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Structure of Vancomycin and a Vancomycin/D-Ala-D-Ala Complex in Solution

Henriette Molinari[‡] and Annalisa Pastore*
EMBL, Meyerhofstrasse 1, 6900 Heidelberg, West Germany
Lu-yun Lian,[§] Geoffrey E. Hawkes,[∥] and Keith Sales[∥]
NMR Biological Center, University of Leicester, Leicester, England, and Queen Mary College, Mile End Road, London E1 4NS, England
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ABSTRACT: Restrained molecular dynamics simulations were used to study the interactions between the glycopeptide antibiotic vancomycin and the dipeptide Ac-D-Ala-D-Ala. Restraints were obtained from a combination of homonuclear and heteronuclear two-dimensional NMR experiments (NOESY, ROESY, $^{1}H-^{15}N$ inverse correlation). The comparison between the structures obtained for vancomycin alone and for the complex suggests a new hypothesis on the binding mode of this system. The numerical simulations were not straightforward because vancomycin is made of building blocks for which standard force-fields are not available. The representation of unusual chemical environments is also mandatory. We believe that our extension of the force-field parameters to our system could be of more general interest. Furthermore, we consider vancomycin and its complex a good example for exploring the more general problem of molecular recognition, a challenge that has been widely approached in the past few years but for which no unique and general methodology has, so far, been recognized.

The glycopeptide antibiotic vancomycin (see structure I) from *Streptomyces orientalis*, first reported in 1956 (McCormick et al., 1956), is of interest not only for its renewed clinical importance as a therapeutic agent in the treatment of methicillin-resistant staphylococcal infections (Karchmer et al., 1983) but also because it represents one of the smallest peptide–peptide binding systems where specific and tight interaction is achieved. The bacteriocidal actions of many antibiotics are associated with the inhibition of cell-wall biosynthesis that results from the binding of the antibiotics to enzymes and intermediates in the cell-wall biosynthetic pathway (Strominger, 1969). It has been suggested (Rogers, 1969; Rogers & Forsberg, 1971) that when cell-wall biosynthesis is inhibited, the natural ongoing autolysis of the cell wall is no longer in balance with the insertion of new cell-wall material. The bacteriocidal effect of vancomycin, in the cell, is associated with the strong binding of vancomycin to the cell wall. This is likely to result in changes in the three-dimensional arrangements of the cell-wall polymers and in the contacts between the cell wall and protoplast membrane, thus affecting the relative rates of cell-wall autolysis and the insertion of new cell-wall material (Best & Durham, 1965). It is therefore of interest to study models of the binding mode of this antibiotic.

The complex between vancomycin and the dipeptide Ac-D-Ala-D-Ala is a simple good model to simulate the binding (Nieto & Perkins, 1971), since this dipeptide represents a common C-terminal unit in the cell walls of several bacterial species (Perkins, 1982). On the basis of the NMR data as well as of molecular modeling and chemical modifications, Williams and Convert, who first investigated the complex, have proposed the hydrogen-bonding pattern in the complex (Williams, 1984; Kannan et al., 1988; Convert et al., 1980). They assumed the rotation of the *N*-Met-leucine side chain as a driving force for the complexation, which would allow a closer intermolecular distance between the dipeptide carboxylate and the vancomycin ammonium group (see structure I).

Popieniek and Pratt (1988) have recently explored in more detail the kinetics and the mechanism of the binding of three

* To whom correspondence should be addressed.
‡ Permanent address: Dipartimento di Chimica Organica e Industriale, Via Golgi 19, 20133 Milano, Italy.
§ University of Leicester.
∥ Queen Mary College.

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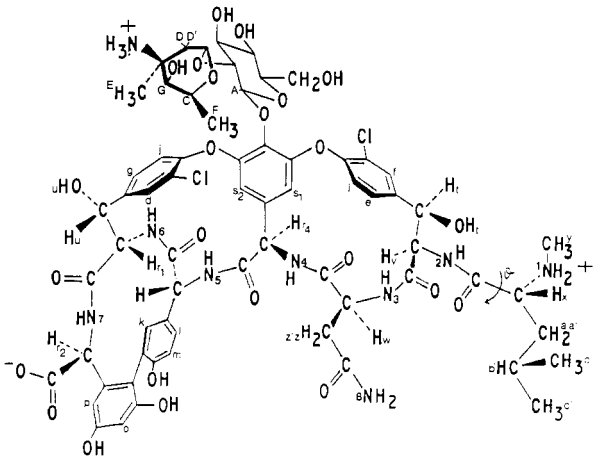

I

antibiotics (vancomycin, ristocetin, and avoparcin), using fluorescence techniques. All these are very similar in structure, but vancomycin presents a mobile *N*-Met-Leu side chain. They found comparable binding rates and the same rate-determining step in all three cases. This would exclude a conformational change specific to vancomycin as the driving force in the binding.

A model able to explain these experimental results is still lacking and a contribution in this direction could arise from a theoretical approach. As the static modeling techniques are not always adequate to provide a comprehensive description of the physicochemical behavior of molecular associations, we carried out restrained molecular dynamics (RMD) simulations on both vancomycin and its complex with D-Ala-D-Ala, in the manner of Fesik's work on the structure of the complex of ristocetin pseudo-aglycon with Ac_2-Lys-D-Ala-D-Ala (Fesik et al., 1986). We indeed thought that a comparison between the free and bound structures of vancomycin would also be helpful in clarifying the model.

We applied both homo- and heteronuclear two-dimensional NMR techniques to obtain quantitative distance information.

The numerical simulations were not straightforward because vancomycin is made of building blocks for which standard force-fields are not available. The representation of unusual chemical environments is also mandatory. We believe that our extension of the force-field parameters to our system could be of more general interest. Furthermore, we consider vancomycin and its complex a good example for exploring the more general problem of molecular recognition, a challenge that has been widely approached in the past few years (de Vlieg et al., 1986; Billeter et al., 1987a,b) but for which no unique and general methodology has, so far, been recognized.

Experimental Procedures

Vancomycin hydrochloride is commercially available from the Lilly Co., Indianapolis, and we used their product without any further purification. Ac-D-Ala-D-Ala was prepared by acetylation of D-Ala-D-Ala using acetic anhydride in water.

Spectra were obtained with a Bruker AM-250, a WP-400-SY, and an AM-500 operating at 250.13, 400.13, and 500.13 MHz, respectively. The solutions were typically 2 mM in DMSO-d_6, both for vancomycin and for the complex. In the case of the complex a ratio of 1:1.5 vancomycin/Ac-D-Ala-D-Ala was generally employed. Experiments were performed in the temperature range 283–330 K. Two-dimensional proton-correlated COSY and DQF COSY spectra were performed by using the standard pulse sequences, in the phase-sensitive mode, acquired on a typical spectral window of 4000–5000 Hz. The 2D data matrix consisted of 2K × 512 spectra, which yielded, after zero-filling in the F_1 dimension and Fourier transformation, a 2K × 1K matrix.

The HOHAHA (Bax & Davis, 1985) experiment was performed by using the MLEV17 composite pulse cycle for the generation of the spin-lock field of (γB_2) = 12.5 kHz, which was applied for 50 ms. At the beginning and at the end of the mixing time two "trim" pulses of 2.5 ms each were applied.

2D phase-sensitive NOESY experiments were performed at six different mixing times, 100, 200, 250, 350, 450, and 550 ms, for two different temperatures, 283 and 298 K. For the complex, only the shortest mixing time was used. The intensities of cross-peaks were taken from single cross sections.

The ROESY experiments were performed by using both the scheme originally described by Bothner-By (Bothner-By et al., 1984) and Bax (Bax & Davis, 1985), employing a continuous spin-lock pulse (γB_2 = 5.6 kHz) for 200 ms, and the scheme described by Kessler (Kessler et al., 1987) with a mixing pulse of 200 ms and a τ/P_1 ratio equal to the duty cycle, to minimize Hartmann–Hahn transfer. The Bothner-By experiment was repeated at different carrier frequencies to check for scalar contributions.

Heteronuclear correlations in the inverse mode were performed by employing the Bird sequence described by Bax (Bax & Subramanian, 1986).

Molecular dynamics calculations were carried out on the EMBL VAX-8650 using the GROMOS package (van Gunsteren & Berendsen). The system was weakly coupled to a thermal bath (Berendsen et al., 1984) of T = 293 K with a temperature relaxation time of 0.01 ps during the first picosecond of thermal equilibration and 0.1 ps during all the following runs. To reduce the number of van der Waals interaction pairs considered both in the dynamics and in energy minimization, interactions were neglected when the atomic separation was larger than 1.0 nm. Such a list was updated every 10 steps of 2 ps during the dynamics and every 10 cycles of the minimization, respectively.

The bond length constraining algorithm (SHAKE) (Ryckaert et al., 1977; van Gunsteren & Berendsen, 1977) was used in the simulations with the solvent but not in the simulations in vacuo. During preliminary simulations, we attempted several times to find the best computational conditions, and we noticed essentially the same behavior for the simulations with and without bond length constraining. The only difference was a larger fluctuation of the energy in the absence of bond length constraining.

Initial velocities for the atoms were taken from a Maxwell distribution at T = 300 K. The MD time step for the integration of Newton's equation was δt = 0.002 ps. Water simulations were performed by simulating a rectangular box (23.588 Å, 26.309 Å, 29.499 Å) containing 538 water molecules. The water model used was the single point-charge SPC2 (Hermans et al., 1984).

Results

NMR assignment of vancomycin and its complex (see Table I) relies on 2D Hartmann–Hahn cross-polarization (HOHAHA), NOESY, and ROESY experiments and generally agrees with the data previously reported (Confert et al., 1980; Williams, 1984; Kannan et al., 1988).

One of the advantages of HOHAHA, compared to COSY-like experiments, is an expected lengthening of the apparent decay constant of spin-locked magnetization owing to the use of the MLEV17 cycle. By use of this scheme, the magnetization is aligned along the static magnetic field for half the time and the relaxation is determined by the average

Table I: ^{1}H NMR Chemical Shifts (ppm) of Vancomycin (1) (Solution 2 mM in DMSO, T = 296 K) and Vancomycin/Ac-D-Ala-D-Ala (2) (Ratio 1:1.5, 2 mM in DMSO, T = 296 K)

^{1}H	1	2	^{1}H	1	2
OHp	9.52	9.43	B	5.32	5.43
OHo	9.20	9.12	s2	5.29	5.29
HN5	8.72	8.69	t	5.19	5.24
HN7	8.56	8.60	u	5.18	5.18
HN4	8.32	8.87	v	4.94	5.24
d	7.90	7.30	C	4.76	4.72
HN2	7.70	11.35	r3	4.50	4.70
e	7.57	7.65	r2	4.50	4.40
g	7.54	7.52	w	4.40	4.75
f	7.43	7.30	r1	4.26	4.49
j	7.42	7.43	x	3.23	4.06
i	7.33	7.00	y	2.42	2.58
k	7.25	7.72	z	2.38	2.31
HN3	6.90	8.40	z′	2.20	2.22
l	6.84	6.86	D	1.94	1.98
m	6.84	6.79	D′	1.81	1.98
HN6	6.69	6.79	b′	1.79	1.68
o	6.48	6.42	a	1.56	1.55
p	6.33	6.29	a′	1.50	1.50
OHu	6.02	6.03	E	1.39	1.32
OHt	5.86	5.86	F	1.16	1.10
r4	5.82	5.91	c	0.97	0.81
s1	5.62	5.50	c′	0.92	0.81
A	5.42	5.54			

of $1/T_1$ and $1/T_{1\rho}$ (Bax & Davis, 1985; Subramanian & Bax, 1987). The HOHAHA experiment was of crucial importance in our case since both the antibiotic itself and the complex especially tend to aggregate and give rise to very broad resonances even in dilute solution.

Details of the NMR assignments are beyond the scope of the present paper.[1] Here we confine ourselves to mentioning that the largest proton chemical shift variation observed when going from vancomycin to the complex is experienced by HN2 (see numbering in structure I), which moves downfield by 4 ppm, thus indicating the presence of a strong hydrogen bonding at that position. HN3 and HN4 move downfield by 1.5 and 0.5 ppm, respectively, HN7 and HN5 exhibit smaller variations, HN6 does not show any appreciable shift, and HN1 of the *N*-Met-leucine side chain is not detectable.

The most important sources of geometric restraints from NMR measurements are short ^{1}H–^{1}H distances, obtained from NOE measurements (Havel & Wüthrich, 1985). In general, the precision of NOESY distance measurements is rather limited and the NOE data are interpreted better as restraints than as precise distance values. To obtain better distance information, different phase-sensitive NOESY experiments were performed, on both vancomycin and complex (see Experimental Procedures), to be able to detect spin diffusion effects (all NOEs are negative). It was apparent that for short mixing times (0.1, 0.2 s) the spin diffusion effects were not important, as expected.

A further check came from the ROESY experiment. It is known (Ernst et al., 1987) that in the extreme narrowing condition the terms W_2 and W_1 dominate the cross-relaxation rate and the term W_0 (which is responsible for the conservative exchange of energy between two spins) is less important. The ROESY experiment simulates the extreme narrowing conditions because molecular reorientation rates are fast compared to the effective Larmor frequency in the rotating frame, and therefore multispin effects will be small (Farmer et al., 1987).

For vancomycin we compared the results obtained with NOESY and ROESY (see Figures 1 and 2). In the ROESY experiment performed with a continuous spin-lock field, two cross-peaks, r1–u and v–t, are missing because they are strongly coupled (in the ROESY experiment magnetization transfer due to scalar coupling is also present) and the HOHAHA cross-peaks have opposite signs with respect to the ROESY cross-peaks.[2]

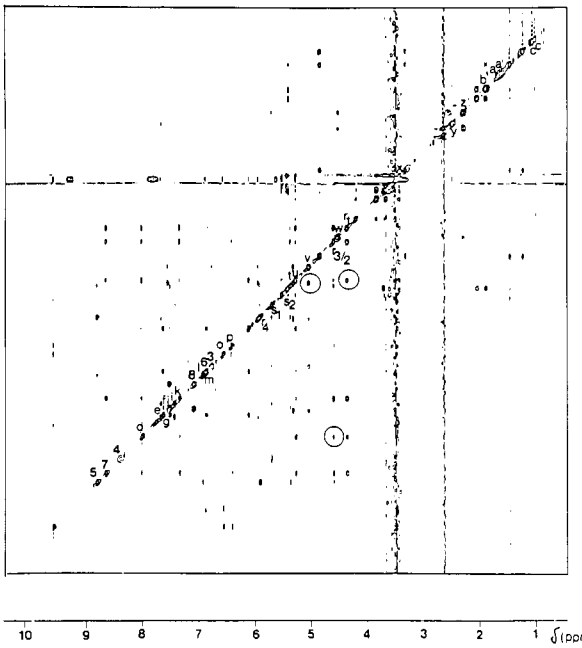

FIGURE 1: Phase-sensitive NOESY spectra of vancomycin, 2 mM in DMSO at 400 MHz. Conditions: T = 299 K, mixing time = 0.2 s, SW = 4588, SI2 = 2048, and NE = 256; zero-filled twice in the F_1 dimension and transformed with a sine-bell shifted 6 in both dimensions. In circles are indicated those cross-peaks missing in the ROESY experiment (see Figure 2).

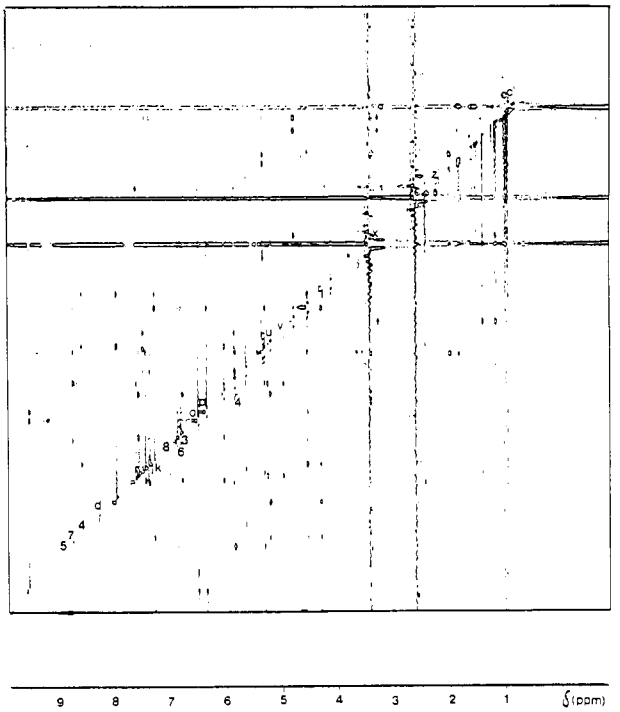

FIGURE 2: ROESY on vancomycin 2 mM in DMSO at 500 MHz. Conditions: the spectrometer was set to the "reverse" mode to have phase coherence among the transmitter-generated hard pulse and the decoupler-generated spin lock train; T = 299 K, spin-lock pulse = 200 ms, SW2 = 5376, SI2 = 2048, NE = 512; zero-filled twice in the F_1 dimension and transformed with a sine-bell shifted 2 in both dimensions.

[1] Additional material is available on request.

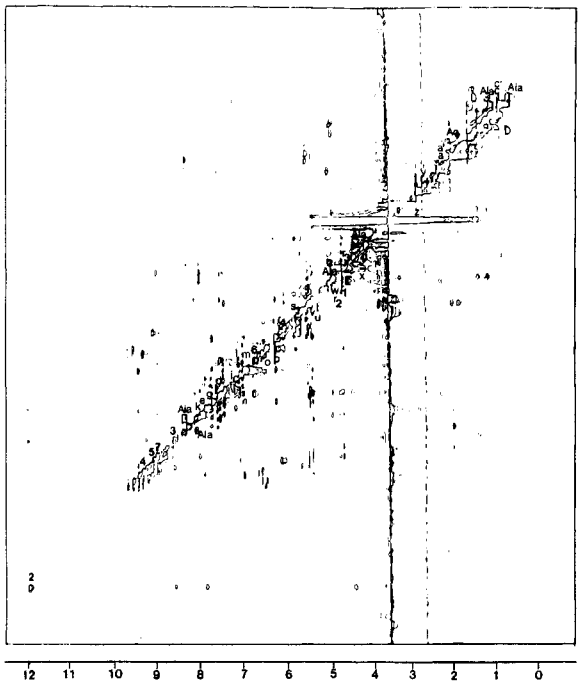

FIGURE 3: Phase-sensitive NOESY spectra of the 1:1.6 complex, 2 mM in DMSO at 400 MHz. Conditions: T = 283 K, mixing time = 0.1 s, SW = 6024, SI2 = 2048, and NE = 256; zero-filled twice in F_1 dimension and transformed with a sine-bell shifted 6 in both dimensions.

When the experiment was performed with a discontinuous spin-lock field, employing the sequence described by Kessler et al. (1987) to minimize Hartmann–Hahn artifacts, the two cross-peaks were present but the quality of the entire experiment was worse, because of the short effective spin-lock mixing time. The same pattern of cross-peaks is present in both the ROESY and NOESY experiments. The only exception occurs for the d–r3 cross-peak missing in both ROESY experiments. It arises from spin diffusion, as is also evident from its buildup rate curve.

For distance evaluation we then applied a method recently described by Esposito and Pastore (1988) that makes use of the ratio between the cross-peak and the diagonal cross-peak and is independent of the initial rate approximation and any calibration. It makes use of the relation

$$r = \{2q[6J(2w) - J(0)]/Rc^{1/6}\}$$

where r is the internuclear distance, $q = 0.1\gamma^4\hbar^2u_0^2$, $J(n\omega) = t_c/[1 + (n\omega t_c)]$, τ_c is the correlation time, $R_c = (k/t_m)$ log $[(1 + x)/(1 - x)]$, with $k = 1$, if $\omega\tau_c > 1$, $k = -1$, if $\omega\tau_c < 1$, and x is the experimental ratio between the cross and diagonal intensities.

In our case, we computed a correlation time τ_c = 3.8 ns using the known distance between two ortho protons in the aromatic ring (2.48 Å). The set of distances obtained was then compared to the values from the initial rate approximation method (Wüthrich et al., 1986), and we found a fairly good agreement between the two sets of data (see Table II). The distance data were transformed into 43 restraints for the free vancomycin.

A comparable accuracy was not obtained for the complex, where, under the best experimental conditions (283 K), the solution was quite viscous even after addition of some CCl_4 and the noise affected the quality of the spectrum (see NOESY in Figure 3). At this temperature, the complex is in the slow exchange regime as reported in the literature (Williams et al., 1981; Kannan et al., 1988) and confirmed by our observation of signals arising both from the free and from the bound peptide.

[2] Relay effects, analogous to spin diffusion in NOESY, may also be observed in ROESY experiments. They give rise to cross-peaks having sign $(-1)^m$, assuming positive diagonal peaks, where m is the number of ROE transfers, as carefully discussed by Farmer et al. (1987).

Table II: Comparison of the Distances between Pairs of Protons Evaluated with the Two Different Methods (1 and 2) and Those Obtained after 40 ps of Dynamic Simulations[a]

	first rate approximation	cross/diag ratio	from MD
e–i	2.48	2.48	2.88
OHp–o	2.50	2.26	2.24
OHo–o	2.26	2.47	2.17
HN5–1	3.15	3.13	3.30
HN5–r4	2.32	2.41	2.86
HN5–s2	2.64	2.63	2.88
HN5–r3	3.14	3.13	2.76
HN7–k	2.28	2.50	2.54
HN7–u	2.68	3.15	2.81
HN7–r3	2.65	3.06	3.26
HN7–r1	2.58	2.53	2.18
HN7–d	3.00	2.74	3.11
HN4–s1	2.20	2.21	1.94
HN4–r4	2.73	2.75	2.75
d–u	2.21	2.48	2.53
d–r1	2.91	3.64	2.58
g–j	2.20	2.60	2.41
g–OHu	2.56	3.00	1.70
f–t	2.48	2.87	2.67
k–r3	2.30	2.64	2.22
k–r1	2.50	2.87	2.64
k–d	3.17	3.56	3.97
OHu–g	2.89	2.74	1.70
OHu–u	2.70	2.90	2.62
OHu–d	3.60	3.65	4.22
OHu–j	3.78	3.84	4.06
OHt–t	2.54	2.71	2.15
r4–s2	3.20	2.90	2.86
r4–s1	3.15	2.90	3.25
B–D	3.03	4.13	2.60
t–v	2.60	2.91	2.40
w–z′	3.72	3.70	2.60
C–E	2.50	2.50	4.40
C–F	2.50	2.60	2.40
r3–r1	2.30	2.88	1.58
r3–s2	2.90	3.46	3.66
D–E	2.69	2.53	2.75
E–G	2.57	2.50	2.98
HN6–r1	3.05	3.30	2.81
HN6–u	3.10	3.30	3.38

[a] Average restrain energy = 0.92 kJ; total restrain energy 39.12 kJ.

Distance restraints were obtained both from a NOESY with 100-ms mixing time and from hydrogen-bond information. We interpreted all the NOESY data only in terms of "strong" (2–2.5 Å), "medium" (2–3 Å), and "weak" (2–4 Å) NOEs (Havel & Wüthrich, 1985): 27 restraints were obtained from the NOESY experiment (Figure 3), and they were mainly intramolecular effects.

However, the dominant restraints for the simulation of the complex were the hydrogen-bond distances from ^1H chemical shift changes and from ^{15}N measurements (see Table III). The ^{15}N chemical shift behavior of both the free antibiotic and the complex provides important additional information on the hydrogen-bond pattern of this system (Live et al., 1984; Hawkes et al., 1987) and is therefore a potential source of restraints for dynamic simulations (Kaptein et al., 1985). ^{15}N chemical shift assignment was obtained by heterocorrelated reverse experiments (see Figure 4A). The analysis of the cross sections allowed an accurate determination of $^3J(^1H–NH)$. Figure 4B shows the spectrum obtained for the complex. Although only four correlations could be detected, because of

Table III: Assignment of ^{15}N Chemical Shifts in the Free Ligand and in the Complex[a]

	free	complex
N1	12.8	19.1
N2	90.3	97.0
N3	97.4	98.4
N4	97.8	96.1
N5	103.7	104.9
N6	86.3	86.0
N7	107.0	106.3
N8	89.7	87.6
N9	36.2	36.0
dipeptide resonances		
AcNH	104.3	106.7
AcAlaNH	98.1	100.0

[a] The assignment of N2 was obtained from a heterocorrelated experiment (Hawkes et al., 1987). N8 is the only amide moiety present in the molecule and was identified from a coupled INEPT experiment (Hawkes et al., 1987).

the unfavorable T_2, it is worth noting that, at the elevated concentration necessary for a direct observation, we were unable to obtain any correlation (^{15}N–^{1}H HETCOR experiment). This experiment, on the contrary, allowed us to identify the signals arising from the dipeptide and from N7, thus confirming our previous tentative assignments, based on titration of vancomycin with increasing amounts of the dipeptide (Hawkes et al., 1987). The ^{15}N chemical shift changes due to complexation are shown in Table III and agree with the hydrogen-bond pattern previously suggested (Sheldrick et al., 1978; Williams, 1984):

HN7---CO–CH_3 (Ala 1) HN5–CO---HN (Ala 2)

HN4---COO^- (Ala 2) HN2---COO^- (Ala 2)

HN3---COO^- (Ala 2) HN1---COO^- (Ala 2)

In particular, ^{15}N data provided direct evidence of the involvement of the *N*-Met-leucine side chain in the hydrogen bonding, on the basis of the large downfield chemical shift observed for HN1. The corresponding amide proton resonance could not be detected in the proton spectra, and the interaction between this charged group and the carboxylate anion of the dipeptide was suggested on the basis of equilibrium constant determinations (Convert et al., 1980). They provided 19 additional restraints [each hydrogen bond would provide two distances, apart from those involving carboxyl groups (three restraints) and HN1, which is an NH_2 group (6). In this case, both the hydrogen/oxygen distances were considered and corrected by an additional quantity].

The two body potential energy terms used in the calculations represent stretching, bond-angle bending, harmonic dihedral bending (puckering), sinusoidal dihedral torsion, and Lennard-Jones (LJ) and Coulomb interactions (EL). In restrained molecular dynamics (RMD) (van Gunsteren & Berendsen; Hermans, 1984), this function also includes a harmonic potential that takes into account the NMR information. This term should, in fact, function as a spring between each pair of atoms and restrain the distance so that their distances lie within the experimentally observed limits. Usually, only the semiharmonic region of the curve describing the attractive interactions is applied (Hermans, 1984), since we assume that the repulsive term is already contained in the van der Waals interactions. We chose a force constant of 210 kJ mol^{-1} nm^{-2}, which should allow 0.1-nm tolerance on the distance between two restrained atoms at a temperature of 300 K (Hermans et al., 1984). The energy minimizations (EM) and molecular dynamics (MD) simulations were performed by the GROMOS package using the 1FP37D4 version of the force-field.

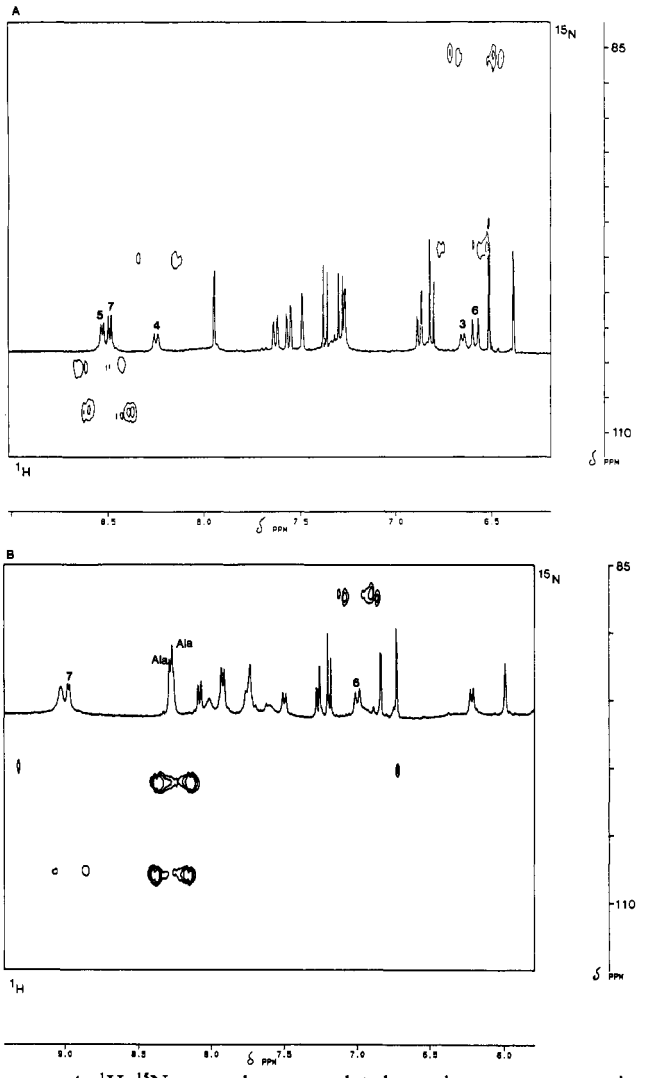

FIGURE 4: ^{1}H–^{15}N reverse heterocorrelated experiment on vancomycin (A) 8 mM and on the complex (B) 10 mM at 400 MHz. Conditions: T = 320 K, SW2 = 1500 Hz, SW1 = 1200 Hz, SI2 = 1024, NE = 32; Bird sequence, D2 = 0.0054 s, D4 = 0.43 s; total acquisition time = 9 h without decoupling in F_2; zero-filled to 1K in the F_1 dimension.

It was not possible to use the normal building blocks contained in the GROMOS library since the vancomycin molecule does not contain standard units. A unique block, involving the whole molecule, was built by using, when possible, standard GROMOS parameters for the force-field. One of the main problems arose from chlorine atoms, which are not included in the GROMOS library. The stretching and bending parameters were taken from Yamamoto and Oki (1985). Other force-fields described in the literature show comparable values (Weiner & Kollman, 1981; Allinger & Yuh, 1985). The Buckingham parameters for the steric functions were taken from Reynolds (1974), Bonadeo and D'Alessio (1973), and Bates and Busnig (1974) and transformed into the Lennard-Jones parameters used in GROMOS by a least-squares fit. The values obtained were 185.8 kJ $Å^6$ mol^{-1} for C6 and 8039.1 kJ $Å^{12}$ mol^{-1} for C12. Other minor modifications included 334.7 kJ mol^{-1} $Å^{-2}$ for the stretching constant between aromatic carbons and the use of the same parameters for atoms experiencing equivalent environments; e.g., carbon atoms belonging to aliphatic or sugar methine groups were considered equivalent when they were not otherwise treated in the GROMOS library. However, the treatment of the electrostatic interactions is not straightforward; indeed, while the GROMOS library included the required atomic charges [except for the value of ±0.106

e for the C–Cl group (Bates & Busnig, 1974)], their use together with a unit dielectric constant led, in our opinion, to a clear underestimation of the role of steric interactions. For instance, after 1000 steps of restrained energy minimization with the conjugate gradient algorithm, the EL and LJ contributions were 226.3 and −11.5 kJ mol^{-1}, respectively, out of a total energy of 486.5 kJ mol^{-1}. The trend above results from the presence of cyclic substructures able to impose some close contacts among atoms carrying significant charge of the same sign. Polar solvents reduce this effect, and we simulated their presence by employing a macroscopic dielectric constant of 50 (Ahlstrom et al., 1987). In this way, the results for vancomycin, after 30 ps of restrained molecular dynamics, look much more reasonable: the electrostatic and steric interactions become indeed of the same order of magnitude, respectively, 7.4 and −18.7 kJ mol^{-1}.

The suitability of our choice was verified independently by running a RMD simulation of a vancomycin molecule in a rectangular box filled with water molecules using periodic boundary conditions. The energy values for the Coulomb and Lennard-Jones contributions after 300 cycles of minimization without restraints were of the same order of magnitude (EL = 117.2 kJ mol^{-1} and LJ = 71.1 kJ mol^{-1}). The absolute values are, of course, much larger than the corresponding values obtained from the simulation in vacuo since the structure optimization is driven by strong solute/solvent attractive forces. The similarity between RMD results in vacuo and the MD ones in water supports the assumption that NMR restraints can be regarded as an effective way of including the effect of the solvent.

No crystallographic data of vancomycin are available, so the coordinates of the vancomycin degradation product CDPI from the Cambridge library (Sheldrick et al., 1978), slightly modified by model building, were used as the starting structure for our calculations. The dipeptide was obtained from standard building blocks on the FRODO program. The two coordinate files were merged in the complex. Initial strain in the molecules due to nonbonded contacts was relieved by performing first 600 cycles of EM using the conjugate gradient method (McCammon & Harvey, 1987). Three different trajectories were followed: two on vancomycin alone, with and without restraints, and one on the D-Ala-D-Ala/vancomycin complex with restraints. On vancomycin, 43 NMR restraints from NOESY experiments were included, while 46 distances (27 from NOESY and 19 from intermolecular hydrogen bonding) were used in the complex. The two runs on vancomycin with and without restraints permitted us to evaluate the actual effect of the restraints in the results.

For each MD run, about 30 ps were calculated at $T = 300$ K, but only 20 ps were used in the following analyses. At the end of this period, an energy minimization was performed again on the averaged structure to find the closest minimum, to be able to compare the resulting energies. In the final structures, violations of the distance restraints were all quite small; the averaged restraint violations were 0.01 nm in vancomycin and 0.02 nm in the complex. A comparison between the structure of vancomycin with (broken line) and without (continuous line) restraints is shown in Figure 5. In spite of its intrinsic rigidity, vancomycin shows quite a few changes under the application of the restraints. In addition, motions allowed in the trajectory without restraints disappear when they are applied, and an overall restriction of the conformational space is observed.

Figure 6 shows the averaged structure of the complex. The largest violation of the interatomic distance restraints between

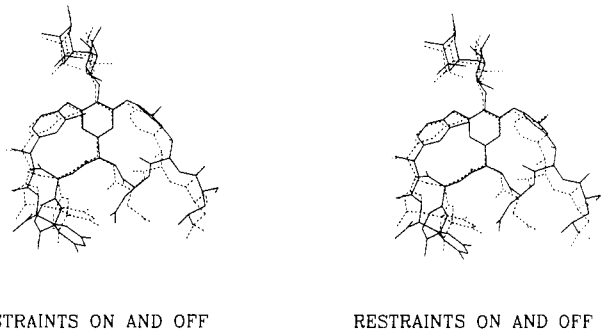

FIGURE 5: Superposition of vancomycin without (continuous line) and with (broken line) restraints. Constant temperature: 300 K. Range for the simulation: 30 ps with an integration step of 0.002 ps. Both structures were averaged over the last 10 ps and energy minimized.

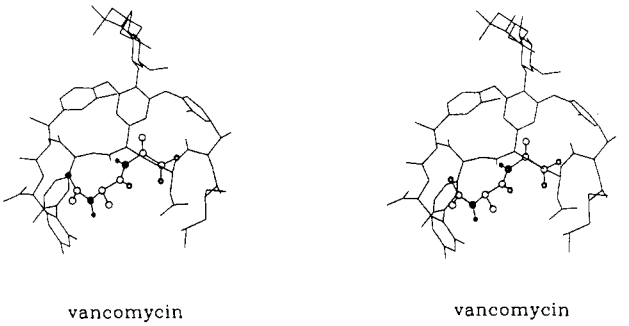

FIGURE 6: Vancomycin/D-Ala-D-Ala with restraints. Constant temperature: 300 K. Range for the simulation: 30 ps with an integration step of 0.002 ps. The structure was averaged over the last 10 ps and energy minimized.

the vancomycin and dipeptide moieties is 0.07 nm. It occurs between HN2 and one of the oxygens of the terminal carboxylic group of the dipeptide. The energy contribution due to the binding was −104.6 kJ mol^{-1}, and the difference between the total potential energy of the complex and the sum of vancomycin and dipeptide at infinite separation is around −41.8 kJ mol^{-1}.

Discussion

For the X-ray structure of CDPI, a degradation product closely related to vancomycin, Sheldrick et al. (1978) inferred that the main interactions between vancomycin and the dipeptide could be due to three hydrogen bonds involving HN7---CO (Ala1), HN5–CO---HN (Ala2), and HN4---COO. Subsequently, this model was confirmed by 1D NOE measurements performed by Williams (1984) and Kannan et al. (1988) on the complex. They further suggested that the carboxylate group of D-Ala-D-Ala would interact with HN1, HN2, HN3, and HN4 to form a cluster. The binding would induce a large conformational change around the θ angle of the *N*-Met-leucine residue in vancomycin to allow a closer electrostatic interaction of the charged groups. Figure 7 clearly shows that binding of vancomycin and the Ac-D-Ala-D-Ala dipeptide does not involve major conformational changes within vancomycin. In particular, the *N*-Met-leucine side chain maintains the same conformation in both systems, as opposed to Williams' hypothesis.

Our simulations indicate that the main structural modification occurring upon complexation is the opening of the two vancomycin "claws" (see broken structure in Figure 7) to allow the inclusion of the dipeptide, driven by the electrostatic interaction between charged groups.

Our model is also consistent with the comparable binding rates of vancomycin and other antibiotics without mobile

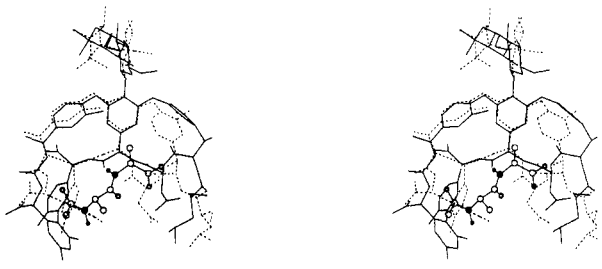

FIGURE 7: Superposition of vancomycin (with restraints, broken line) and the complex (continuous line).

N-Met-Leu side chain published by Popienek and Pratt (1988). Indeed, too much attention has been given in the literature to the studies of the complex, but it was only by comparing the structures of both unbound vancomycin and the complex that it was possible to determine the dynamics of the binding.

On the basis of both homonuclear and heteronuclear NMR data and restrained molecular dynamics calculations, we have been able to determine the experimental structure of vancomycin and of its complex, which differs in important respects from that suggested by molecular modeling alone and which rationalizes the observed binding of a series of antibiotics related to vancomycin. We are now confident that the same procedure can be applied in other examples of drug molecular recognition.

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