The Binding of Erythromycin A to Bacterial Ribosomes: A ^{1}H Transferred NOE Study

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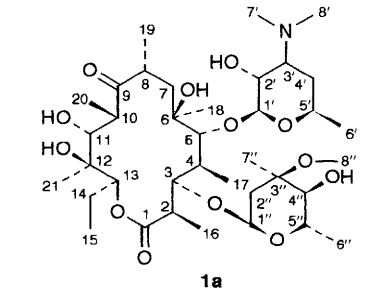
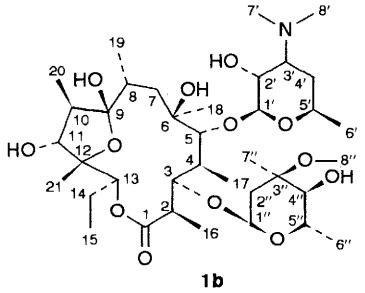
Transferred nuclear Overhauser effects are used to determine the conformation of the antibiotic erythromycin A (ketone) when bound in fast exchange to bacterial ribosomes.

Erythromycin A **1** is a clinically important antibiotic, which exists in aqueous solution as a mixture of the 9-ketone **1a** and the 9-12 hemiketal **1b**.[1] We have shown that the ketone, but not the hemiketal, is able to bind to *Escherichia coli* ribosomes,[2] specifically 50S subunits,[3] in a fast exchange process on the NMR timescale. When ribosomes are titrated into an excess of erythromycin A, selective line broadening in the ^{1}H NMR spectrum of the drug is observed. The most extensively broadened signals, which may be assumed to be due to those protons most intimately involved in binding, form



1a



1b

a distinct surface on the three-dimensional (minimised crystal) structure of the molecule.[3]

In the present study we have sought to obtain information about the conformation of the bound drug, taking advantage of the phenomenon of transferred nuclear Overhauser effects. When the NOESY experiment is performed on ligand–macromolecule complexes in fast exchange with the ligand in excess, the cross-peaks which dominate the spectrum are not those due to the free ligand, but those due to the bound complex, transferred to the free ligand.[4,5] These NOEs are negative, they appear when relatively short mixing times are used, and may represent ligand–ligand or macromolecule–ligand connectivities. The erythromycin–ribosome complex lends itself especially well to this approach since the technique favours very large macromolecules (or macromolecular complexes), and because free erythromycin A at room temperature in aqueous solution exhibits NOEs that are close to zero.

Ribosomes were prepared, as described previously,[6] from frozen cell paste of *E. coli* MRE 600 (Porton Down). Fully deuteriated ribosomes were prepared from cultures of MRE 600.pCP40.pCI857, grown on a medium based on [$^2H_4$]-succinate and D_2O. (The plasmids were present because these cells were also used to produce other deuteriated proteins.) Ribosomal 'core' particles were prepared as previously described,[6] by treating 50S ribosomal subunits with 1.3 mol dm^{-3} lithium chloride solution.

To a solution of erythromycin A (4 mmol dm^{-3}) in buffered D_2O at pH 7.6, were added 70S ribosomes, in a small volume of the same buffer, to a final concentration of 12 μmol dm^{-3}. The 600 MHz 1D ^1H NMR spectrum was run to confirm that the expected selective line broadening occurred, then the NOESY experiment with a mixing time of 150 ms (optimised for macromolecules) was recorded. (A second NOESY spectrum, mixing time 75 ms, was also measured in a control for spin diffusion. The data were qualitatively the same but with a poorer signal to noise ratio.) The experiment was repeated (mixing time 150 ms) using fully deuteriated ribosomes, to allow ligand–ligand and ribosome–ligand NOEs to be distinguished. Ribosome–ligand NOEs would not appear in the latter experiment, and, in addition, spin diffusion in the macromolecule would be eliminated so this experiment served as a second control. Two further controls, the NOESY experiments on erythromycin alone and an erythromycin in the presence of 19.5 μmol dm^{-3} 'cores' were also run, under the same conditions. Previously, we have shown that 'cores' interact very weakly and non-specifically with erythromycin A, causing slight uniform broadening in the ^1H NMR

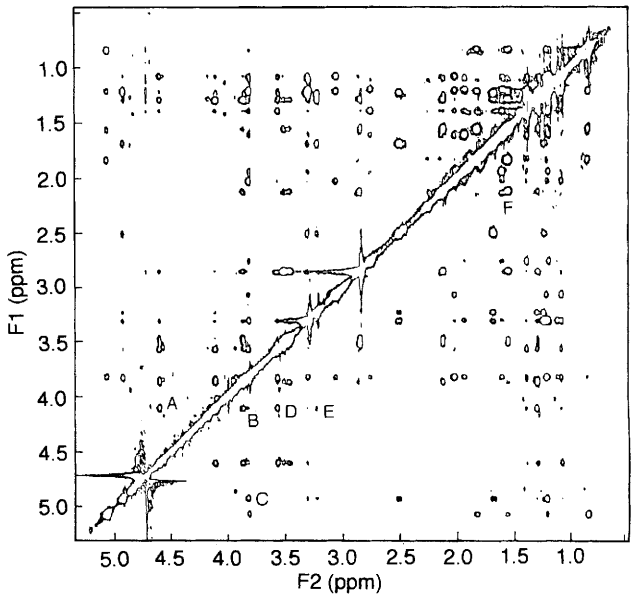


Fig. 1 600 MHz NOESY spectrum of 4 mmol dm^{-3} erythromycin A in the presence of 12 μmol dm^{-3} *E. coli*: ribosomes, mixing time 150 ms. Selected ^1H–^1H crosspeaks are marked as follows: A 5″-1′, B 5″-5′, C 1″-3, D 5″-5, E 5″-4″, F 4′eq-4′ax, all marked signals are due to the 9-ketone.

spectrum of the drug. These two control NOESY spectra were almost identical and almost blank, showing just 15 very weak cross-peaks. By contrast, there were 128 cross-peaks in the spectrum of erythromycin A–ribosomes (Fig. 1), many of them strong. All of these were ligand–ligand cross-peaks; the only difference between this spectrum and the erythromycin–deuteriated ribosomes spectrum was that the signal to noise ratio was better (this probably reflects a difference in the activity of the ribosomes, which were isolated from rather different cell preparations). All but a very few weak correlations were due to the 9-keto form of the drug, confirming that the weak binding between erythromycin A and ribosomes[2] is associated exclusively with this structure.

A NOESY spectrum of free erythromycin A ketone in $CDCl_3$ has been reported, and interpreted in terms of two rapidly interconverting conformations coexisting in this solvent.[7] In particular no cross-peak 5″–5′ was observed, although this would have been expected from the crystal structure of the drug.[8] This cross-peak appears strongly in our experiment suggesting that the ribsome may be able to 'freeze' a particular conformation of the drug.

Fig. 2(*a*) shows the three-dimensional minimised crystal structure of the drug, derived from 9-[*O*-(2,5-dioxahexyl)oxime] erythromycin A hydrate.[8] NOE distance constraints, large cross-peak (*e.g.* 4′ax–4′eq) ≤ 2.5 Å, medium (*e.g.* 1″–3) ≤ 3.5 Å, small (*e.g.* 5″–4″) ≤ 5.0 Å, were then applied to this structure. Where methyl groups were observed, distance constraints of ≤ 5.7, ≤ 4.2 and ≤ 3.2 Å were applied to the corresponding carbon atom. Polygen QUANTA/CHARMm software was used to minimise the structure within these constraints. The resulting structure is shown in Fig. 2(*b*). In both Figs. 2(*a*) and (*b*), the binding 'surface', representing the most extensively broadened ^1H signals on binding of the drug to ribosomes, is indicated using van der Waals surfaces. These structures suggest that on binding to ribosomes the conformation of the drug is distorted. The sugar rings twist, so that overall the binding surface expands and flattens, though the desosamine ring becomes more closely associated with the surface. The absence of ribosome–drug cross-peaks indicates, however, that there may be some residual conformational lability in the bound complex.

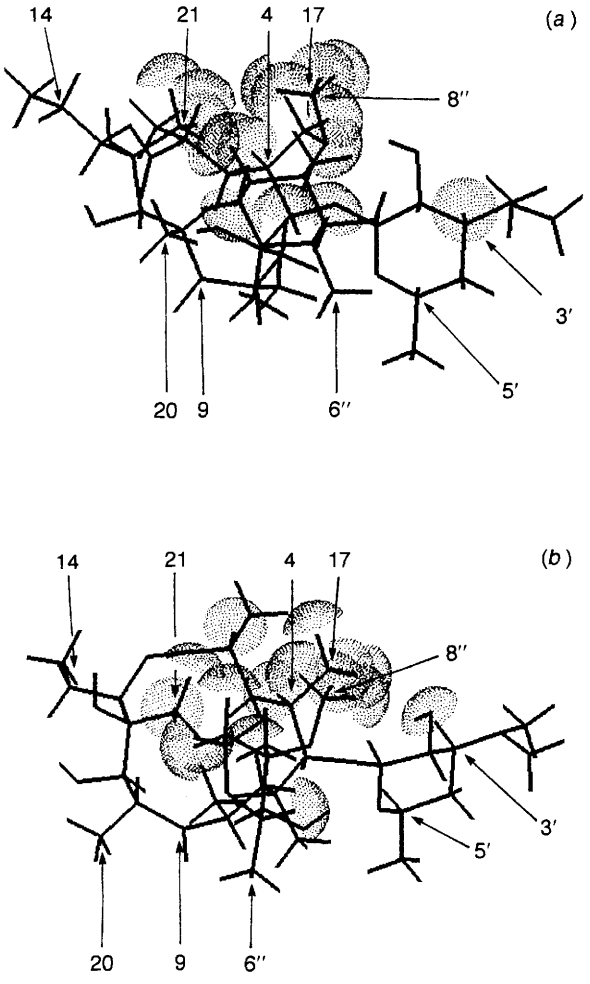


Fig. 2 Erythromycin A ketone: (*a*) minimised crystal structure (unbound); (*b*) minimised structure from transferred NOE data (bound to ribosomes). van der Waals radii indicate the binding 'surface' of the drug molecule as deduced from ^{1}H NMR line broadening.[3] Selected carbon atoms are numbered.

Weak specific binding of erythromycin A ketone to bacterial ribosomes has now been amply demonstrated and considerable detail about the nature of the interaction has also been obtained. A measure of caution must be employed in the interpretation of variations in line broadening, the binding 'surface' indicated appears within a relatively rigid part of the molecule,[7] which might be expected to give short T_2 values on binding. The correspondence between the 'surface' and the rigid part of the molecule is not exact, however. Energy minimisation has been used to refine structures **2a** and **b**, but the refinement is quite small. The information present in these structures derives overwhelmingly from experimental data. The overall picture, therefore, is of weak specific binding focused on those parts of the molecule that are known to be essential for its antibacterial action, and of a plausible conformational change on binding to the bacterial ribosome.

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References

1 J. Barber, J. I. Gyi, G. A. Morris, D. A. Pye and J. K. Sutherland, *J. Chem. Soc., Chem. Commun.*, 1990, 1040.
2 D. A. Pye, J. I. Gyi and J. Barber, *J. Chem. Soc., Chem. Commun.*, 1990, 1143.
3 J. Barber, J. I. Gyi and D. A. Pye, *J. Chem. Soc., Chem. Commun.*, 1991, 1249.
4 D. Neuhaus and M. P. Williamson, *The nuclear Overhauser effect in structural and comformational analysis*, New York: VCH, 1989, pp. 175–181.
5 F. Ni, Y. Konishi and H. A. Scheraga, *Biochemistry*, 1990, **29**, 4479.
6 H. E. Homann and K. H. Nierhaus, *Eur. J. Biochem.*, 1971, **20**, 249.
7 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1659.
8 B. Bachet, C. Brassy and J. Mornon, *Acta Crystallogr. Sect. C*, 1988, **44**, 112.