Sequence-Specific Peptide−Carbohydrate Interactions in an Asparagine-Linked Glycopeptide

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Asparagine glycosylation (N-glycosylation) of the Asn-Xaa-Ser/Thr sequence is a cotranslational process that can affect protein structure and function.[1,2] Little is known, however, about glycosylation's influence on the structure of flexible *N*-glycopeptides.[3−7] Fluorescence studies show that N-glycosylation can alter peptide backbone conformation.[6] Circular dichroism also indicates that N-glycosylation can perturb α-helical structure.[7] In this work, we use NMR to determine details about *N*-glycopeptide structure in an organic solvent. Significantly, glycopeptide Ac-Asn(β1-*N*-GlcNAc)Leu-Thr-NH_2 (**1**) shows long-range $^1H-^1H$ NOEs between the peptide and the *N*-acetylglucosamine (GlcNAc) sugar attached to Asn1. Besides these peptide-sugar NOEs, a comparison of $^3J_{\alpha,\beta}$ coupling constants and NH temperature coefficients in glycopeptide **1** and aglycosyl peptide Ac-Asn-Leu-Thr-NH_2 **3** indicate that N-glycosylation stabilizes peptide conformation.

Until recently, NMR studies showed that N-glycosylation caused little change in peptide structure.[3,4] Peptide−sugar NOEs have not been observed in flexible *N*-glycopeptides. Our studies of a glycodecapeptide in H_2O indicated nonrandom conformation near the N-glycosylation site but showed no intramolecular peptide−sugar NOEs.[3e] We have now examined tripeptides containing one sugar, Ac-Asn(β1-*N*-GlcNAc)Leu-Thr-NH_2 (**1**) and Ac-Gln(β1-*N*-GlcNAc)Leu-Thr-NH_2 (**2**). The "unnatural" glycopeptide **2** has an Asn1-Gln1 mutation that allows one to determine if glycopeptide structure is sequence-specific. Glycopeptide **1** and aglycosyl peptide **3** contain an N-glycosylation consensus sequence. Comparison of **1** and **3** reveals glycosylation's influence on peptide conformation.

Glycopeptides **1** and **2** were synthesized from β-1-amino-GlcNAc and the corresponding Asp and Glu peptides.[8] The ^1H resonances in **1**−**3** were assigned using TOCSY, DQF-COSY, and ROESY experiments.[9] NMR data indicated that glycopeptide **1** was not structured in DMSO-d_6 at 298 K. To narrow the conformational distribution and favor low-energy structures, NMR analysis of **1**−**3** was done at low temperature.

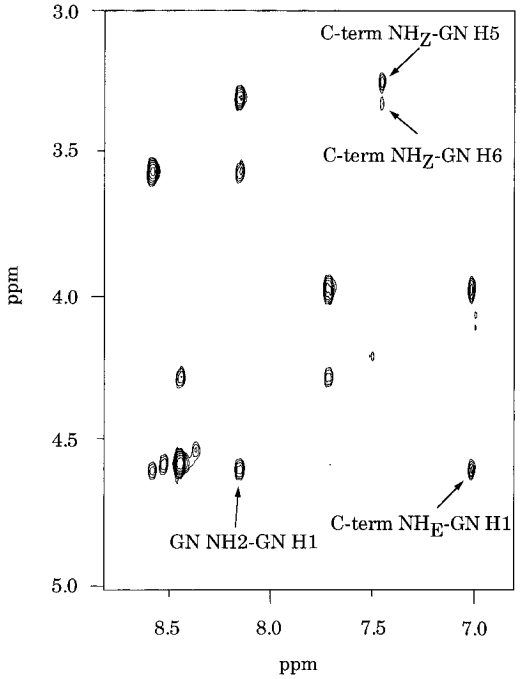
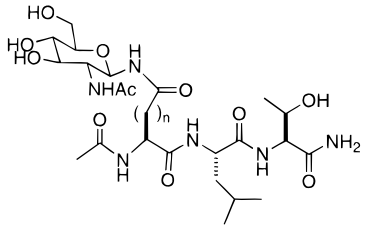


Figure 1. Contour plot of a region of the 500 MHz ROESY spectrum of Ac-Asn(β1-*N*-GlcNAc)-Leu-Thr-NH_2 (**1**) (2.0 mM) recorded at 233 K in 30% DMSO-d_6/70% CD_2Cl_2. The spin locking time was 200 ms. Key peptide−sugar NOEs are indicated.



1 Ac-Asn(β-1-N-GlcNAc)-Leu-Thr-NH_2 n=1

2 Ac-Gln (β-1-N-GlcNAc)-Leu-Thr-NH_2 n=2

ROESY experiments at 233 K in 30% DMSO/70% CD_2Cl_2 revealed that glycopeptide **1** (2.0 mM) had unique intramolecular peptide−sugar NOEs. The aglycosyl peptide **3** showed no long-range NOEs under identical conditions. As shown in Figure 1, the C-terminal trans amide NH (δ 7.01) has an NOE to GlcNAc H1 (δ 4.60) while the C-terminal cis amide NH (δ 7.45) has NOEs to GlcNAc H5 (δ 3.26) and a GlcNAc H6 (δ 3.33). Importantly, NMR spectra of glycopeptide **1** in 30% DMSO/70% CD_2Cl_2 at 233 K indicated no change in the NH resonances over a 5.0−0.05 mM concentration range. The concentration independence of the NH chemical shifts verified that the peptide−sugar NOEs were intramolecular.

The peptide−sugar NOEs significantly limit glycopeptide conformation since, under these conditions, dipolar interactions are not observed for interproton distances >3.1 Å.[10] These intramolecular peptide−sugar interac-

(1) (a) Kornfeld, R.; Kornfeld, S. *Ann. Rev. Biochem.* **1985**, *54*, 631. (b) Gilmore, R. *Cell* **1993**, *75*, 589.
(2) Varki, A. *Glycobiology* **1993**, *3*, 97.
(3) For NMR of *N*-glycopeptides: (a) Ishii, H.; Inoue, Y.; Chujo, R. *Int. J. Pept. Protein Res.* **1984**, *24*, 421. (b) Ishii, H.; Inoue, Y.; Chujo, R. *Polym. J. (Tokyo)* **1985**, *17*, 693. (c) Wormald, M. R.; Wooten, E. W.; Bazzo, R.; Edge, C.; Feinstein, A.; Rademacher, T. W.; Dwek, R. A. *Eur. J. Biochem.* **1991**, *198*, 131. (d) Davis, J. T.; Hirani, S.; Bartlett, C.; Reid, B. R. *J. Biol. Chem.* **1994**, *269*, 3331.
(4) N-glycosylation can alter Pro cis/trans and Cys thiol/disulfide equilibria: Rickert, K. W.; Imperiali, B. *Chem. Biol.* **1995**, *2*, 751.
(5) O-Glycosylation influences peptide conformation: (a) Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 3352. (b) Liang, R.; Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1995**, *117*, 10395.
(6) Imperiali, B.; Rickert, K. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 97.
(7) (a) Otvos, L.; Thurin, J.; Kollat, E.; Urge, L.; Mantsch, H. H.; Hollosi, M. *Int. J. Pept. Protein Res.* **1991**, *38*, 476.
(8) Cohen-Anisfeld, S. T.; Lansbury, P. T., Jr. *J. Am Chem. Soc.* **1993**, *115*, 10531.
(9) (a) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820. (b) Piantini, U; Sorenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800. (c) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

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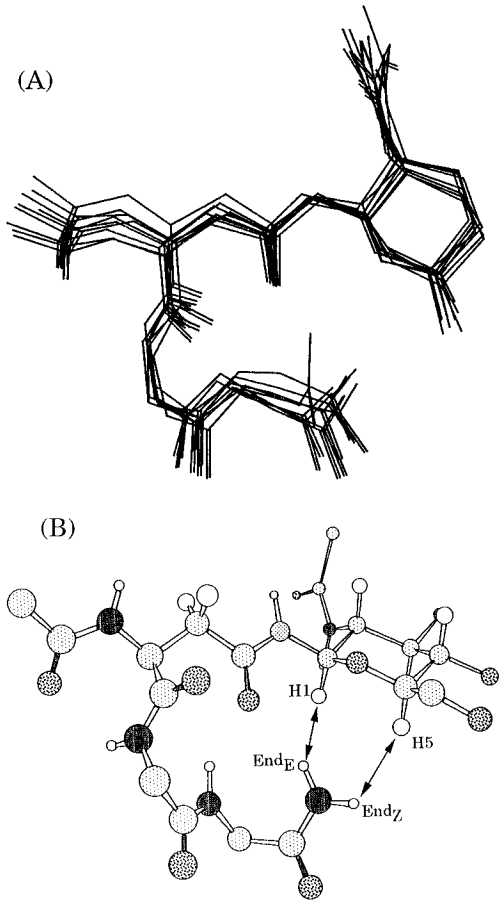

Figure 2. Structural model for glycopeptide **1**. (A) Overlay of 10 structures from simulated annealing/energy minimization protocol, showing the peptide main chain and GlcNAc residue. (B) The lowest energy structure for glycopeptide **1**. Key interproton NOEs are indicated. For clarity the Leu2 and Thr3 side chains are not shown.

tions are specific for Asn glycopeptide **1**. The "unnatural" Gln glycopeptide **2** had no sugar−peptide NOEs under identical conditions. Apparently, the longer Gln side chain in **2** does not allow the same intramolecular contacts that favor folded structures for Asn glycopeptide **1**.[11] Sequential NOEs between Leu2 NH and Thr3 NH and between Thr3 NH and the C-terminal trans NH and a Thr3 NH-Asn1 H_α NOE also indicate a folded backbone structure in glycopeptide **1**.

Thirty NOE distance constraints were used in molecular dynamics calculations to determine the structure of glycopeptide **1** (Sybyl 6.1). An extended conformation for **1** was heated to 1000 K for 1 ns and exponentially cooled to 0 K over 10 ns. The simulated annealing process was repeated 10 times, and the resultant structures were minimized using a conjugate gradient method with 500 iterations. The 10 minimum energy structures, overlaid in Figure 2A, all show the peptide main chain folded toward the GlcNAc sugar. All 10 structures, including the minimum energy structure in Figure 2B, have Thr3 NH hydrogen bonded to the Asn1 γ-carboxamide. In addition, the C-terminal trans NH is within hydrogen bond distance of the Asn γ-carboxamide and/or the GlcNAc endocyclic oxygen. The Asn1-Thr3 hydrogen bond forms an Asx-turn.[12,13] Under these conditions, the Asx-turn is stabilized by peptide-sugar interactions.

Other NMR data support our model. Both ^3J coupling and NH variable-temperature data indicate that N-glycosylation stabilizes the Asx-turn in glycopeptide **1** relative to the aglycosyl peptide **3**. N-Glycosylation clearly restricts Asn χ_1, as reflected by the greater $^3J_{\alpha,\beta}$ differences for glycopeptide **1** compared to **3**. At 233 K, $^3J_{\alpha,\beta}$ for glycopeptide **1** were 11.1 and 3.4 Hz, while peptide **3** had $^3J_{\alpha,\beta}$ values of 9.2 and 4.9 Hz. The $^3J_{\alpha,\beta}$ coupling data and stereospecific Asn NH-β,β' and Asn α-β,β' NOEs indicate that the glycopeptide's predominant Asn1 χ_1 rotamer is trans (±180°), consistent with a type I_t Asx-turn.[12a,14]

Temperature dependence of amide NH chemical shifts ($-\Delta\delta/\Delta T$) can identify hydrogen bonds.[15] Usually, amides with $-\Delta\delta/\Delta T$ values less than 3 ppb/K in competitive solvents are solvent shielded. Temperature coefficients were measured for glycopeptide **1** and aglycosyl peptide **3** in 30% DMSO/70% CD_2Cl_2. For the aglycosyl peptide **3**, all amides had $-\Delta\delta/\Delta T$ values greater than 6.7 ppb/K, except for the C-terminal trans NH (1.9 ppb/K), suggesting its involvement in secondary structure.[16] Glycopeptide **1** also had a C-terminal trans NH with a low $-\Delta\delta/\Delta T$ value of 1.6 ppb/K. N-Glycosylation dramatically lowered the Thr3 NH $-\Delta\delta/\Delta T$ value, from 6.7 ppb/K in **3** to 1.3 ppb/K in glycopeptide **1**. The low $-\Delta\delta/\Delta T$ values for the glycopeptide's Thr3 NH and C-terminal trans NH indicate that these two protons are hydrogen bonded. Again, the Asn1 γ-carbonyl is a likely acceptor for Thr3 NH, while the C-terminal trans NH can hydrogen bond with the GlcNAc endocyclic oxygen and/or the Asn γ-carboxamide.

We have provided the first detailed evidence for long-range peptide−sugar interactions in a flexible N-linked glycopeptide. Under these conditions, N-glycosylation stabilizes a main-chain to side-chain turn. Since turns have been implicated as folding initiation sites, our results are significant in understanding how N-glycosylation might influence peptide folding.

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Supporting Information Available: Experimental procedures, Figures 1−9, and Tables 1−3 (17 pages).

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(10) Since GlcNAc is in a $^4C_1$ conformation, diaxial interproton distances are 3.0−3.1 Å. With a 200 ms spin-locking time NOEs were not observed between the GlcNAc H1 (σ 4.60 ppm) and GlcNAc H2 (σ 3.57 ppm). Thus, a 3.1 Å upper limit was put on the distance between protons that did show NOEs.

(11) Baker, E. N.; Hubbard, R. E. *Prog. Bioophys. Molec. Biol.* **1984**, *44*, 97.

(12) Abbadi, A.; Mcharfi, M.; Aubry, A.; Premilat, S. Boussard, G.; Marruad, M. *J. Am. Chem. Soc.* **1991**, *113*, 2729.

(13) (a) Imperiali, B.; Shannon, K. L. *Biochemistry* **1991**, *30*, 4374. (b) Imperiali, B.; Shannon, K. L., Rickert, K. W. *J. Am. Chem. Soc.* **1992**, *114*, 7942. (c) Imperiali, B.; Spencer, J. R.; Struthers, M. D. *J. Am. Chem. Soc.* **1994**, *116*, 8424.

(14) Rotamer populations for **1** (P_t = 78%, P_{g^+} = 15, P_{g^-} = 7%) were calculated as described by: Kover, K.; Jiao, D.; Fang, S.; Hruby, V. J. *J. Org. Chem.* **1994**, *59*, 991.

(15) Interpretation of NH temperature coefficients can be problematic in some apolar solvents (Gellman, S. H.; Dado, G. P.; Liang, G.; Adams, B. R. *J. Am. Chem. Soc.* **1991** *113*, 1164). However, the use of NH temperature coefficients to identify hydrogen bonds in flexible peptides in DMSO is more reliable; see: Kemp, D. S.; Curran, T. P.; Boyd, J. G.; Allen, T. J. *J. Org. Chem.* **1991**, *56*, 6683.

(16) Boc-Asn-Xaa-Ser-NHMe peptides show an Asx-turn overlapped by a β-turn between the C-terminal NH and the Asn C=O; see ref 12a.