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Comparison of the Solution Structure of Vancomycin with Its X-ray Crystallographic Structure

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Abstract Since pathogens resistant against vancomycin occur rapidly, the development of a new drug is needed. To make a new drug based on a rational drug design, the structural study of vancomycin is necessary. Accordingly, this study reports on a comparison of the solution structure of vancomycin determined by NMR spectroscopy, which was performed in the present work, with the X-ray crystallographic structure previously deposited in the Protein Data Bank (PDB).

Key words: Vancomycin, NMR spectroscopy, X-ray crystallography, solution structure

Vancomycin (Fig. 1) is a secondary metabolite obtained from soil microorganisms such as *Nocardia orientalis* or *Streptomyces orientalis* [7]. Vancomycin has been used for treating Gram-positive infections, especially infections caused by methicillin-resistant *Staphylococcus aureus* for the past forty years [10], and because of its important roles, it has also been the focus of many studies. Vancomycin is a glycopeptide that inhibits biosynthesis of the cell wall of bacteria [11]. Despite its wide use compared with other glycopeptide antibiotics, pathogens that are resistant against vancomycin occur rapidly, therefore, the development of a new drug is needed [8]. Unlike previously, recent drug discovery has been based on rational drug design, therefore, the structural study of vancomycin is very important. Several X-ray crystallographic structures for complexes of vancomycin with its ligands have already been studied, and deposited in the Protein Data Bank (PDB) [5, 6, 9, 13]. However, in order to obtain the solution structure of vancomycin, a three-dimensional study using NMR spectroscopy is required. Accordingly, this study reports on a comparison of the solution structure of vancomycin obtained from NMR spectroscopy with its X-ray crystallographic structure.

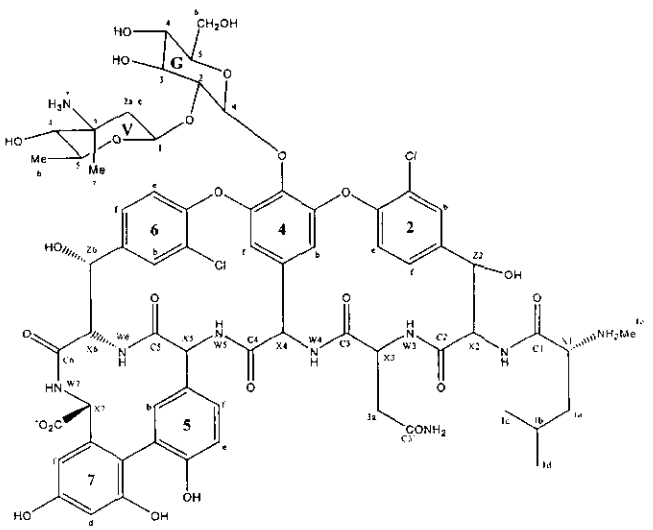

Fig. 1. Structure of vancomycin and its numbering.

The X-ray crystallographic structure was obtained from the PDB and the solution structure was determined by the current study using NMR spectroscopy. The NMR experiments were carried out on a Bruker ARX400 NMR spectrometer (9.4 T). The NMR spectra including the ^{1}H-NMR, ^{13}C-NMR, distortionless enhancement of polarization transfer (DEPT) [4], correlated spectroscopy (COSY) [2], homonuclear hartmann hahn spectroscopy (HOHAHA) [1], nuclear overhauser exchanged spectroscopy (NOESY) [14], and heteronuclear multiple quantum coherence (HMQC) [3] were collected in DMSO-d_6. The concentration of the sample was 50 mM and the experimental temperature was 298 K. The sample of vancomycin produced by *Nocardia orientalis* was supplied by the Cheiljedang R&D Center (Ichun, Kyunggi-Do, Korea). Because the purity of the vancomycin was 99.0% based on an HPLC analysis, no further purification was necessary. For the ^{1}H-NMR experiments, 32 transients were acquired with a 1 sec relaxation delay using 32 K data points, and the 90° pulse

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was 9.7 μsec, with a spectral width of 6,500 Hz. For the ^{13}C-NMR and DEPT experiments, 1,024 transients were acquired with a 2 sec relaxation delay using 64 K data points, and the 90° pulse was 9.8 μsec with a spectral width of 31,250 Hz. The two-dimensional spectra were acquired with 2,048 data points in t2 increments and 256 in t1 increments. All the computational calculations were performed using MSI software (San Diego, U.S.A.) on a Silicon Graphics INDY R4400 workstation.

The ^{1}H chemical shifts of vancomycin as determined by ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, COSY, and HOHAHA are listed in Table 1. The interproton distances, r, were calculated based on the integration of the NOESY cross peaks. A mixing time of 1 sec was employed for the NOESY data. The 116 cross peaks observed in the two-dimensional NOESY spectrum were assigned and the results are listed in Table 2.

Table 1. ^{1}H chemical shifts of vancomycin assigned based on ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, COSY, and HOHAHA.

δ (^{1}H/ppm)	Assignment
1.07	V6
1.35	V7
0.95	1c
0.85	1d
1.70	1b
2.45	1e
1.72	V2ax
1.89	V2eq
2.42	3a
2.12	3a'
1.48	1a'
1.51	1a
4.35	X3
4.45	X5
5.78	X4
4.42	X7
4.90	X2
3.50	G6a'
3.68	G6a
4.05	G6-OH
3.25	X1
4.20	X6
4.70	V5
3.25	G4
5.13	G4-OH
3.18	V4
5.43	V4-OH
5.18	Z2
5.89	Z2-OH
5.12	Z6
5.95	Z6-OH
3.48	G3
5.38	G3-OH
327	G5
3.53	G2
5.25	V1
5.29	G1
6.45	7d
5.22	4f
6.29	7f
5.55	4b
6.72	5e
7.35	6e
7.25	2e
6.77	5f
7.58	2f
7.45	6f
7.88	6b
7.42	2b
7.18	5b
6.62	W3
6.67	W6
7.93	W2
8.25	W4
8.48	W7
8.64	W5
9.12	OH
9.42	OH

Because the cross peak intensity is proportional to a reciprocal value of r^6 as in the following equation [12], the volumes of the NOESY cross peaks were measured using the Felix software (MSI, SanDiego, U.S.A.) and the interproton distances calculated.

$$\frac{\eta_{reference}}{\eta_{sample}} = \frac{(r_{sample})^2}{(r_{reference})^2}$$

where η and r denote the nuclear Overhauser effect (nOe) and distance, respectively, which are listed in the second and third column of Table 2.

In order to obtain the calculated structure using the interproton restraints, distance geometry was applied using the DGII program (MSI, San Diego, U.S.A.). The structures

Table 2. Assignments, volumes, and distances of 116 cross peaks observed in two-dimensional NOESY.

Assignment	Volume	Distance (Å)
1e/1c	0.7064E+06	4.054
V6/V7	0.4132E+07	2.905
V4/V6	0.9547E+07	2.730
G4/V6	0.6962E+06	4.224
G3/V6	0.2010E+07	3.540
G6a'/V6	0.2155E+07	3.500
G2/V6	0.2366E+07	3.444
V5/V6	0.9827E+07	2.717
V2eq/V7	0.4135E+07	3.140
V2ax/V7	0.1958E+07	3.555

Table 2. Continued.

Assignment	Volume	Distance (Å)	Assignment	Volume	Distance (Å)
V4/V7	0.8671E+07	2.829	Z6/X5	0.4583E+07	3.086
G4/V7	0.1074E+07	3.932	4f/X5	0.8994E+07	2.758
G3/V7	0.4310E+07	3.117	X4/X5	0.2011E+07	3.540
G6a'/V7	0.3428E+06	4.753	Z6-OH/X5	0.1185E+07	3.863
G2/V7	0.2435E+07	3.430	7f/X7	0.5759E+07	2.970
V5/V7	0.8672E+07	2.717	5e/X5	0.1721E+07	3.633
1a/1a'	0.3119E+06	4.829	5f/X5	0.3721E+07	3.195
1b/1a'	0.5254E+06	4.428	5b/X5	0.1448E+07	4.180
V2ax/V2eq	0.4294E+07	3.120	6b/X5	0.1051E+08	2.687
V5/V4	0.7007E+07	2.875	W7/X7	0.6276E+07	2.928
G6a'/G4	0.1346E+08	2.577	W5/X7	0.1242E+07	3.830
G6a/G5	0.3202E+07	2.934	V1/V5	0.1096E+07	3.914
V2eq/G3	0.2409E+07	3.434	G1/V5	0.1608E+07	3.673
V2ax/G3	0.1418E+07	3.751	Z2/X2	0.7521E+07	2.841
V5/G3	0.1326E+07	3.792	4b/X2	0.7302E+06	4.125
V2eq/G2	0.1525E+07	3.709	2b/X2	0.4721E+07	3.070
V2ax/G2	0.5590E+06	4.382	2f/X2	0.7356E+06	4.098
G6a'/G6a	0.9420E+07	2.736	Z6-OH/Z6	0.5798E+07	2.967
V5/G2	0.1190E+07	3.858	5e/Z6	0.4567E+07	3.492
G5/G6-OH	0.2530E+07	3.407	5b/Z6	0.6142E+07	2.939
G3/G6-OH	0.4850E+07	3.057	6f/Z6	0.2182E+07	3.087
G6a'/G6-OH	0.1088E+07	3.920	6b/Z6	0.2298E+08	2.359
G6a/G6-OH	0.1830E+07	3.596	W7/Z6	0.3217E+07	3.273
X7/X6	0.6876E+07	2.884	2b/Z2	0.8851E+07	2.720
3a'/X3	0.9388E+06	3.994	2f/Z2	0.1993E+07	3.564
1c/X2	0.3792E+07	3.185	X4/4f	0.5106E+07	3.030
V1/V6	0.1880E+07	3.580	5b/4f	0.3130E+07	3.301
G1/V6	0.1755E+07	3.623	W5/4f	0.3513E+07	3.226
2e/V6	0.1676E+07	3.647	2e/V1	0.3477E+07	3.230
V1/V7	0.4262E+07	3.123	6e/G1	0.1317E+07	3.802
G1/V7	0.2340E+07	3.451	X4/4b	0.5945E+07	2.955
6e/V7	0.1676E+07	3.442	2e/4b	0.4571E+07	3.089
V1/V2eq	0.7367E+07	2.851	2f/4b	0.1358E+07	3.778
V1/V2ax	0.4968E+07	3.044	W5/4b	0.9379E+06	4.019
Z6/G4	0.2687E+07	3.372	5f/X4	0.4870E+07	3.054
G3-OH/G4	0.2249E+07	3.474	W5/X4	0.8552E+07	2.781
V1/G5	0.1336E+08	2.776	5e/Z6-OH	0.4730E+07	3.069
G1/G5	0.1393E+08	2.776	5b/Z6-OH	0.1289E+07	3.811
Z6/G3	0.7826E+07	3.372	6e/Z6-OH	0.4977E+07	3.043
Z2/G3	0.3321E+07	3.293	6f/Z6-OH	0.8946E+07	2.746
G3-OH/G3	0.5875E+07	2.960	6b/Z6-OH	0.5067E+07	3.032
V4-OH/G3	0.3100E+07	3.293	W7/Z6-OH	0.8258E+07	4.105
Z2-OH/G3	0.5508E+07	2.992	5b/7f	0.7255E+06	4.196
Z6-OH/G3	0.7359E+07	2.851	W7/7f	0.8258E+06	4.105
G3-OH/G6a'	0.1874E+07	3.583	6e/5e	0.4537E+07	3.090
V1/G2	0.1189E+08	2.632	6f/5e	0.9988E+07	2.710
G1/G2	0.7140E+07	2.866	6b/5e	0.1868E+07	3.583
Z6/G6a	0.1674E+07	3.651	W5/5f	0.1432E+07	3.747
Z6/X6	0.4474E+07	3.098	6b/5b	0.9306E+07	2.742
4f/X6	0.3699E+07	3.198	W7/5b	0.5526E+07	2.990
5b/X6	0.8296E+07	2.795	W5/5b	0.5886E+06	4.344
6b/X6	0.9085E+07	2.753	2f/2e	0.1631E+08	2.500
W7/X6	0.2935E+07	3.3323	W7/6b	0.2943E+07	3.323

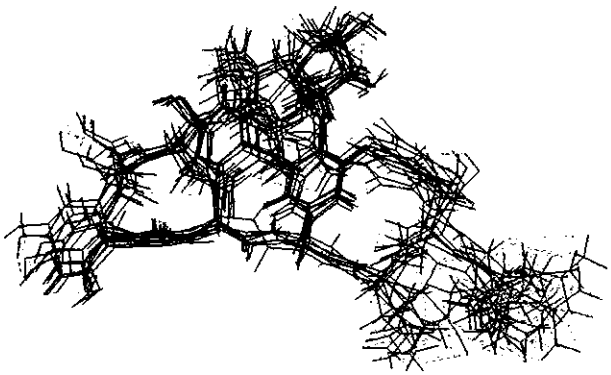

Fig. 2. Best-fit superposition of 10 solution structures obtained using NMR spectroscopy.

were calculated on the basis of the 71 nOe restraints, and, as a result, the 10 structures exhibiting the lowest total energy were selected. A best-fit superposition of the 10 structures is shown in Fig. 2. Unlike the central ring area, the first residue and vancosamine could not be superposed very well, because they were continuously moving in the solution.

The X-ray crystallographic structure was obtained from the PDB, which was coded as 1C0Q and deposited by P. J. Loll *et al.* [5]. Among the 12 vancomycin derivatives in the PDB, 9 structures had been determined by X-ray crystallography. All of them included ligands as well as a dimer. The only structure obtained by NMR spectroscopy was a 4-epi-vancosaminyl derivative. The other two structures were determined by theoretical calculations. As a result, all the structures in the PDB were different from the solution structure determined in the current work. Since the crystallographic structure used for comparison, 1C0Q, was a dimer and included lactic acid as a ligand, the molecule 2 of the dimer and ligand were deleted.

When the crystallographic structure was compared with our 10 solution structures, the average value of the root mean squares (rms) was found to be 1.58 Å. Two central rings composed of five residues and three phenyl rings exhibited a good superposition. However, as shown in a best-fit superposition of the solution structure, the first residue and vancosamine were not superposed very well. In addition, the glucose did not overlap either. Therefore, even though a few parts of the solution structures fit the crystallographic structure well, those of the averaged solution structure did not fit. In conclusion, the sugar parts such as the vancosamine and glucose differed between each structure. While the sugar parts of the solution structure covered the two central rings, those of the crystallographic structure were far away from the central region. In the latter case, the reason for this can be explained based on the existence of a ligand. Since the ligand of the crystallographic structure is positioned beside the central region, it protects the sugar parts from covering the two central rings. In future work, the current authors intend to confirm this theory by establishing the solution structure of vancomycin including a ligand.

Acknowledgments

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