1H-NMR Spectroscopic Characterization of Dansyl Glyco-asparagines Derived from Hen Egg White Glycoproteins

HERMAN VAN HALBEEK1, JOHANNES F G VLEGENTHART1,*, HITOO IWASE2, 3, SUCHEN LI2 and YU-TEH LI2

1Department of Bio-Organic Chemistry, University of Utrecht, Croesestraat 79, NL-3522 AD Utrecht, The Netherlands
2Department of Biochemistry, Delta Regional Primate Research Center, Tulane University, New Orleans, Louisiana 70112, USA
3Present address: Department of Biochemistry, School of Medicine, Kitasato University, 145-1 Sagami, Kanagawa 228, Japan

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Dansyl glyco-asparagines were prepared from a partially fractionated mixture of hen egg white glycoproteins. Reverse-phase high performance liquid chromatography (HPLC) on a silica-based octadecyl column yielded ten such derivatives in a virtually pure state. The detailed structures of the glyco-asparagines were identified by 500-MHz 1H nuclear magnetic resonance (NMR) spectroscopy. Two of them were found to be of the oligomannoside N-type, four were of the intersected-hybrid N-type and another four were of the intersected multi-antennary N-type. In monogalactosylated, intersected structures the galactose residue was proved by 1H-NMR to be attached in β(1-4)-linkage to the GlcNAcβ1-4Manα1-3 branch.

Dansyl glyco-asparagines turned out to be suitable derivatives for 1H-NMR spectroscopic analysis. The combination of HPLC and high-resolution NMR spectroscopy of such derivatives proved to be a powerful technique in studying the (micro-)heterogeneity of sugar chains in glycoproteins.

Abbreviations: dns, dansyl (5-dimethylaminonaphthalene-1-sulfonyl); ODS, octadecyl-silica; WEFT, water-eliminating Fourier transform; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate; OVA, ovalbumin; OVM, ovomucoid; OVT, ovotransferrin.

*Author for correspondence
Complete elucidation of the structure of the carbohydrate chain(s) of a glycoprotein is a prerequisite for understanding its biological function. One of the most perplexing features of glycoproteins is the heterogeneity of their carbohydrate chains. Although quite often readily detectable, the detailed characterization of the heterogeneity of sugar chains in glycoproteins is largely restricted by the fractionation and identification techniques available. Hen egg white glycoproteins are known to be extremely heterogeneous in their carbohydrate chains [1, 2]. We now report the fractionation and characterization of their glycans without laborious pre-purification steps (cf. [3-20]). We have used reverse-phase HPLC to fractionate the dansyl derivatives of the glyco-asparagines obtainable from ovalbumin, ovomucoid and ovotransferrin. The separation by HPLC is based on hydrophobic interaction of dansyl groups with silica-bonded octadecyl (ODS) groups [21]. The structures of the dansyl glyco-asparagines isolated by reverse-phase HPLC were subsequently identified by 500-MHz $^1$H-NMR spectroscopy.

Materials and Methods

Materials

Glycoproteins prepared from pooled hen egg whites according to [22] were partially fractionated as described [23], yielding a mixture of ovalbumin (OVA) and ovomucoid (OVM), and ovotransferrin (OVT). Pronase® was a product from Calbiochem (San Diego, CA, USA), 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride) from Pierce (Rockford, IL, USA), and acetonitrile from Mallinckrodt (Grossostheim, W. Germany). All solvents used for HPLC were filtered through a Millipore filter (pore size 0.45μ).

Preparation of Dansyl Glyco-asparagines

Glyco-asparagines from the partially purified OVA/OVM mixture and from OVT were prepared by repeated Pronase digestion. The OVA/OVM compounds were separated into five fractions (A-E) by Dowex-50 anion-exchange chromatography, as described [24]. Dansylation of glyco-asparagines was carried out according to the method of Gray [25].

Fractionation of Dansyl Glyco-asparagines by Reverse-phase HPLC

The HPLC system consisted of an Altex (Rochester, N.Y., USA) Model 110A solvent delivery system, an LKB (Bromma, Sweden) Model 11300 Ultragrad solvent gradient maker, an Altex Ultrasphere ODS reverse-phase column (5 μ; 25 cm x 1 cm i.d.) protected by a Bio-Rad (Richmond, CA, USA) Microguard column (5 cm x 4 mm i.d.) packed with Bio-Rad Bio-Sil ODS reverse-phase hydrocarbon (10 μ), and the following components from Perkin Elmer: a Model 7105 valve sample injector, a Model 650 fluorescence monitor, and a Model 56 recorder. For analytical purposes 2-5 nmol of dansyl glyco-asparagines in 5 μl water, and for preparative purposes, about 500 nmol in 50 μl water were applied to the column. The column was eluted with a linear gradient from water to 75% acetonitrile.