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Forces in molecular recognition: Comparison of experimental data and molecular mechanics calculations

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SUMMARY

NMR studies of the rotation barrier of the disaccharide of the glycopeptide antibiotic vancomycin have been used to test the performance of computer simulation techniques using molecular mechanics. In the absence of any solvated water, no correlation could be found between experiment and calculation. By introducing solvent water molecules into the binding region of the antibiotic, the NMR results could be simulated both qualitatively and quantitatively within experimental error without using massive computational resources.

INTRODUCTION

Computational methods are in widespread use to predict the low energy conformations of molecules, their intra- and inter-molecular interactions, and their mobility [1]. In most cases, the simulations are performed without consideration of the solvent, which, particularly in the case of water, may perturb the energy of the system to such an extent that the calculated gas-phase conformational preferences are overwhelmed. In order to assess the accuracy with which the COSMIC computational chemistry package [2] may be used to predict the conformational preferences of water soluble molecules, we have attempted to simulate molecular motion within the glycopeptide antibiotic vancomycin [3].

Vancomycin (see Fig. 1) consists of a heptapeptide aglycone glycosidically linked to a disaccharide, and contains both hydrophobic and hydrophilic domains. As depicted in Fig. 1, the front face is the one that binds the peptide target for antibiotic action and will subsequently be referred to as the 'binding face'. Proton NMR studies, particularly using nuclear Overhauser effects (see below) have shown that within vancomycin there exist regions of relative inflexibility (owing to

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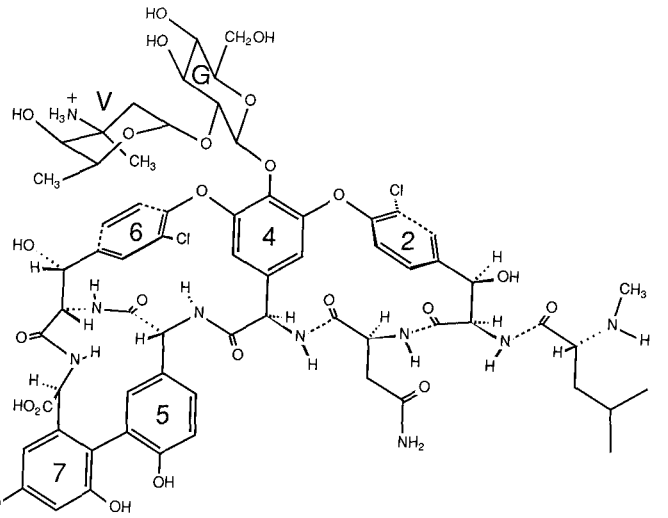


Fig. 1. The structure of vancomycin viewed from the face to which bacterial cell-wall peptides bind as the front face.

the extensive cross-linkage of the amino acid side chains), and regions of higher mobility [4,5]. For example, the rotational frequency of the side chain of the N-terminal residue, N-methylleucine, is faster than the overall rotational frequency of the molecule. The isopropyl terminus undergoes the fastest rotation with a reduction in frequency towards the peptide backbone. Similarly, the rotational frequency of the disaccharide with respect to the aglycone is higher than the overall molecular rotational frequency. Thus, vancomycin is a useful system with which to test experimental methodology for two main reasons:

(i) Unlike in completely flexible molecules, the motion of the flexible regions may be measured with respect to inflexible, conformationally restricted regions. In other words, it is possible to determine the position of flexible portions of the molecule relative to known positions within the inflexible regions. For example, the conformations populated by the disaccharide of vancomycin relative to the aglycone may be studied by observing the proximity of hydrogen nuclei within the disaccharide to hydrogen nuclei within the conformationally restricted triaryl system involving rings 2, 4 and 6 of the aglycone (see Fig. 1 and 2).

(ii) Vancomycin contains both hydrophobic and hydrophilic regions and so the populations of the various conformations available to freely rotatable regions are considerably influenced not only by other parts of the antibiotic, but also by the solvent.

The interconnection of the glycone and the aglycone portions of the molecule is the region of high mobility investigated in the following studies. These studies concentrate on the information that may be derived using moderately powerful computing systems (VAX 11/780).

A powerful means of determining the rate of molecular rotations faster than the overall rotational frequency of the molecule using NMR spectroscopy is through the rate of build-up of nuclear Overhauser effects (NOEs) [6]. The rate of build-up is related to the frequency of the rotation via the rate of change of a vector connecting two hydrogen nuclei perturbed by the rotation (the correlation time). It is also related to the time-averaged distance (r) between these nuclei (specifically to r^{-6}). Knowledge of the time-averaged distance between nuclei allows a qualitative estimate of

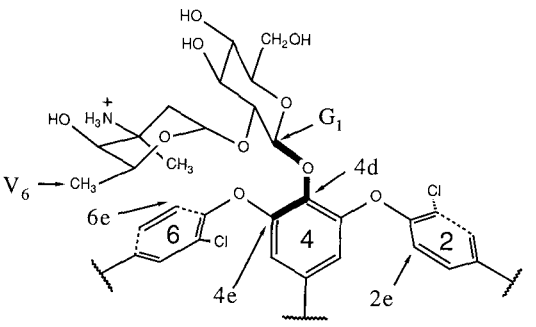


Fig. 2. The torsional angle (shown in bold) varied during the simulation of the relative motion of the glycone and aglycone portions of vancomycin.

the population (and hence the relative energy) of the various conformers visited during the rotation. It is possible to separate the variables 'correlation time' and 'time-averaged distance' by measuring the rate of build-up of NOEs in the absence and presence of a spin-locking field [7].

NOE studies [5] of vancomycin in dimethyl sulphoxide solution revealed that the rotation of the disaccharide with respect to the aglycone portion may be described in terms of the population of two major conformers. The term 'conformer' is used here to describe one time-averaged position on the fast rotation profile. Furthermore, within the energetic boundaries of the conformation, oscillatory behaviour is probable. In one conformer, the 6-methyl group (V_6) of vancosamine – the amino sugar – occupies the binding face of vancomycin, its protons sharing an NOE with the proton at position 2e (for nomenclature see Fig. 2). In this conformer, the glucose unit occupies the back face (e.g. its anomeric proton G_1 shares an NOE with the proton at 6e). The second conformer is the ca. 180° rotamer (about the glycone-aglycone glycosidic bond) of the first conformer which places V_6 on the back face near to proton 6e, and glucose on the binding face with G_1 close to 2e. Accurate populations of the two conformers cannot be determined from the rate of build-up of the NOEs between the disaccharide and the aglycone because of the likelihood of dynamic behaviour within each conformer. However, the presence of fast-building NOEs for both conformers means that in qualitative terms, the populations appear to be similar. The relative motion of the disaccharide and the aglycone, as deduced from the correlation time of the above NOEs, occurs at a frequency of up to approximately 400 MHz. Hence, the barrier to the rotation of the disaccharide is approximately 6 kcal·mol^{-1}.

METHODS

The simulation of the rotation of the disaccharide of vancomycin, relative to the aglycone portion, was performed using the SPIN01 program of COSMIC [2]. The torsional angle involving the anomeric carbon of glucose, the glycosidic oxygen between glucose and ring 4, and carbons 'd' and 'e' of ring 4 was incremented at 10° intervals. The bonds involved are highlighted in Fig. 2. After each increment, the above torsional angle is restricted to the set angle by holding the appropriate atoms in a steep-sided energy well. The remaining atoms are allowed full relaxation at each torsional angle using a variant of the MIN05 type combined atom/torsional minimiser [2]. Total calculation time was of the order of 24 h. Partial charges were included in the simulation and were

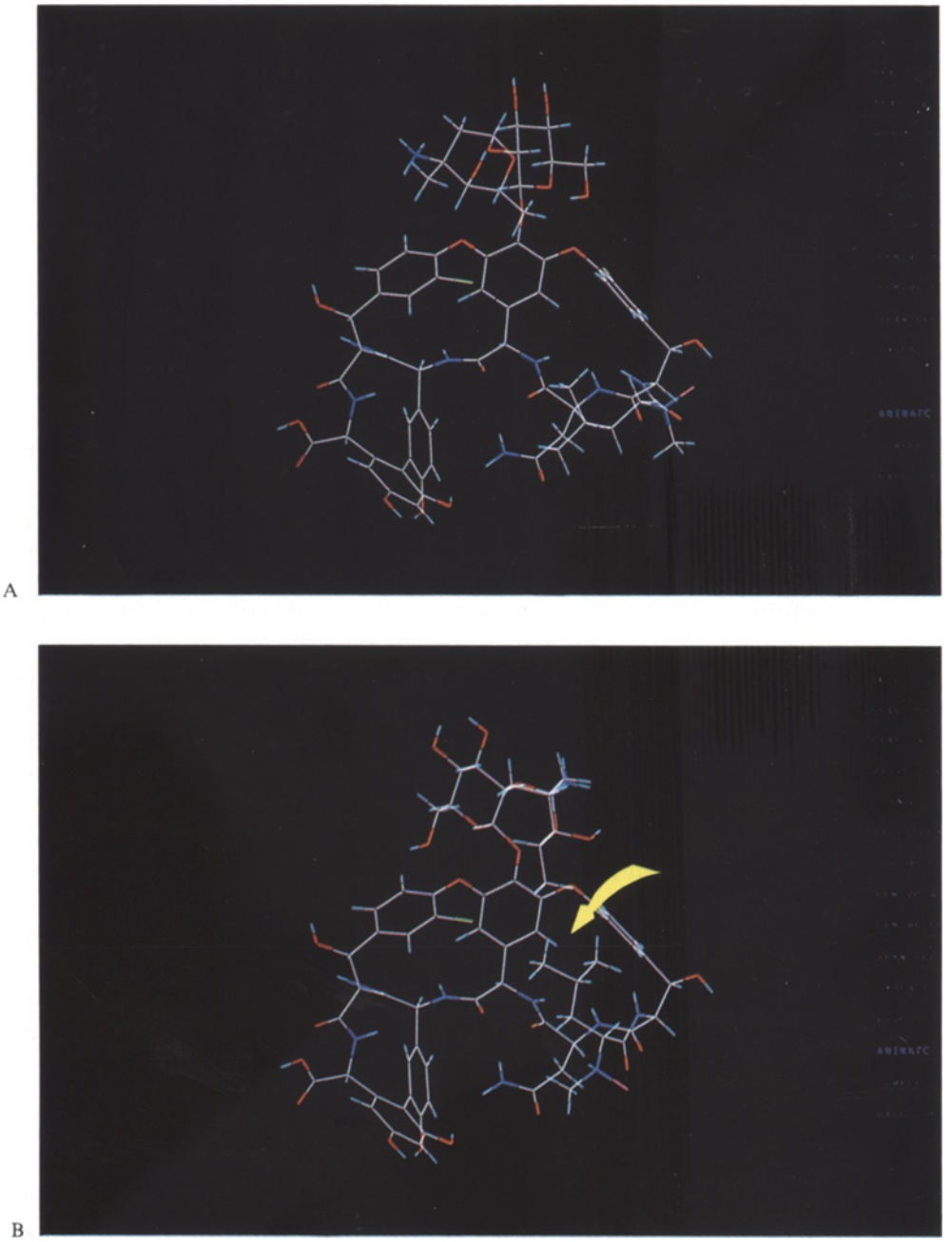
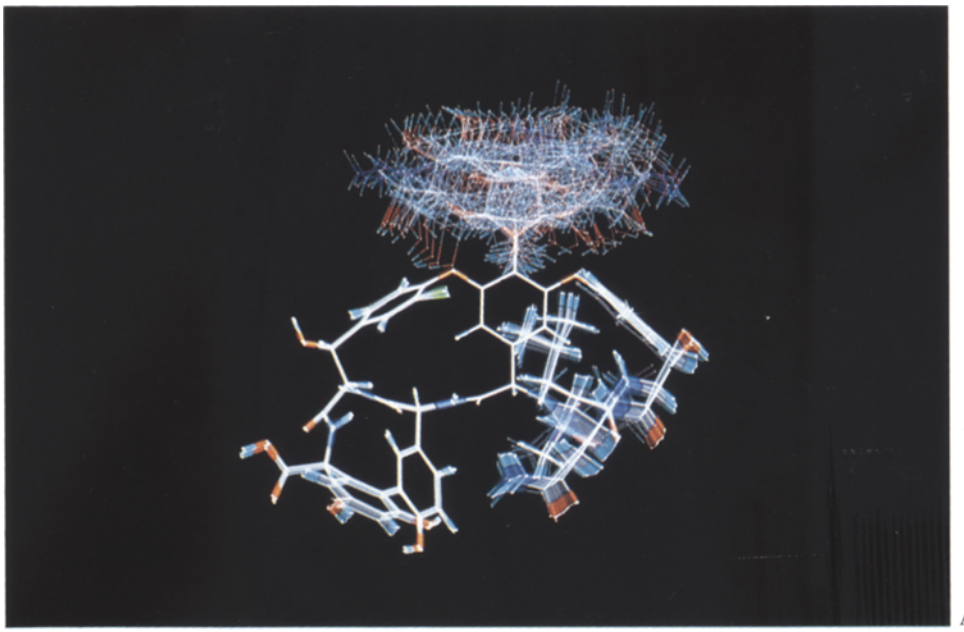


Fig. 3. Conformers of vancomycin produced during the simulation of the rotation of the disaccharide unit when no water molecules are present in the binding pocket. (A) Starting position. (B) After ca. 90° rotation. Note that the 6-methyl group of vancosamine and the *N*-methylleucine side chain (arrowed) are blocking solvent access to the amide groups of the binding pocket.



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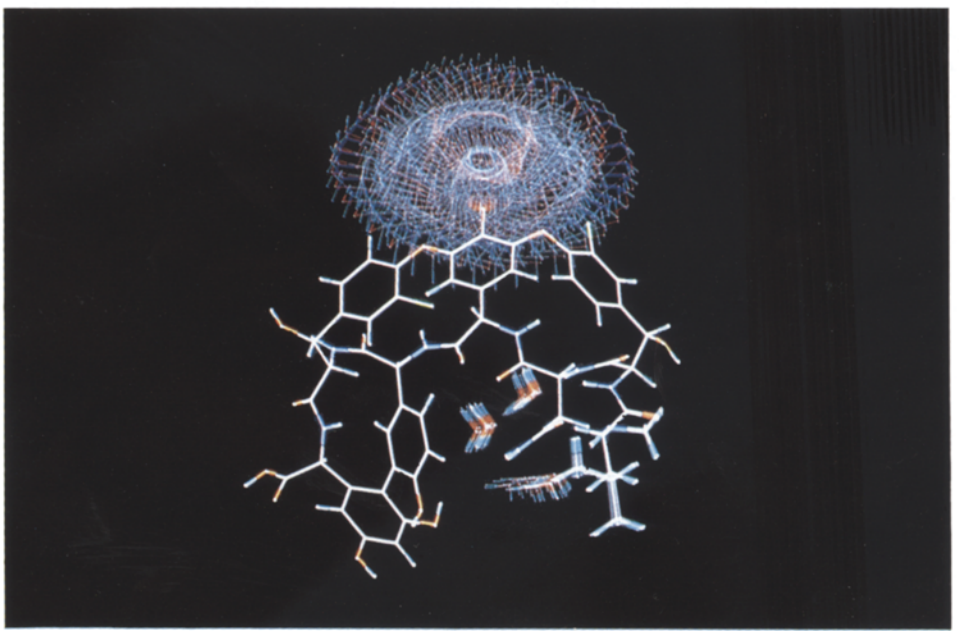

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Fig. 4. Full rotation profile from simulations of the rotation of the disaccharide of vancomycin: (A) in the absence of water; (B) in the presence of water in the binding pocket. Note the increased cooperative movements associated with the unsolvated structure during rotation compared with the water complex. Also note the large movements associated with each water molecule in (B) as the sugar moiety spins.

calculated using the COSMIC CNDO utility. Later it was found that the LIVERPOOL [8] method could be used with no significant change of effect. A dielectric constant of unity was used throughout.

As a starting point for the rotation, the disaccharide of vancomycin was placed in the conformation observed in the crystal structure of CDP-I [9], a degradation product of vancomycin. This system was minimised fully prior to rotation which resulted in only small atom movements. In later spins (see below), it was found that a similar conformation to this starting conformation was simulated at the appropriate torsional angle when the simulation was continued for more than 360°.

RESULTS AND DISCUSSION

Initial attempts to simulate the rotation of the disaccharide portion of vancomycin were performed on a molecule without partial charges attributed to any atoms. Therefore, no coulombic terms were involved. During the rotation, the *N*-methylleucine side chain moved from its original position – projecting freely into the solvent (Fig. 3A) – to a position over the three amide protons of the binding pocket (Fig. 3B). There is no NMR evidence to support the population of such a conformation, and it is expected that the blocking of access of the polar solvent to these amide groups would energetically severely disfavour this conformer. Furthermore, the 6-methyl group of vancosamine (V_6) falls into the binding pocket (over the face of ring 4) during the initial stages of the rotation. Once in the binding pocket, the minimiser draws the V_6 group and the *N*-methylleucine isopropyl group together. The results of this as the rotation continued was to turn the vancosamine unit over (i.e., to rotate the vancosamine unit relative to the glucose unit about the glycosidic C-O bonds) before it crossed ring 2. Again, there is no NMR evidence to suggest any population of such a conformer (i.e., where vancosamine is orientated upside-down relative to the starting position). The simulation was repeated after the addition of partial charges to the atoms, but the *N*-methylleucine side chain and the V_6 group still folded in over the binding pocket, resulting in the flipping of vancosamine (Fig. 4A – full rotation).

The introduction of charges and therefore the presence or absence of the coulombic term makes little qualitative difference to the overall behaviour of rotation and the 'dip and flip' is still seen. The introduction of charges also leads to the introduction of a potential inaccuracy in the simulation, i.e., the pulling together of atoms to form intramolecular hydrogen bonds. The gas-phase approximation neglects the favourable interaction hydrogen bond donators and acceptors have with the solvent, which in many cases overwhelms any possible intramolecular interactions. Indeed, the molecular design of vancomycin is such that the electrostatic interactions that participate in complex formation (at the site of action) are not exposed to the solvent, but are strengthened by being in a hydrophobic environment. [J.P. Waltho, D.H. Williams and J.G. Vinter, manuscript in preparation].

All the problems in the above simulations may be avoided by adding solvent (in this case, water) to the system. Attempts to immerse entire systems in a bath of water have been reported [10], but these experiments require large amounts of supercomputer time. Instead, the strategy used here was to place single water molecules near the sites of interest on the vancomycin model and allow them to find low-energy positions using molecular mechanics minimisation methods. Specifically, one water molecule was hydrogen bonded to the N-terminal amine NH proton, one

to the three amine NH protons of the carboxylate binding pocket and one was positioned over the binding pocket face of ring 4, hydrogen-bonded to the second water molecule. Energy minimisation of the entire system is allowed to continue within the rotation simulation and normally a self-consistent conformation of the non-spinning part of the antibiotic and the solvent molecules is attained during the first revolution. With the three water molecules in position, the simulation of the rotation of vancosamine proceeds without either the V_6 group or the *N*-methylleucine side chain folding into the binding pocket (Fig. 5). Consequently, vancosamine is not flipped by 'colliding' with ring 2 during the rotation (Fig. 4B – full rotation).

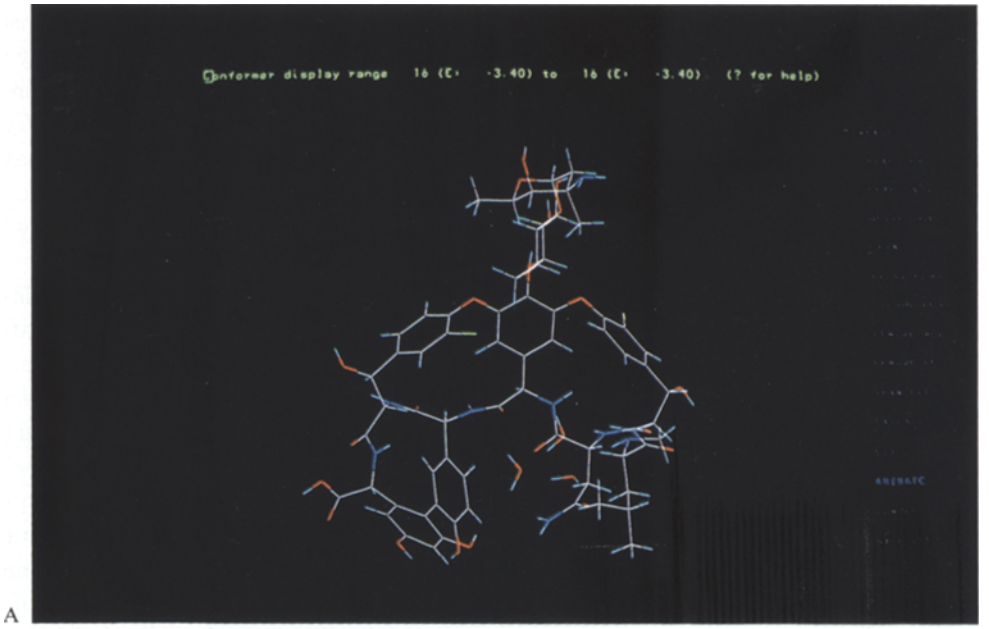
The energy profile obtained for the rotation of vancosamine, shown in Fig. 6A, consists of an energy 'well' with a minimum energy conformation (by approximately 8 kcal·mol^{-2}) after a rotation of 200° from the starting position. This contradicts the NMR observation of approximately equal populations of the conformers with vancosamine over the binding face and with vancosamine over the back face of the antibiotic. The profile of the energy well is similar to the profile of the coulombic energy contribution to the total energy of the system.

The coulombic energy well mainly results from the electrostatic attraction between the protonated amine of vancosamine and the non-binding face of the aglycone when this sugar is over the back face of the molecule, and conversely, repulsion when vancosamine is over the binding face. The positively polarised nature of the binding face of vancomycin is an integral part of its antibiotic activity: three adjacent amide NH groups are used to bind a carboxylate anion in the target peptide. These NH groups have a combined charge approximately equivalent to a formally charged group and so contribute strongly to the coulombic interactions. Correspondingly, the negatively polarised carbonyl groups of these amide units all occupy the back face of vancomycin and lead to a net electrostatic attraction with the amino group of vancosamine.

When the coulombic term is mathematically removed from the energy calculation, (i.e., without re-running the simulation) the energy profile is modified to that shown in Fig. 6B. This is qualitatively similar to the profile expected from NMR studies in that the energy of the conformer with vancosamine over the binding face is equivalent to that of the conformer with vancosamine over the back face, and there is a small barrier for the sugars to exchange between these conformers (i.e., to cross rings 2 and 6).

As the protonated amino group on vancosamine was a major influence on the coulombic energy profile, the simulation was repeated with the charged state reduced to a neutral amine. The rationale for this is that being fully exposed to the solvent, repolarisation of the solvent molecules disperses the centre of charge of the protonated amine (i.e. it is subject to a high dielectric constant in the determination of the experimental data). Other workers have also adopted this approximation for charged species in solutions of high dielectric [11]. The resulting energy profile is shown in Fig. 6C and, like the manipulated profile in Fig. 6B, satisfies the NMR data, i.e., the equivalence in energy of conformers with vancosamine over the binding face and back face of the antibiotic. Furthermore, the calculated value of the energy barriers to interconversion of these conformers (5.7 kcal·mol^{-1}) reproduces that deduced from NMR correlation time observations. The two low-energy conformers are those with vancosamine over the binding face (Fig. 5A) and the back face (Fig. 5B).

Although the calculated and experimental rotational barriers are similar, it should be noted that the calculated values neglect three solvent-related factors: hydrogen-bonding, van der Waals and hydrophobic interactions between the rotating groups and the solvent. The underfaces of



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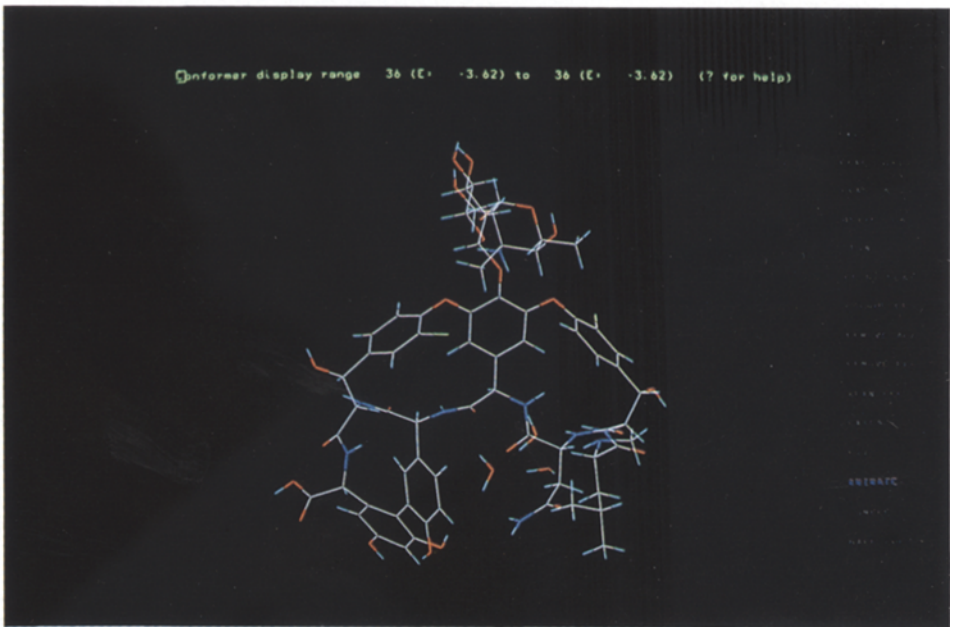

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Fig. 5. Conformers of vancomycin produced during simulation of the rotation of the disaccharide unit when water molecules are present in the binding pocket. (A) Over the binding pocket. Note that the 6-methyl group is no longer attracting the *N*-methylleucine side chain. (B) Over the back face.

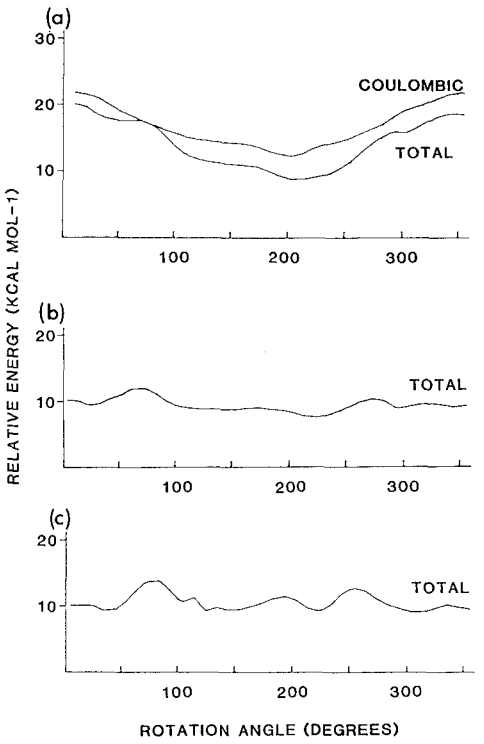

Fig. 6. Energy profiles from simulations of the rotation of the disaccharide of vancomycin (in each case there are three water molecules associated with NH protons of the peptide backbone): (A) with an NH_3+ group on vancosamine, showing both the total energy and the coulombic contribution; (B) as (A) but showing the total energy with the coulombic contribution removed (*after* the simulation); (C) with an NH_2 group on vancosamine, showing the total energy (the coulombic contribution is again included).

both monosaccharide units (i.e., the faces closest to the aglycone portion of the antibiotic during the rotation) are relatively hydrophobic and contain no hydrogen-bond donators or acceptors. Thus, the potential for hydrogen-bonding between the disaccharide and the solvent is not interrupted by the rotation. However, the van der Waals and hydrophobic interaction of the rotating atoms, which in some conformers lie over the aglycone but in others are exposed to the solvent, must influence the real system. At the top of the simulated barriers to rotation, the hydrophobic underfaces of vancosamine and glucose lie over the aromatic rings 2 and 6 of the aglycone. The calculated energy for these conformers includes attractive van der Waals interactions as a result of the proximity of these atoms. In the low-energy conformers between the barriers, the underfaces of the monosaccharide units lie perpendicular to the face of ring 4, and hence are exposed to the solvent. This gives rise to van der Waals attractions between the antibiotic and the solvent, but also to unfavourable hydrophobic group–solvent interactions. The former interaction is an enthalpic contribution and the latter mainly an entropic contribution to the free energy of these conformers and neither are included in the calculations. In terms of free energy, these interactions are of opposite sign. The calculated stability of the low-energy conformers is too low because van der Waals attractions between the underfaces of the disaccharide and the solvent are not considered.

However, the calculated stability of the low-energy conformers is too high because the increased area of hydrophobic regions exposed to the solvent (relative to when the underfaces of the disaccharide lie above rings 2 and 6) should raise the free energy of the system. It is only because the above negative and positive contributions to the free energy of the conformers are of similar magnitude that a reasonable barrier to the rotation of the disaccharide is calculated.

In an attempt to remove the discrepancy of the calculation containing intramolecular, but not most of the intermolecular (antibiotic-solvent) van der Waals attractions of the system, the simulation was repeated with the attractive component of the van der Waals contribution to the total energy removed from the molecular mechanics calculations. This assumes that the intra- and intermolecular attractive van der Waals interactions are equal. However, this strategy led to unreasonable reorientations of the disaccharide during the rotation, for example the falling of the V_6 group over the face of ring 4 as the sugar finished crossing rings 2 or 6. It is likely that these problems are a result of the repulsive component of the van der Waals interactions causing conformational changes that are normally hindered because of van der Waals attractions. For example, as the leading edge of vancosamine moves from the above ring 2 to above the space between rings 2 and 6, the trailing edge is pushed upward because of repulsive van der Waals interactions with ring 2. This results in the reorientation of vancosamine about its glycosidic linkage with glucose such that the V_6 methyl group occupies the space over the face of ring 4. In the above simulation attractive van der Waals forces limit the pushing effect of, for example, ring 2 on vancosamine and prevent vancosamine from turning upside-down. In the real system (but not in the simulation) there is a penalty to the 'upside-down' conformation of vancosamine because of the disruption of the hydrogen-bonding potential of the sugar.

CONCLUSION

It appears that the COSMIC computational chemistry package may be used to study the motion of groups within polar molecules, but there are certain limitations. Unreasonable folding of hydrophobic groups over strongly hydrophilic regions may be avoided by adding individual water molecules to the appropriate part of the system. Thus, the size of the calculation is not increased vastly, as is necessary using complete solvation models. If the rotating system contains very hydrophilic regions, the simulation will only be accurate where the solvation of these groups is not interrupted by the rotation. The rotation of the disaccharide of vancomycin is a good example of this – it is the hydrophobic faces that pass over each other during the rotation leaving, for example, the protonated amine open to full solvation throughout. A related point is that in some *simulated* conformers, intramolecular hydrogen bonds may contribute significantly to the stability of the conformer. In solution, competition with solvent molecules would weaken, and even overwhelm the intramolecular interaction. Intramolecular van der Waals attractions are included in the energy calculations (removal leads to unreasonable conformational distortions) but as solvation is incomplete, van der Waals attractions of parts of vancomycin with solvent molecules are underestimated. However, in our case, the hydrophobic nature of the groups which change their interaction with the solvent during the rotation counterbalances the underestimation of van der Waals attraction with the solvent. Finally, it appears in this system that the intramolecular interactions of formally charged groups are more accurately simulated by their neutral analogue than the gas-phase ion.

Throughout these simulations, no enhancements to the basic potentials or force-field parameters within COSMIC[2] were done. This is, therefore, one example in which careful attention to the physical science of a process rather than to the enhancement or 'fitting' of the mathematics of the problem has resulted in a satisfactory outcome. We are convinced that many computer-generated simulations using simple molecular mechanics fail, not because of poorly constructed mathematical models but because of poorly understood concepts of physical events.

ACKNOWLEDGEMENTS

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