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Chemistry

Intermolecular anti-parallel β sheet: Comparison of predicted and observed conformations of gramicidin S

(conformational analysis/oligopeptide structure/differential-geometric comparison/dimerization/β structure)

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ABSTRACT **A recently determined x-ray structure of the hydrated gramicidin S–urea complex is compared with a structure predicted by conformational energy minimization. It is shown that the two structures are in good general agreement, including the prediction of a hydrogen bond between the side-chain amino group of ornithine and the backbone carbonyl of phenylalanine. This agreement demonstrates the power of empirical potential energy methods in conformational analysis and illustrates one method for solution of the multiple-minimum problem for small peptides. It is noted that, in the crystal, gramicidin S is a dimer that forms an *intermolecular* antiparallel four-stranded β sheet and that differences between the predicted and x-ray structures can be explained by this intermolecular interaction. The residual conformational asymmetry of the x-ray structure is shown to be due to the formation of the complex with urea.**

The prediction of polypeptide and protein conformations requires first the development of appropriate empirical potential energy functions and then the solution of the "multiple-minimum" problem—i.e., the location of the global minimum among all those occurring in the multidimensional plot of energy vs. conformational variables for the molecule under consideration. This problem has been solved (1) for small open-chain and cyclic peptides and for synthetic analogs of fibrous proteins but not for globular proteins. The solution achieved in the former cases involves minimizing the conformational energy of a sufficiently large number of different starting conformations to ensure the complete coverage of conformational space.

One example of a small molecule for which this problem has been solved is the cyclic decapeptide antibiotic gramicidin S [cyclic(-Pro-Val-Orn-Leu-D-Phe-)$_2$], for which an initial 10,541 conformations were examined, with C_2 symmetry imposed in the computations (ref. 2, hereafter referred to as D-G-S). The structure of lowest energy was designated as M1 in ref. 2; the existence of at least two similar structures (M2 and M3) having slightly higher energies suggests that the gramicidin S molecule is somewhat flexible. This molecule has been of considerable theoretical (2–11) and experimental (12–18) interest; the results of several of the experimental studies were used in selecting some of the initial conformations used for energy minimization.

Recently, the x-ray crystal structure of a hydrated gramicidin S–urea complex has been solved (19), and it is therefore of interest to compare the cartesian coordinates of this structure with the ones computed for M1 given in table VIII of ref. 2. The dihedral angles (ϕ_i,ψ_i) of the residues in the x-ray and several computed structures have been compared by Liquori and De Santis (20). We shall show that agreement between the D-G-S and the x-ray structures is generally good and that the principal differences are due to a remarkable type of bimolecular association that occurs in the crystal. This conclusion differs from that of Liquori and De Santis (20), who suggested that irregularities in the x-ray structure are due to intramolecular effects.

Comparison of calculated and x-ray structures

Two separate comparisons were carried out by using the differential-geometric method (21, 22). In the first, the D-G-S-predicted structure was compared with the x-ray structure. In the second, the two halves (residues 1–5 and 6–10) of the x-ray structure were compared to determine its degree of asymmetry.

The results of the first comparison are shown in Fig. 1. The conformational distance, ρ_i, at site i, between the two molecules, is actually the conformational distance between the virtual-bond backbone segments $C^\alpha_{i-1} - C^\alpha_{i+2}$ in the two molecules (21, 22). It should also be remembered that, because gramicidin S is a cyclic decapeptide, $C^\alpha_i \equiv C^\alpha_{i+10}$, a fact that is used in calculating ρ_9, ρ_{10}, and ρ_1.

Thus, the folding of the backbone of the DGS structure differs from that of the x-ray structure mainly at four sites—two neighboring residues, Val-3 and Orn-4, and the chemically symmetric Val-8 and Orn-9 (as noted in ref. 22, the conformations of four C^α segments for which $\rho < 0.1$ are probably identical within the accuracy of the x-ray data). These residues fall within the extended strands that together make up the β-sheet section of the molecule. Also, there is quite good correspondence between the predicted and observed conformations in the other regions of the molecule. The bends are particularly well predicted, as shown by the low values of ρ_6 and ρ_1.

In the computations (2), the predicted structure was required to be C_2 symmetric on the basis of NMR results, which show no splitting of the various peaks. It is therefore of interest to determine the degree of backbone symmetry exhibited by the x-ray structure. As shown in Fig. 2, in which the ρ_is are calculated for the comparison of residues 2–6 and 7–1 (≡11), the x-ray structure is slightly asymmetric. The principal asymmetry is between the two segments centered at Leu-Phe.

It is of interest to compare the side-chain conformations of the D-G-S and the x-ray structures. Certain side chains are found to have quite similar conformations in the two structures. These are the two D-Phe side chains and *one* of the two Orn side chains. It is particularly significant that D-G-S correctly predicts an Orn-Phe side-chain-backbone hydrogen bond. In the x-ray structure, this occurs only on one side of the molecule; we shall suggest a reason for this below. It also appears that the dis-

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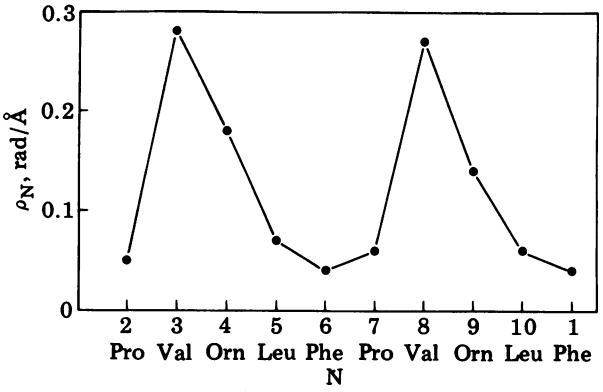

FIG. 1. Differential-geometric comparison of D-G-S and x-ray structures of gramicidin S. ρ is the differential-geometric distance parameter. N, residue number.

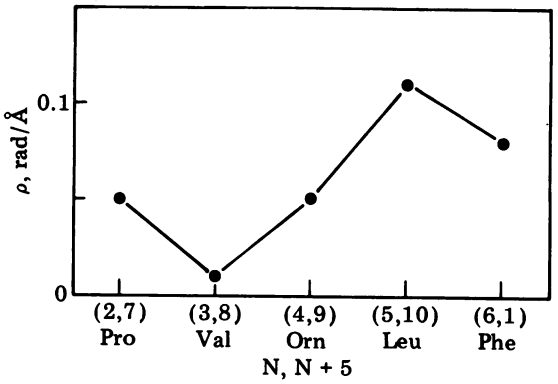

FIG. 2. Differential-geometric comparison of the two chemically identical halves of the x-ray structure of gramicidin S.

crepancies that exist between the two structures in the conformations of other side chains are not as significant as might be thought. Recent NMR data (16–18) suggest that, in *solution*, there are multiple side-chain conformations in gramicidin S, although the evidence favors a rigid backbone. (This experimental conclusion was predicted by D-G-S on the basis of the energy minimization studies.) In those cases where there is disagreement between the χ^1 values of D-G-S and those of the x-ray structure, the χ^1 value of each structure is in fair agreement with *one* of the several χ^1 values possible in solution. It appears that crystal forces select a particular set of χ values out of the ensemble present in solution. As pointed out in ref. 18, the D-G-S values are in reasonable agreement with the rotamer distribution observed in solution.

Comparison of Fig. 8 of D-G-S (2) with figure 1a of Hull *et al.* (19) shows the correct prediction by D-G-S of the observed *intra*molecular hydrogen-bonding pattern, particularly that of the Orn-Phe side-chain-backbone hydrogen bond.

In view of the apparent flexibility of side-chain conformations and the fact that there is no evidence for asymmetry in solution, one must look beyond side-chain-backbone interactions for an explanation of the differences between the D-G-S and the x-ray structures. We shall focus on the role of the influence of crystal interactions in determining the x-ray structure.

As pointed out by Hull *et al.* (19), one of the salient features of the x-ray structure is the presence of an *intermolecular* hydrogen bond between backbone atoms of two gramicidin S molecules that are related by a twofold axis. We shall refer to the two symmetry-related molecules as M and M′. Analysis of the x-ray coordinates suggests that there is a *pair* of hydrogen bonds, one between the carbonyl oxygen of Orn-4 of M and the backbone amino group of Orn-4 of M′, and the other the reverse. We also draw attention to the location of the urea molecules, which fall in the immediate neighborhood of Phe-1 and Leu-10 of both M and M′.

In Figs. 3 and 4, we show stereoscopic views of the x-ray structure of the M–M′ pair and the associated urea molecules. As shown in Fig. 3, in the crystal, M and M′ undergo a remarkable type of association, forming a *four-stranded, antiparallel, intermolecular β sheet*. The overall twist of this four-stranded bimolecular sheet is similar to those of the separate molecules (see Fig. 4). This twist, which is a general feature of β sheets, is necessary to ensure the proper mutual registration of the individual strands and readily explains the deviations shown in Fig. 1. The major peaks in ρ_i occur at Val-3–Orn-4 and the chemically symmetric Val-8–Orn-9. Detailed consideration of the differential-geometric parameters shows that these peaks indeed arise from differences in the twist of the extended structure at Val-3–Orn-4 and at Val-8–Orn-9. Although only the Val-3–Orn-4 side of the molecule is required to register with the corresponding part of the other molecule (i.e., it is the 1–5 portion of each molecule that is in contact in the dimer), the conformational constraint imposed by the cyclic nature of the structure suggests that a corresponding twist will occur at the chemically symmetric point on the other side of the molecule. It therefore seems clear that the major deviations between the x-ray and predicted structures, which occur precisely in the region of the molecule where intermolecular β-sheet association occurs, are due to intermolecular interactions. These interactions perturb the single-molecule structure, which is quite close to the D-G-S structure.

Similar considerations explain the slight conformational asymmetry apparent in the x-ray structure (see Fig. 2). This

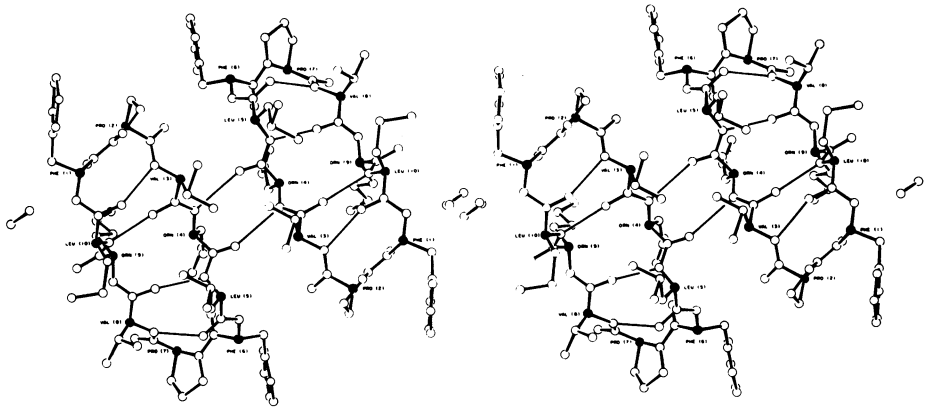

FIG. 3. Stereo ORTEP diagram of the M–M′ pair viewed from above. Half of the two associated urea molecules are also shown. Inter- and intramolecular hydrogen bonds are indicated by thin lines. The antiparallel, four-stranded β sheet formed by the dimer is visible. [The $C^{\delta 2}$ atom of Leu-10 was not visible in the x-ray structure (19).]

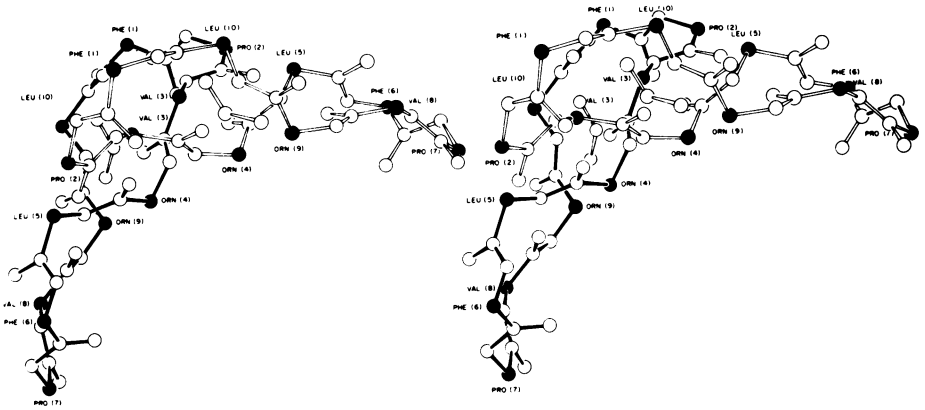

FIG. 4. Stereo ORTEP diagram of the M–M′ pair (including urea molecules) viewed from the side. The backbone of one of the gramicidin S molecules is shown in black. Hydrogen bonds and side chains are not shown. The twist of the β sheet is seen in this view.

arises at Leu-Phe, precisely in the region where, on one side, the molecule interacts with a urea molecule. Because of the small size of the urea molecule, which enables it to adapt to the conformation of the larger gramicidin S molecule, this interaction can be expected to induce a relatively small perturbation of the overall symmetry of the molecule; this is in fact observed (see Fig. 2). This small perturbation is sufficient to rotate the carbonyl group of the Phe residue slightly, thus making the formation of the Orn-Phe side-chain-backbone hydrogen bond on this side of the molecule impossible.

It should be noted that, in crystals of *N*-acetyl gramicidin S (23) (in which the formation of a small-molecule complex does not occur), asymmetry is not observed. This provides support for the view that hydrogen bonding to urea molecules induces the asymmetry in the hydrated gramicidin S–urea complex.

Summary

To summarize, we have pointed out that, in the crystal of its hydrated urea complex (19), gramicidin S undergoes an intermolecular association to form a four-stranded antiparallel β sheet. The conformational differences between the observed x-ray structure of a single molecule and the structure predicted by energy minimization (2) can be explained as arising from this bimolecular association and from the gramicidin S–urea interaction. This result demonstrates the power of conformational analysis with empirical potential functions and illustrates a solution of the multiple-minimum problem for small peptides.

It is interesting to speculate as to whether this intermolecular β sheet formation occurs in solution. It has been suggested (18) that the C_2 symmetry suggested by solution NMR is only a time-averaged effect. It may be that some of the characteristic vibrational modes of a β sheet, operating in such an oligopeptide bimolecular complex, are capable of producing an apparent C_2 symmetry. Clearly this question deserves further experimental clarification, because such a solution complex would constitute a particularly simple system for the study of β-sheet formation.

The possible presence of a four-stranded β-sheet complex (at very low concentrations) in solution may have implications for the mechanism of antibiotic action, because the bimolecular β sheet may be the species responsible for binding to receptor sites. It therefore seems appropriate to ask whether this type of structure formation through molecular association is a general phenomenon in peptide antibiotics.

We thank the York group [Hull *et al.* (19)] for providing the x-ray coordinates of the hydrated gramicidin S–urea complex. We are grateful to Prof. E. Benedetti and Dr. G. Némethy for helpful discussions and to S. M. Rumsey for her patient and skilled production of the ORTEP diagrams. This work was supported by research grants from the National Institute of General Medical Sciences of the National Institutes of Health (GM-24893) and from the National Science Foundation (PCM79-20279). S.R. was a National Institutes of Health Postdoctoral Fellow, 1977–1978.

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