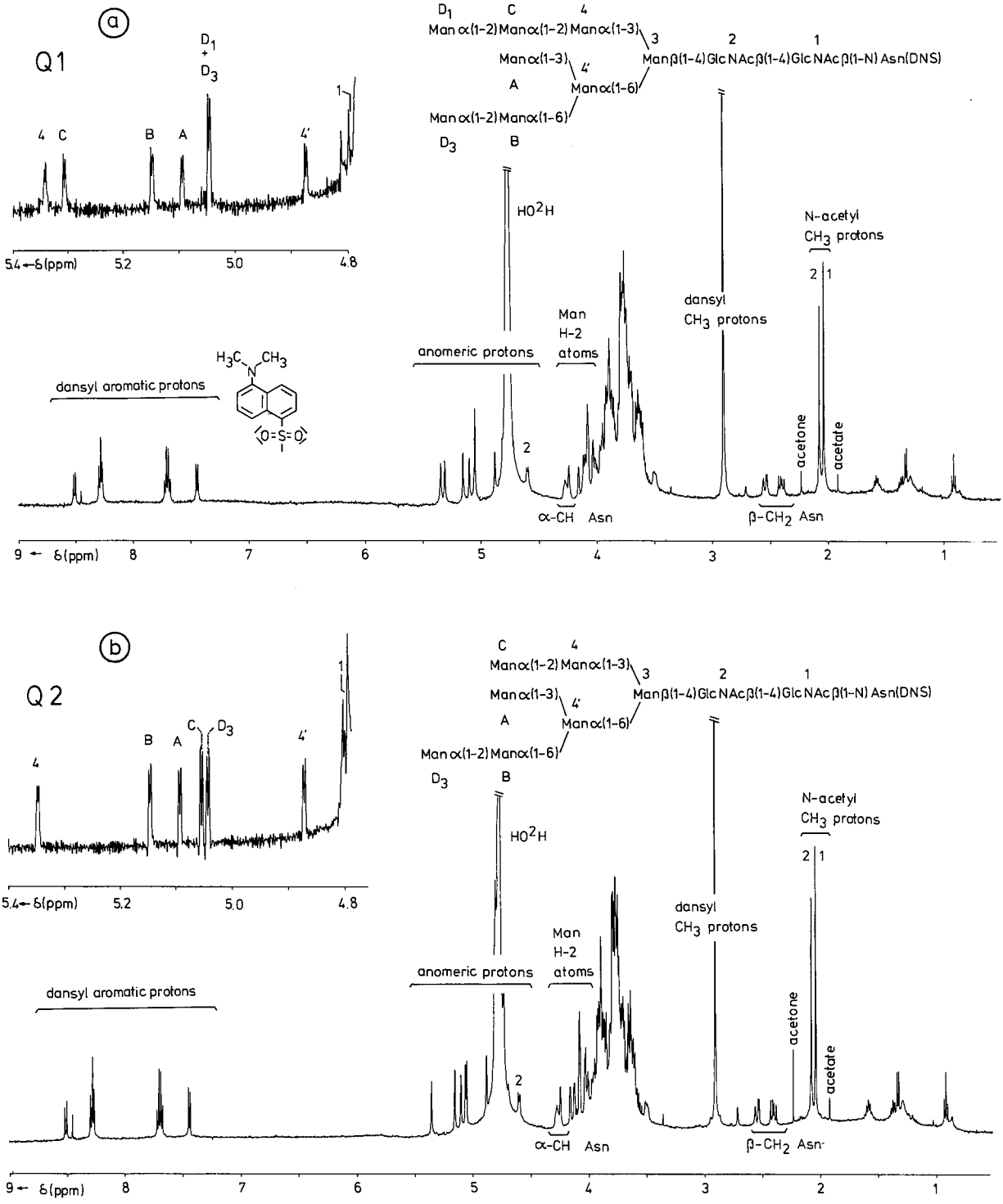


Fig. 2. 500-MHz ^{1}H-NMR spectra (^2H_2O; 27°C) of dansyl glyco-asparagines Q1 (a) and Q2 (b) obtained from quail ovalbumin. In the insets, the expanded α-anomeric proton regions are shown after resolution enhancement. The numbers and letters in the spectra refer to the corresponding residues in the structures.

with a linear gradient of 2.0–8.0% acetonitrile in 25 mM sodium borate buffer (pH 7) at a flow rate of 1.0 ml/min for 8 h. The effluent was monitored by a ultraviolet detector (UVIDEC-100IV, Japan Spectroscopic Co., Ltd.) at 213 nm.

Prior to ^{1}H-NMR spectroscopic analysis, samples (about 0.5 μmol) were repeatedly treated with ^2H_2O with intermediate lyophilization. For analysis, the samples were redissolved in 400 μl ^2H_2O (99.96 mol%, Aldrich). ^{1}H-NMR spectroscopy was performed on a Bruker WM-500 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, Nijmegen University, The Netherlands), operating at 500 MHz and 27°C in the Fourier transform mode [4]. Chemical shifts (δ) are expressed in ppm down-field from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), but were actually measured by reference to internal acetone (δ 2.225 ppm in ^2H_2O at 27°C), within an accuracy of 0.002 ppm.

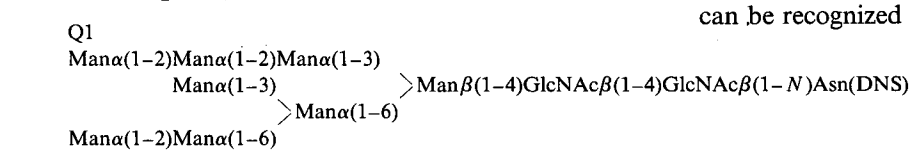
Results

Glycopeptides from quail ovalbumin (50 mg) were prepared by repeated pronase digestion as described [5]. Amino acid analysis of the glycopeptide mixture revealed aspartic acid along with traces of some other amino acids such as threonine and serine. The glycopeptides were dansylated and subsequently fractionated by reversed-phase HPLC [2,3]. The HPLC profile (Fig. 1) showed two main fractions (Q1 and Q2), together accounting for 90% of the total carbohydrate of the glycoprotein. The minor component (Q3) was not available in sufficient amounts for NMR analysis to be performed. However, on the basis of the HPLC behaviour, it was proposed [2] that a $Man_6GlcNAc_2$Asn(DNS) structure was involved.

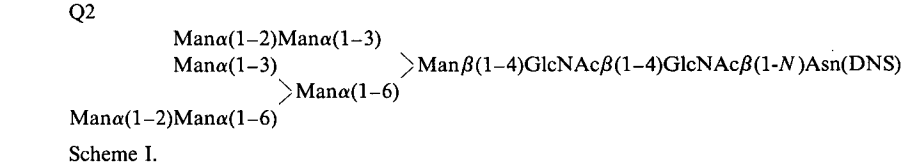
The 500-MHz ^{1}H-NMR data of Q1 and Q2 are given in Table I and the spectra are compared in Fig. 2. The carbohydrate moiety of Q1 and Q2 appeared to be of oligomannoside type, containing 8 and 7 D-mannose residues, respectively. The arrangement of the mannose residues in Q1 could be inferred directly from the close similarity of its ^{1}H-NMR spectrum with that of $Man_8GlcNAc_2$Asn from immunoglobulin M obtained from a patient with Waldenström's macroglobulinemia (compound 70 in Ref. 4). Similar reasoning holds for Q2 when compared with $Man_7GlcNAc_2$Asn from immunoglobulin M (compound 69 in Ref. 4). Therefore, it could be concluded that the structures of Q1 and Q2 are as shown in Scheme I.

Careful comparison of the chemical shift data for the dansyl derivatives Q1 and Q2 with those for the corresponding glycopeptides (compounds 70 and 69 in Ref 4) demonstrated that the presence of the dansyl group at the *N*-terminus of Asn has a few, but limited, effects on chemical shifts of neighbouring protons. First of all, effects were noticed on the resonance positions of the protons of the amino acid itself and secondly, on the structural-reporter group signals of the core GlcNAc-residues 1 and 2. The H-1 signal of GlcNAc-1, characterized by its typical, relatively large $J_{1,2}$ value (9.8 Hz), is shifted upfield from δ 5.02 to δ 4.79. The chemical shift of H-1 of GlcNAc-2 is influenced by dansylation, changing from δ 4.62 to 4.59. The *N*-acetyl signals of both residues shift down-field upon dansylation of Asn: for GlcNAc-1, from δ 2.008 to 2.029, and for GlcNAc-2 from δ 2.054 to 2.068. It is noteworthy that the chemical-shift values of the structural-reporter groups of the mannose residues are close to those observed for the corresponding glycopeptides. In the NMR spectra, the dansyl group can be recognized from the set of aromatic proton

Q1



Q2



Scheme I.

signals in the region between δ 7.6 and 8.9, and from the relatively high signal at δ 2.900 equivalent to six protons, stemming from the dimethylamino function.

Discussion and Conclusions

The observation [1] that quail ovalbumin binds more tightly to Con-A-Sepharose than does chicken ovalbumin, can now be understood, since the affinity of a carbohydrate chain towards concanavalin A is correlated with the number of α-mannose residues, unsubstituted at C-3, C-4 and C-6 [7,8]. The carbohydrate chains of chicken ovalbumin are of the hybrid and oligomannoside type, the latter of which are bearing five or six mannose residues [9,10]. As shown in this study, quail ovalbumin contains oligomannoside type of chains bearing seven or eight mannose residues, and consequently, binds more tightly to concanavalin A.

Finally, it should be emphasized that HPLC in combination with high-resolution ^{1}H-NMR spectroscopy is a powerful tool in the analysis of mixtures of dansyl glyco-asparagines and therefore in the description of (micro)heterogeneity in terms of well-defined structures.

Acknowledgements

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