Solution Conformations of a Biantennary Glycopeptide and a Series of Its Exoglycosidase Products from SequentialTrimming of Sugar Residues*

(Received for publication, July 26, 1995, and in revised form, October 25, 1995)

Pengguang Wu†, Kyung Bok Lee, Yuan Chuan Lee, and Ludwig Brand§

From the Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218

Linkages between sugar residues in branched oligosaccharides exhibit various degrees of flexibility. This flexibility, together with other forces, determines the overall solution conformation of oligosaccharides. We used the method of time-resolved resonance energy transfer to study the solution conformations of a biantennary glycopeptide and its partially trimmed products by exoglycosidases. The N-terminal of the glycopeptide was labeled with 2-naphthyl acetic acid as a fluorescent donor. Either terminal sugar residue, Galβ1, on the branch bearing 6-linked Man (antenna 6), or Neu5Ac on the branch bearing 3-linked Man (antenna 6) was labeled with 5-dimethylaminonaphthalene-1-sulfonil as an acceptor. The distance and distance distributions between the terminals were measured. In the intact biantennary glycopeptide, the donor-acceptor distance distribution of antenna 6 is bimodal with a majority of the population in the extended conformation and that of antenna 6 in one very broad population. The Neu5Ac on antenna 6 is oriented toward the N-terminal at low temperature and adopts a more extended form at high temperature. The removal of individual sugar residues along one of the two antenna in the biantennary oligosaccharide has a small effect on the distance distribution of the remaining antenna for both antennae 6 and 6. Together with previous studies of the triantennary glycopeptides (Rice, K. G., Wu, P. G., Brand, L., and Lee, Y. C. (1993) Biochemistry 32, 7264–7270), our results suggest that both steric hindrance and inter-residue hydrogen bonding are very important in the folding pattern in oligosaccharide structures.

Conformational flexibility of linkages between sugar residues is an integral part of oligosaccharide structure. This is demonstrated in X-ray structures as multiple conformations (2) or conformations selected according to protein-carbohydrate interactions (3–5). Oligosaccharides in solution are subject to thermal fluctuations and consequently they sample various conformations, which leads to heterogeneity in the structure. Great efforts both in the experimental and theoretical areas have been devoted to the determination of solution structure and conformation of oligosaccharides (6–8).

In previous work, we used the method of time-resolved resonance energy transfer to determine the solution conformations of complex triantennary glycopeptides. Both extended and folded conformations of carbohydrate chains were observed (9) and the conversion between the two forms was temperature-dependent (10). These results suggested that some linkages between sugar residues can rotate to a large extent under certain conditions. When the triantennary glycopeptides were treated with exoglycosidases to produce single chain isomers, the extended conformation was the dominant form (1). Thus the intrinsic flexibility of linkages themselves does not automatically lead to a large conformational heterogeneity in oligosaccharides with multiple antennae. Other factors such as steric hindrance and hydrogen bonding may play roles in the solution behaviors of oligosaccharides.

In this work we present results on a biantennary glycopeptide and its partially digested products by exoglycosidases. We used time-resolved resonance energy transfer methods to determine the average end-to-end distances and distance distributions between the N-terminal and each one of the sugar terminals. Our aim is 3-fold. (i) How does the intact biantennary behave in solution compared to the triantennary glycopeptides reported recently? (ii) How does the sugar residue Neu5Ac behave at the end of the carbohydrate chain? (iii) How do the properties of the biantennary glycopeptides change when one or more sugar residues on one antenna are removed?

MATERIALS AND METHODS

Selective Fluorescent Labeling of the Biantennary Glycopeptides—The details for preparation of the doubly fluorescing labeled glycopeptide has been published elsewhere (11). A brief description of the selective introduction of a fluorophore at a specific branch on a biantennary glycopeptide is described below. The monosialyl biantennary glycopeptides derived from bovine fibrinogen were treated with galactose oxidase to convert the terminal Galβ1 on the Man(1–6)Man branch into 6-oxo-galactose. The oxidized glycopeptides (C6 aldehyde of galactose) were coupled with 2-(dansylamido)ethylamine by reductive amination and 2-naphthylacetic acid was attached to the N terminus of the peptide. Alternatively, 2-naphthylaetic acid was first attached to the N terminus of the peptide portion on the monosialylated glycopeptide and the sialic acid on the Man(1–3)Man branch was oxidized with periodate. The oxidized glycopeptide (exo-sialic acid) was coupled with 2-(dansylamido)ethylamine by reductive amination. All of the doubly fluorescent labeled glycopeptides were purified to homogeneity on reverse phase-high performance liquid chromatography and characterized by monosaccharide and amino acid analysis.

Time-resolved fluorescence decays were measured on a photon-counting instrument with a picosecond dye laser system as described earlier (10). The overall instrument response is about 60 ps. The excitation wavelength was set at 290 nm and the emission wavelength of donor naphthyl was set at 340 nm with a polarizer oriented at the magic angle 54.7°. A dilute Ludox (silica) scattering solution was used to collect the instrument response. The temperature of the cuvette was controlled by a circulating water bath. Duplicate or triplicate data sets were collected. The decay data were analyzed by nonlinear least squares as described (9, 10). Briefly, the donor decay in the absence of
acceptor dansyl\(^1\) was analyzed by a sum of exponentials:

\[
I(t) = \sum \alpha_i \exp \left(-\frac{t}{\tau_i}\right) \quad \text{(Eq. 1)}
\]

where \(\alpha_i\) and \(\tau_i\) are the amplitude and lifetime of component \(i\). The quality of fit was judged by the reduced-\(x^2\), weighted residuals, and the autocorrelation of the residuals. A good fit was accepted when the reduced-\(x^2\) was between 1.0 and 1.2 with random residuals and autocorrelation of the residuals. The average lifetime was calculated from \(\tau = \sum \tau_i / 2\). The donor decay in the presence of dansyl was also analyzed empirically by Equation 1 to obtain the average lifetime. Once the average lifetimes of the donor in the absence and presence of the acceptor were obtained, the energy transfer efficiency was calculated by \(E = 1 - \frac{\tau_{DA}}{\tau_{0}}\). The average distance between donor naphthyl at the N-terminal and acceptor dansyl at the antenna terminal was calculated according to the Förster distance (22 Å at 20°C) of the donor-acceptor pair evaluated as before (9). The donor decay in the presence of the acceptor was analyzed by a model of distance distributions,

\[
I_{DA}(t) = \sum \alpha_i \sum \exp \left[ \frac{-1}{\pi} \left( \frac{1}{\tau_i} + \frac{1}{\tau_D} \right) \right] \exp \left( -\frac{t}{\tau_D} \right) \quad \text{(Eq. 2)}
\]

where the first sum refers to the number of populations, each with a concentration of \(\alpha_i\), and a distance distribution where \(\tau_D = \tau_i\). The distance distribution was modeled either as a Gaussian,

\[
p(r) = \frac{1}{\sqrt{2\pi\sigma}} \exp \left[ -\frac{(r - \bar{r})^2}{2\sigma^2} \right] \quad \text{(Eq. 3)}
\]

or by a Lorentzian

\[
p(r) = \frac{1}{\pi \sigma} \left( 1 + \frac{(r - \bar{r})^2}{\sigma^2} \right)^{-1} \quad \text{(Eq. 4)}
\]

where \(\bar{r}\) is the average distance and \(\sigma\) is the standard deviation. When the distance distribution is asymmetric, an asymmetry parameter was used as before (12). Equation 2 was used to fit experimental data by nonlinear least squares, with the average distance, the standard deviation, and the concentration as the adjustable parameters.

**RESULTS**

Our previous results showed that there is a large fraction of folded conformation in both antennae 6 and 6' in the triantennary structure (9, 10). When two antennae were completely removed (resulting in three single chain isomers), there is a substantial increase in the average donor-acceptor distance in antennae 6 and 6' (1). Starting with a biantennary structure in this work, one might expect either that there has already been a large conformational change from a triantennary to a bi-antennary structure or that significant changes occur only from a biantennary to a single chain structure. The following results show that conformational changes are relatively minor from a biantennary to a single chain structure, thus implying that a substantial conformational change may have already occurred from a triantennary to a biantennary structure.

**Average Distances as a Function of Temperature and Cleaved Sugar Residues**—In this section we compare the average distances between N-terminal naphthyl and the dansyl group attached to either Gal' or antenna 6' or Neu5Ac on antenna 6 (see Fig. 1 for residue and antenna assignment). These distances were measured in the intact biantennary structure as a function of temperature, in a series of partially digested bi-antennaries, and in the single chain isomers. The results are shown in Tables I and II and can be summarized as follows.

(a) With a stepwise removal of sugars in either antenna 6 or 6', the change in average distances is small from the biantennary to the single chain structures and is not uniform (or monotonic) in that the removal of either GlcNAc 5 or 5' leads to a minor decrease. Thus we do not expect a large shift in conformation from a biantennary to a single chain isomer.

(b) For antenna 6', the average distance changes are 1.7 Å in the biantennary and 1.6 Å in the single chain structure from 1 to 40°C. The decrease in distance is comparable with that of a similar single chain glycopeptide containing only a variation in the amino acids (1), but not to that of antenna 6' in the triantennary glycopeptide, at 1, 20, and 40°C, respectively (from Ref. 1).

(c) In antenna 6, the average distance is 17.2 Å in the biantennary glycopeptide at 20°C. This is about 3 Å shorter than that of the same antenna without Neu5Ac in the triantennary and about 6 Å shorter than that of the single chain isomer 6.

---

\(^1\)The abbreviation used is: dansyl, 5-dimethylaminonaphthalene-1-sulfonyl.
lacking Neu5Ac (1). Since there is one more sugar unit in antenna 6 of the biantennary structure, the end to end distance should be longer were all sugars in straight conformation. The shortening in the distance in this antenna implies that the linkage between Neu5Ac and Gal6 must be oriented such that Neu5Ac folds back toward the N-terminal in order to reduce the effective end to end distance. This is corroborated by the temperature dependence of the average distances. The change in the average distances from 1 to 40 °C in antenna 6 of the biantennary and single chain is about 0.5 Å, which is much smaller than that of antenna 6'. As shown earlier (1, 10), the smaller change is due to some conformational change. In this case it can only occur between Neu5Ac and Gal6.

Distance Distribution of the Biantennary Glycopeptides—It has been shown recently that both antennae 6 and 6' in a triantennary glycopeptide can adopt a substantial fraction of folded conformation at or below room temperature (9, 10). It is interesting to examine how these two antennae behave in the biantennary structure in solution. The distance distribution fits are shown in Fig. 2 for antenna 6 and in Fig. 3 for antenna 6'. The decay of donor naphthyl with the acceptor attached to Neu5Ac in antenna 6 can be described by one asymmetric Lorentzian (also by one asymmetric Gaussian. Since we have used Lorentzian form earlier, this will be the form used throughout the paper.) If we fit with two distance distributions

---

**Fig. 2.** Distance distributions (peak-normalized) of antenna 6 in the intact biantennary glycopeptide as a function of temperature. One asymmetric distance distribution was used in the fit.

**Fig. 3.** Distance distributions of antenna 6' in the intact biantennary glycopeptide as a function of temperature. Two distance distributions were used in the fit. The distributions are plotted in two ways: in A, the population of each distribution is proportional to the area; in B, is proportional to the peak value.

**Fig. 4.** Distance distributions (peak-normalized) of antenna 6 in the partially digested biantennary glycopeptides as a function of sugar residues removed from antenna 6'. Solution temperature was at 20 °C. Sugar residues trimmed are indicated in the figure.

**Fig. 5.** Distance distributions (peak-normalized) of antenna 6' in the partially digested biantennary glycopeptides as a function of sugar residues removed from antenna 6. Solution temperature was at 20 °C. Sugar residues trimmed are indicated in the figure.
and superimpose them, we then get essentially one asymmetric distribution back. Since the distribution is quite broad, it is difficult to separate two populations under most circumstances. The collapse to one population is not entirely due to the Neu5Ac sugar unit since we can also fit the decay of a similar biantennary sample lacking Neu5Ac (and with three more amino acids) in antenna 6 with one asymmetric Lorentzian distance distribution. The decay of naphthyl with dansyl attached to Gal6 in antenna 6 of the biantennary on the other hand requires two distance distributions, similar to the case of antenna 6 in the triantennary glycopeptide (9). The concentration of the folded form is nonetheless greatly diminished in the biantennary and is thus much less sensitive to temperature modulation than its counterpart in the triantennary (10). Thus substantial changes have already occurred from the triantennary to the biantennary structures.

Changes in Distance Distribution in the Partially Digested Biantennary Glycopeptides—In order to determine what the factors are that modulate the conformational changes in the carbohydrate chains, we used exo-glycosidases to remove one sugar residue at a time and monitored the changes in the distance distribution.

Fig. 4 shows the change in distance distribution of antenna 6 when sugar residues Gal6, GlcNAC5, and Man4 on antenna 6 are sequentially removed. The removal of Gal6 has no noticeable effect on the distance distribution on antenna 6 and the cleavage of GlcNAC5 slightly broadens the distribution. Cutting Man4 leads to a small decrease in the width of the distribution. Nonetheless the distance distribution of the single chain 6 is quite broad.

DISCUSSION

The end to end distances of the biantennary glycopeptide and those of its single chain isomers show some interesting features compared to those of a triantennary glycopeptide and its single chain isomers (9, 10). In the triantennary glycopeptide, the presence of antenna 8 apparently forces antennae 6 and 6 to fold back half of the time (9). In the biantennary glycopeptide, on the other hand, the major portion of the population is in the extended conformation in both antenna 6 and 6. This implies that antenna 8 in the triantennary glycopeptide has substantial steric effects on the solution behaviors of other antennae. Thus steric hindrance is important in determining the overall folding pattern of carbohydrate chains. In antenna 8, all sugars
except for Man4-Man3 are β1,4-linked, which are known to be conformationally rigid. The rigidity of antenna 8, in tri-, bi-, and mono-antennary structures suggests that α1,3-linkage in the Man4-Man3 is also a rigid one. Therefore antenna 8 appears more rigid and forces other antennae to adopt an alternative conformation in a crowded structure such as a triantennary. A schematic illustration of the conformational flexibilities in triantennary and biantennary glycopeptides is shown in Fig. 6. In the triantennary structure, there is an equilibrium between the fold-back and open conformation in either antenna 6 or antenna 6′, while in the biantennary structure, the equilibrium is predominantly shifted toward the open form.

Our results show that Neu5Ac has orientations more toward the N-terminal. This can be viewed as folded-back conformations as shown in Fig. 7. The α2–6-linkage, like α1–6-linkage (14, 15), is flexible arising from the rotation of several single bonds. Since this sugar residue is at the end of the antenna, it is unlikely that steric hindrance is the determining factor for the conformation observed. Another factor may be hydrogen bonding between sugar residues. Molecular dynamics simulations of the biantennary glycopeptide up to 200 ps have shown that Neu5Ac can form many hydrogen bonds with various inner sugar residues (13). If a sufficient number of hydrogen bonds are formed, a folded-back conformation can be stabilized. As solution temperature increases, more motions are produced, which leads to breakage of hydrogen bonds. This in turn results in a more extended conformation for Neu5Ac. The shift to an extended conformation compensates the temperature-enhanced intramolecular diffusion between the two ends (which reduces apparent distances when this motion is not included in data analysis) so that a smaller apparent change in average distances is detected.

The wide distance distribution of the single chain 6 can be understood in the following. From the results of Rice et al. (1), the single chain 6 lacking Neu5Ac has quite a wide distance distribution. This can be attributed to the flexibility of β1,2-linkage between GlcNAc5 and Man4. The linkage between Neu5Ac and Gal6 is the α2–6 type, which is also expected to be flexible. The combination of the two factors are thus responsible for the asymmetric distance distribution.

REFERENCES

1. Rice, K. G., Wu, P., Brand, L., and Lee, Y. C. (1993) Biochemistry 32, 7264–7270
2. Wright, C. S. (1992) J. Biol. Chem. 267, 14345–14352
3. Shaanan, B., Lis, H., and Sharon, N. (1991) Science 254, 862–866
4. Bourne, Y., Rouge, P., and Cambillau, C. (1992) J. Biol. Chem. 267, 197–203
5. Weis, W. I., Drickamer, K., and Hendrickson, W. A. (1992) Nature 260, 127–134
6. Carver, J. P. (1991) Curr. Opin. Struct. Biol. 1, 716–720
7. Bush, C. A. (1992) Curr. Opin. Struct. Biol. 2, 655–660
8. Rice, K. G., Wu, P. G., Brand, L., and Lee, Y. C. (1993) Curr. Opin. Struct. Biol. 3, 669–674
9. Rice, K. G., Wu, P., Brand, L., and Lee, Y. C. (1991) Biochemistry 30, 6646–6653
10. Wu, P., Rice, K. G., Brand, L., and Lee, Y. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9355–9359
11. Lee, K. B., and Lee, Y. C. (1999) J. Biol. Chem. 274, 1462–1469
12. Wu, P., and Brand, L. (1992) Biochemistry 31, 7593–7597
13. Dauchez, M., Mazurier, J., Monteuil, J., Spik, G., and Veroten, G. (1992) Biochimie (Paris) 74, 63–74
14. Cumming, D. A., and Carver, J. P. (1987) Biochemistry 26, 6676–6683
15. Homans, S. W., Dwek, R. A., and Rademacher, T. W. (1987) Biochemistry 26, 6571–6578