Aspects of Molecular Recognition: Use of a Truncated Driven Pseudo-NOESY Experiment to Elucidate the Environment of Intermolecular Electrostatic Interactions in Vancomycin

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The folding-in of a hydrophobic sidechain, to complete a wall of a peptide carboxylate anion binding pocket, in the vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex has been unambiguously determined using a series of nuclear Overhauser effect difference spectra, presented as a two-dimensional contour plot.

The vancomycin group of glycopeptides are clinically important as antibiotics and constitute an excellent small natural system with which to study intermolecular interactions.[1] The electrostatic interactions that bind these antibiotics to models of their natural target, bacterial cell wall precursor peptides with the C-terminal sequence -L-X-D-Ala-D-Ala, have been elucidated over a number of years.[2—4] Further, it has been shown in this system how the complementarity of hydrophobic and electrostatic interactions serves to strengthen each type of interaction.[5]

In earlier work, we have reported evidence[2] for the 'folding-in' of the sidechain of the N-terminal *N*-methyl-leucine residue of vancomycin in its complex with di-*N*-acetyl-L-Lys-D-Ala-D-Ala. This conclusion was partly based on a large steady-state intermolecular nuclear Overhauser effect (n.O.e.) (30%) between the bound sidechain methyl groups of *N*-methyl-leucine (LeuMe) and the bound A_NMe group of the peptide (Figure 1). The conclusion was supported by smaller intramolecular n.O.e.s between the LeuMe groups and both the methylene (10%) and the backbone amide proton (9%) of asparagine (termed $AsnCH_2$ and AsnNH, respectively). In our recent studies of vancomycin complexes using NOESY experiments,[4] the intramolecular n.O.e.s $AsnCH_2 \longleftrightarrow$ LeuMe and AsnNH $\longleftrightarrow$ LeuMe were again observed. However, a 30% intermolecular n.O.e. between the LeuMe and the A_NMe groups seemed unreasonably large. In a two-dimensional CAMELSPIN spectrum[6] of the vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex, the identity of the cross-peak at the frequency of the LeuMe group in one dimension and that of the A_NMe group in the other dimension was confirmed, but since this crosspeak is opposite in sign to the genuine n.O.e. peaks, it must in this case result mainly from saturation transfer. The resonances of the LeuMe groups of unbound vancomycin (saturation transfer partners for the bound LeuMe groups) are coincident with that of the A_NMe group of bound peptide (see Figure 2).

In view of the above conclusion, it was necessary to devise an experiment to search for an n.O.e. between the LeuMe and

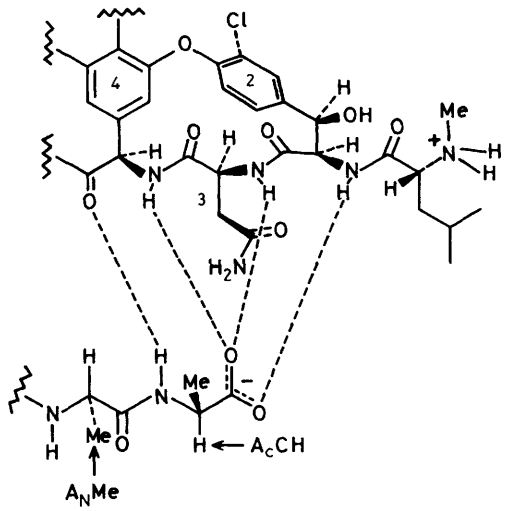

Figure 1. The N-terminal region of vancomycin and the C-terminal region of a bacterial cell wall peptide model -D-Ala-D-Ala. The broken lines indicate intermolecular hydrogen bonds formed on complexation.

A_NMe groups in the vancomycin complex. The ^1H n.m.r. spectrum of the vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex is shown in Figure 2 along with simulated subspectra of the relevant resonances. Clearly, it is necessary to look for a correlation between the resonances A_NMe(free) and LeuMe(bound), using saturation transfer *via* the resonance A_NMe(bound). The overlap of resonances prevents the unambiguous solution of this problem using one-dimensional methods. However, no crosspeak is observed in n.O.e. spectra (see Figure 3), and it is important to consider why this is so. The appropriate one-dimensional analogy for a NOESY experiment is the transient n.O.e. experiment, not the truncated driven n.O.e. experiment,[7] the difference between the two being in the absence or presence of selective irradiation during the development period of the n.O.e. In the truncated driven experiment, the n.O.e. develops during a period of continuous irradiation of a selected resonance. However, in the transient experiment, the resonance is either inverted or saturated by the selective irradiation. Following this, n.O.e.s develop in the absence of any irradiation during a 'mixing time'. If the perturbation of the magnetization caused by the selective irradiation is lost during this time by efficient relaxation processes other than the dipolar relaxation which leads to the n.O.e. enhancement, then the crosspeak in the n.O.e. spectrum may not be observed. A series of n.O.e. difference experiments (truncated driven) where the frequency of the pre-irradiation is incremented in discrete steps over a region of interest[8] can be data-processed to give a two-dimensional 'truncated driven pseudo-NOESY' spectrum. The discrete incrementation of the pre-irradiation frequency is analogous to constructing the F_1 frequency dimension using t_1 in a two-dimensional experiment.[9] Assembling the set of n.O.e. difference spectra in the form of a two-dimensional data set and performing only the Fourier transformation with respect to the t_2 domain, yields a two-dimensional 'truncated driven pseudo-NOESY' spectrum. High digital resolution in the F_1 frequency domain is easily and quickly obtained over small F_1 spectral widths. However, this is also true of the transient NOESY experiment.[10]

It should be stressed that the series of n.O.e. difference spectra contains exactly the same information as the 'truncated driven pseudo NOESY' spectrum, and in that sense there is no advantage. The real advantage is in data presentation and analysis. It is far more convenient to extract data from a two-dimensional spectrum than a large number of one-dimensional spectra. Thus, this approach benefits from the extra sensitivity of a driven experiment and the presentation of data as a two-dimensional plot. This allows systems unsuited to transient n.m.r. experiments to be studied in two dimensions.

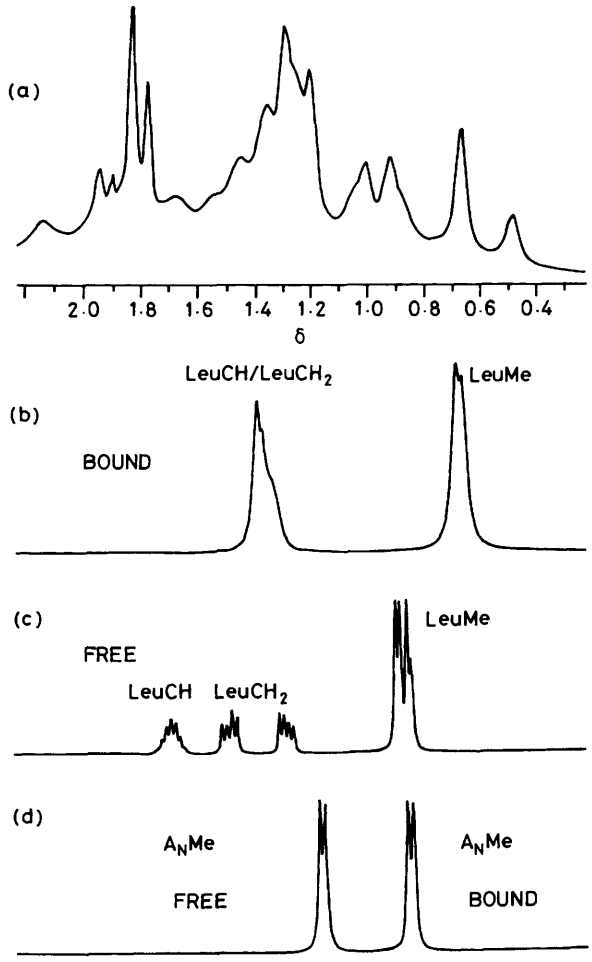

Figure 2. (a) Upfield region of a 400 MHz proton n.m.r. spectrum of a 1:1 vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex (solution *ca.* 10 mM in each component) in 2:1 [$^2H_6$]dimethylsulphoxide (DMSO) CCl_4 at 280 K. (b) Simulated subspectrum of the sidechain protons of the N-terminal residue of vancomycin (*N*-methyl-leucine) when bound to di-*N*-acetyl-L-Lys-D-Ala-D-Ala and (c) when unbound. (d) Simulated subspectra of the methyl group resonances of the non-C-terminal alanine residue of di-*N*-acetyl-L-Lys-D-Ala-D-Ala (A_NMe groups) in both free and bound states.

The 'truncated driven pseudo-NOESY' spectrum of the vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex,† produced by incrementing the decoupler across the resonance of A_NMe(free) (see Figure 4), clearly shows a crosspeak to the LeuMe(bound) resonance. It has the same F_1 frequency and linewidth as the saturation transfer crosspeak A_NMe(free) → A_NMe(bound). Thus, it follows that the A_NMe and LeuMe

† Irradiation time was in the range 0.3 to 0.6 s to avoid spin diffusion complications in the spectra.

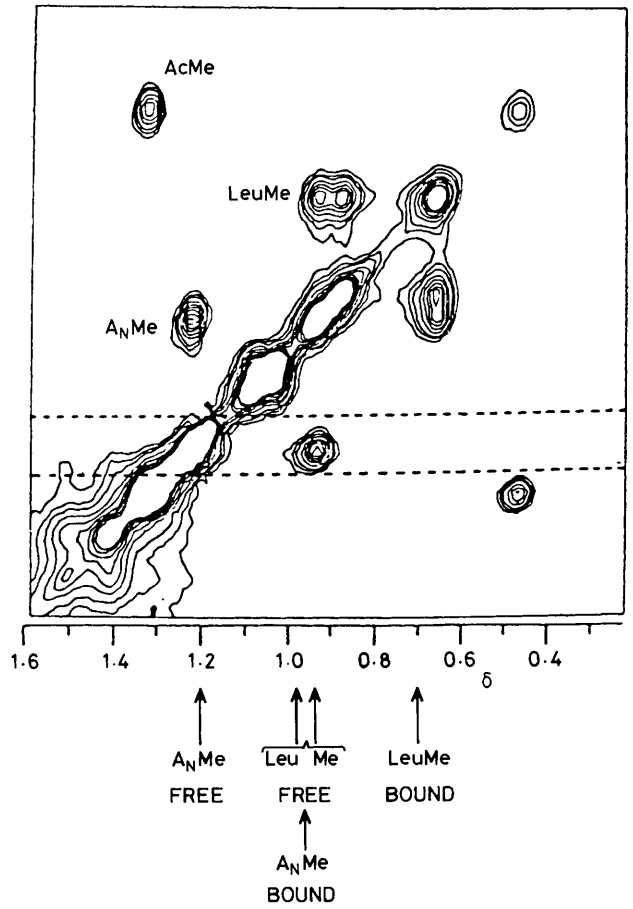

Figure 3. Upfield region of a NOESY spectrum (mixing time 0.4 s) of the 1:1 vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex. The three labelled crosspeaks are all the result of saturation transfer between free and bound resonances. The crosspeaks LeuMe ⟷ A_NMe are not observed above the thermal noise. The region in the box is that shown in Figure 4.

groups are close in space in the complex. Incrementing the decoupler through the LeuMe(bound) resonance produced the corresponding LeuMe(bound) → A_NMe(free) crosspeak. It is noteworthy that the ambiguous interpretation of a one-dimensional n.O.e. difference spectrum, which arises due to overlap of resonances, is removed by the contour display which permits the frequencies of peak maxima to be simply correlated. The driven experiment also highlighted an n.O.e. supportive of the population of the folded-in conformer, *i.e.* LeuMe ⟷ A_CCH (data not shown). The failure of transient n.O.e. experiments to reveal the above correlations shows that it is imperative to study certain systems using the described methods.

Restriction of solvent access by a hydrophobic group to the electrostatic interactions occurring within the binding pocket is a favourable process both enthalpically (the dielectric effect of the surrounding medium being reduced) and entropically (the organisation of water about a polar interaction destabilises the interaction). Furthermore, the removal of one face of

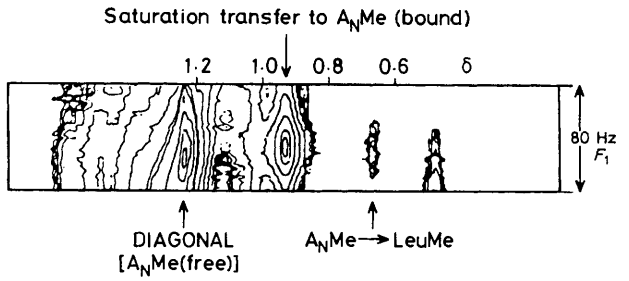

Figure 4. Truncated driven pseudo-NOESY spectrum (pre-irradiation time 0.5 s) of the 1:1 vancomycin–di-*N*-acetyl-L-Lys-D-Ala-D-Ala complex conversing the same region as the box in Figure 3. The decoupler was incremented in steps of 2 Hz over an 80 Hz region centred on the A_NMe(free) resonance.

residue 1 (*i.e.* a hydrophobic region) from the solvent should contribute to the stability of the complex.

We have clearly demonstrated the usefulness of combining a truncated driven n.O.e. experiment with a two-dimensional representation of the final data set as a contour plot. In this way, problems associated with the loss of magnetization before evolution of the n.O.e. are minimised, and at the same time, the assignment of the n.O.e.s is facilitated by use of the contour plot. This procedure was found to be the only way to resolve the problem described above.

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