SHORT COMMUNICATIONS

The Intramolecular Side Chain Interaction in the Triplet Sequence -Asn-Gly-Ser- Studied by ^1H NMR Spectroscopy

Hiroshi ISHII, Akihiro SUZUKI, Yoshio INOUE,
and Riichirô CHÛJÔ

*Department of Polymer Chemistry, Tokyo Institute of Technology,
12-1 Ookayama 2-chome, Meguro-ku, Tokyo 152, Japan*

(Received April 7, 1983)

KEY WORDS Glycopeptide / Glycoprotein / Hydrogen Bond / ^1H NMR / Conformation /

In glycoproteins, *N*-glycosidic linkages appear at the asparagine residues located in the triplet sequence -Asn-X-Ser (Thr-).¹ Here X stands for an arbitrary amino acid residue. It has been proposed that the triplet sequence is a prerequisite for glycosylation, on the basis of a relationship between the primary structure of the model peptides containing a triplet sequence and their susceptibility to enzymatic glycosylation.²⁻⁴ The role of this triplet sequence in the biosynthesis of glycoproteins should be clarified. On the basis of consideration using the space-filling model for this sequence, Marshall¹ proposed a catalytic mechanism of the enzymatic glycosyl transfer in the triplet sequence, where a hydrogen bond is formed between the side chain hydroxyl hydrogen of hydroxyl amino acid and the side chain carbonyl oxygen of asparagine. Recently Bause and Legler⁵ proposed another mechanism in which the hydrogen bond is formed between the hydroxyl oxygen of hydroxyl amino acid and the side chain amide hydrogen of asparagine. This proposal was derived from a study of the enzymatic *N*-glycosylation of model peptides.

In this paper we report on the side chain-side chain interaction within the triplet sequence in solution. For this purpose, we synthesized two peptides, Boc-Asn-Gly-Ser-OMe and Boc-Asn-Ser-NHMe, each consisting of three peptide units, although the central residue corresponding to residue X was absent in the latter.

EXPERIMENTAL

The peptides were synthesized by the liquid phase method. The starting materials H-Ser-OH, H-Gly-OH, and Boc-Asn *p*-nitrophenyl ester (Boc-Asn-ONp) were purchased from the Protein Research Foundation, Japan.

Boc-Asn-Ser-NHMe

Boc-Ser-NHMe was first prepared from Boc-Ser-OH by the mixed anhydride method,⁶ the Boc group was then eliminated by trifluoroacetic acid (TFA), and the resulting TFA·Ser-NHMe was allowed to react with Boc-Asn-ONp in the presence of *N*-methylmorpholine. The solvent was evaporated *in vacuo* and crude Boc-Asn-Ser-NHMe was recrystallized from an ethylacetate solution.

Boc-Asn-Gly-Ser-OMe

Dicyclohexylcarbodiimide was added to a mixture of HCl·Ser-OMe, *N*-methylmorpholine, and Boc-Gly-OH in DMF. After deblocking the Boc group by TFA, the resulting TFA·Gly-Ser-OMe was allowed to react with Boc-Asn-ONp. The product was purified by recrystallization.

The peptides were identified by ^1H and ^{13}C NMR and thin layer chromatography. No trace of *p*-nitrophenol was found.

^1H NMR spectra were recorded on a JEOL JNM FX-270 spectrometer (270 MHz) at 23–53°C. The peptides were dissolved in perdeuterated dimethyl-

sulphoxide (DMSO- d_6) at a concentration of 0.03 M. Tetramethylsilane was used as the internal standard.

RESULTS AND DISCUSSION

Figure 1 shows the ^1H NMR spectrum of Boc-Asn-Gly-Ser-OMe along with the assignment of the resonances. The resonances for the backbone amide protons were assigned on the basis of the selective homo-spin decoupling. The two amide protons of the asparaginyl side chain were observed as two well-separated lines for both peptides and the lower and upper field lines were assigned to the *trans* and *cis* protons relative to the carbonyl group, respectively.⁷ The two β protons of asparagine and serine

were assigned in the usual way⁸; *i.e.*, the higher field resonance was identified as the prochiral R β -proton.

The temperature dependence of the amide proton chemical shifts was measured to estimate intramolecular hydrogen bonding.⁷ Upfield shifts were observed for all amide protons with increasing temperature from 23 to 53°C. The resonances for the *cis* and *trans* amide protons of the asparaginyl side chain broadened on raising temperature, but collapse to a single resonance was not found. This may be due to the hindered rotation about the C^γ-N^δ bond within the temperature range investigated.

In Table I are tabulated the temperature coefficients ($d\delta/dT$) of the amide proton chemical shifts. For the asparaginyl side chain, the $d\delta/dT$ value of the

Figure 1. The 270 MHz ^1H -NMR spectrum of Boc-Asn-Gly-Ser-OMe in DMSO- d_6 solution.

Table I. Temperature coefficients of the amide proton chemical shifts and the backbone dihedral angle ϕ measured in DMSO- d_6 solutions

Compound	$d\delta/dT \times 10^3$ (ppm · deg ⁻¹)					³ J(HNC ^α H) (ϕ(°)) ^a		
	Asn N ^α H	Asn N ^δ H (<i>trans</i>)	Asn N ^δ H (<i>cis</i>)	Ser N ^α H	Gly N ^α H (NHMe)	Asn	Gly	Ser
Boc-Asn-Ser-NHMe	-5.70	-4.02	-5.41	-4.70	-4.72	7.25 (-89, -151)		7.25 (-89, -151)
Boc-Asn-Gly-Ser-OMe	-4.91	-3.64	-5.32	-5.00	-4.02	7.91 (-96, -144)	5.27 (38, 82, -68, -172)	7.26 (-89, -151)

^a The dihedral angle ϕ values shown in parenthesis were calculated from proton coupling constant $^3J(\text{HNC}^*\text{H})$ using the relationship of Demarco *et al.* (1978).

trans amide proton is larger than that of the *cis* proton. This difference in both peptides is probably due to the difference in the effect from the carbonyl group. Also, there are some differences in $d\delta/dT$ between the two peptides. The values of $d\delta/dT$ for *cis* amide protons are comparable, while that of $d\delta/dT$ for the *trans* one is larger in Boc-Asn-Gly-Ser-OMe than in Boc-Asn-Ser-NHMe. Thus, the ratio *cis:trans* of $d\delta/dT$ is 1.35 and 1.46 for Boc-Asn-Ser-NHMe and Boc-Asn-Gly-Ser-OMe, respectively. This difference indicates that the *trans* amide hydrogen participates in a particular intramolecular interaction in Boc-Asn-Gly-Ser-OMe. A probable explanation for the difference in $d\delta/dT$ is the formation of a hydrogen bond involving the *trans* amide hydrogen of the asparaginyl side chain. Since both peptides have three peptide units, it is unlikely that the difference in the ratio of $d\delta/dT$ is due to the intramolecular hydrogen bonding between the *trans* amide hydrogen of asparagine and the peptide backbone. In Boc-Asn-Gly-Ser-OMe, the *trans* amide hydrogen of the asparaginyl side chain may be hydrogen bonded to the seryl side chain as proposed by Bause and Legler,⁵ where the hydroxyl amino acid side chain acts as a hydrogen

bond acceptor and the amide group of asparagine as a hydrogen bond donor. At present, there is no evidence for the formation of the hydrogen bond between the carbonyl group of the asparaginyl side chain and the hydroxyl hydrogen of serine.

We analyzed the side chain conformation in order to determine the type of the hydrogen bond. The side chain conformation of asparagine and serine vary with rotation about the $C^\alpha-C^\beta$ bond. Three stable rotamers about the $C^\alpha-C^\beta$ bond and their notation are indicated in Figure 2. In the triplet sequence -Asn-Gly-Ser- where asparagine and serine are in (tg)- or (gg)-rotamer and (gt)- or (gg)-rotamer, respectively, both side chains spatially face with each other. In this case, the side chains of asparagine and serine come close enough to interact if the backbone of the peptide takes on a suitable conformation.

Table II lists the vicinal proton coupling constants $^3J(HC^\alpha C^\beta H)$ and the rotamer populations calculated in two ways, using Pachler's⁹ and Feeney's¹⁰ approximations. The rotamer populations in Boc-Asn-Gly-Ser-OMe are not the same as those in Boc-Asn-Ser-NHMe. In the former, the population P_{tg} is larger than P_{gt} for the asparaginyl side chain. Since the most preferable rotamer is (gt) for asparagine in protein,¹¹ the result for Boc-Asn-Gly-Ser-OMe is rather unexpected. For seryl side chain, although the difference is comparable to experimental error, P_{gt} and P_{gg} are slightly larger in Boc-Asn-Gly-Ser-OMe than in Boc-Asn-Ser-NHMe. The (tg)-rotamer, in which the side chain of serine can not face that of asparagine, is sparsely populated in Boc-Asn-Gly-Ser-OMe. These results indicate that the two side chains face each other, and support the idea that hydrogen bonding be-

Figure 2. Three stable rotamers about $C^\alpha-C^\beta$ bond and their notation.

Table II. Vicinal proton coupling constants $^3J(HC^\alpha C^\beta H)$ and the rotamer populations^a measured in DMSO- d_6 solutions

Compound	Asn					Ser				
	J_{AX}	J_{BX}	P_{gt}	P_{tg}	P_{gg}	J_{AX}	J_{BX}	P_{gt}	P_{tg}	P_{gg}
Boc-Asn-Ser-NHMe	—	7.25	— ^b (—)	0.42 (—)	— ^b (—)	5.27	4.62	0.24 (0.14)	0.18 (0.17)	0.58 (0.69)
Boc-Asn-Gly-Ser-OMe	5.93	8.57	0.30 (0.26)	0.54 (0.62)	0.16 (0.12)	5.61	3.58	0.27 (0.32)	0.09 (0.00)	0.64 (0.68)

^a The population values were calculated using Pachler's and Feeney's (in parenthesis) approximations.

^b Not determined due to spectral overlapping with solvent.

tween the *trans* amide hydrogen of the asparaginyl side chain and the seryl side chain in Boc-Asn-Gly-Ser-OMe.

By CPK model building, it was found that the formation of a suitable conformation for the side chain interaction in a triplet sequence depends not only on side chain isomerism but also on backbone conformation which we also analyzed. The $d\delta/dT$ values were small for all backbone amide protons, indicating that there was no particular intramolecular interaction which folds the peptide backbone. The values of the dihedral angle ϕ listed in Table I were calculated from the vicinal coupling constant $^3J(\text{NHC}^*\text{H})$ using a Karplus-type relationship.¹² It is difficult to choose appropriate ϕ values, but the ϕ values are in a range considered reasonable from the Ramachandran plot.¹³ The peptides may take on a relatively extended backbone conformation.

The simulation with the CPK model revealed that hydrogen bonding is possible between the *trans* amide hydrogen of asparagine and the hydroxyl oxygen of serine for Boc-Asn-Gly-Ser-OMe with a relatively extended backbone conformation. The CPK model is consistent with the results from NMR study.

From an circular dichroism study using a small model peptide containing an -Asn-Gly-Ser- sequence, it has been found that the peptide chain does not arrange itself in any specially organized structure in water, but an ordered structure favorable for glycosylation in an aqueous solution containing DMSO.¹⁴ Thus, our results in DMSO may reflect a favorable conformational feature in Boc-

Asn-Gly-Ser-OMe.

In summary, our NMR study shows that a hydrogen bond is formed between the *trans* amide hydrogen of asparagine and the hydroxyl oxygen of serine in the triplet sequence. This is the first instance in which intramolecular side chain-side chain hydrogen bond is formed in Boc-Asn-Gly-Ser-OMe, and should provide a basis for studying the mechanism of the enzymatic biosynthesis of N-linked glycopeptides.

REFERENCES

1. R. D. Marshall, *Ann. Rev. Biochem.*, **41**, 673 (1972).
2. C. Ronin, S. Bouchilloux, C. Granier, and J. V. Rietscoten, *FEBS Lett.*, **96**, 179 (1978).
3. E. Bause, *FEBS Lett.*, **103**, 296 (1979).
4. E. Bause, H. Hettkamp, and G. Legler, *Biochem. J.*, **203**, 761 (1982).
5. E. Bause and G. Legler, *Biochem. J.*, **195**, 639 (1981).
6. J. C. Howard, A. Ali, H. A. Scheraga, and F. A. Momany, *Macromolecules*, **8**, 607 (1975).
7. P. H. V. Dreele, I. D. Rae, and H. A. Scheraga, *Biochemistry*, **17**, 956 (1978).
8. J. Kobayashi and U. Nagai, *Biopolymers*, **17**, 2265 (1978).
9. K. G. R. Pachler, *Spectrochim. Acta*, **20**, 581 (1964).
10. J. Feeney, *J. Magn. Reson.*, **21**, 473 (1976).
11. J. Janin, S. Wodak, M. Levitt, and B. Maigret, *J. Mol. Biol.*, **125**, 357 (1978).
12. A. Demarco, M. Llinas, and K. Wüthrich, *Biopolymers*, **17**, 637 (1978).
13. G. N. Ramachandran and V. Sasisekharan, *Adv. Prot. Chem.*, **23**, 283 (1968).
14. J. P. Aubert, N. Helbeque, and M. H. F. Lefebvre, *Arch. Biochem. Biophys.*, **208**, 20 (1981).