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HYDROGEN BONDING INVOLVING THE ORNITHINE SIDE CHAIN OF GRAMICIDIN S

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SUMMARY: The side-chain and backbone hydrogen bonding pattern is identical in the computed minimum-energy conformation of gramicidin S and in the recently determined X-ray crystallographic structure of the hydrated urea commplex of this peptide. The identity of hydrogen bonding (except where it is affected by intermolecular interactions in the crystal) and the close agreement of the overall backbone conformation attest to the validity of the conformational energy computations used to determine the unique low-energy conformation of this cyclic peptide.

Conformational energy computation is one of the important approaches for gaining an understanding of the interactions that determine the folding of polypeptide chains (1,2). Conformational studies of small peptides serve as a critical test of the methods because predicted conformations can be checked easily against observations of conformational properties. Many experimental and theoretical studies of the cyclic decapeptide antibiotic gramicidin S have been carried out. Its amino acid sequence is cyclo(L-Pro-L-Val-L-Orn-L-Leu-D-Phe)$_2$.

An extensive theoretical conformational study of gramicidin S has been reported by Dygert et al. (3). They examined over 10,500 initial conformations with C_2 symmetry (corresponding to the symmetry of the amino acid sequence), and used energy-minimization techniques to find the conformation of lowest energy. This conformation (shown in Fig. 3 of ref. 3) can be described as a two-stranded antiparallel β-sheet formed by the Val-Orn-Leu sequences, with β-bends at the D-Phe-Pro sequences on both ends.

Subsequently, the crystal structure of the hydrated gramicidin S-urea complex was determined by Hull et al. (4). The backbone structure of the molecule in the crystal agrees closely with that of the lowest-energy computed structure (5). The conformation of the molecule in the crystal departs

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slightly from C_2 symmetry because it is present as a dimer in the crystal and because of complexing with urea (5).

In the computed conformation (3), the $N^{\epsilon}H$ group of both Orn side chains forms hydrogen bonds with the C=O groups of the D-Phe residues that precede them in the sequence. When the theoretical structure was reported in 1975, it was stated that "the ornithine side chain may be free to occupy more than one rotational state" and that there is "a hydrogen bond between a δ-NH_2 proton of Orn and the backbone CO of Phe. There is no experimental evidence indicating the existence of this interaction" (3). When the X-ray structure was determined in 1978, it was stated that "there is an intramolecular hydrogen bond between one of the ornithine side-chain atoms and the D-phenylalanine carbonyl oxygen atom which had not been predicted" (4). In the crystal structure, one of the Orn side chains forms a hydrogen bond with the C=O group of the D-Phe residue that follows it in the sequence.The other ornithine side chain has a different conformation in the crystal because the Orn N^{ϵ} atom has a close contact with a water molecule (4); therefore, it cannot form a hydrogen bond with the backbone.

While similar kinds of backbone and side-chain hydrogen bonds occur in the previously computed and the observed structures, the Phe partners of the Orn side chains are interchanged in these two structures. This has prompted us to reexamine the conformational freedom (see above) of the Orn side chain in the lowest-energy computed backbone conformation.

We have carried out the computations with a modification (3) of the recently updated ECEPP/2 version (6) of the "Empirical Conformational Energy Program for Peptides"·(7) algorithm. This modification, which had been used previously, generates exactly closed cyclic peptide structures with C_2 symmetry and minimizes the intramolecular energy as a function of backbone and side-chain dihedral angles. In the initial conformations selected, the dihedral angles of the backbone and of all side chains except those of Orn were taken to be the same as those in the lowest-energy conformation computed earlier (structure M1 of ref. 3). The Orn side chain was initially set into all conformations in which side-chain atoms are staggered, i.e. all combinations, 60°, 180°, and -60°, were used for the dihedral angles χ^1, χ^2, χ^3 and χ^4. The energy was then minimized by varying <u>all</u> side-chain and backbone dihedral angles.

In addition to the structure reported earlier (M1 of ref. 3), a low-energy conformation was found in which the $N^{\epsilon}H$ of each Orn side chain forms a hydrogen bond with the C=O group of the D-Phe residue that <u>follows</u> it in the sequence, i.e. the Orn side chain essentially flips from the D-Phe residue on one side to the corresponding residue on the other side in going from M1 to this alternative structure. This hydrogen-bonding pattern agrees fully with that found in the X-ray structure, as shown in Fig. 1. The N...O distances of the backbone hydrogen bonds are as follows: 2.93 and 2.90 Å for the Val-

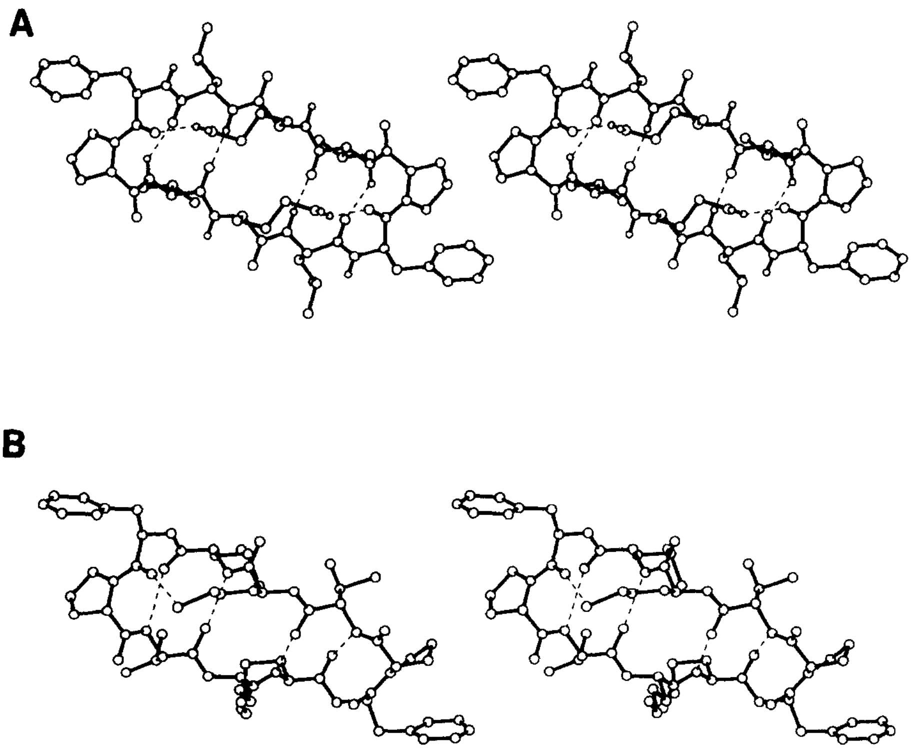

Figure 1. Stereoscopic drawing of the computed (A) and X-ray (B) structures of gramicidin S. In addition to the four backbone hydrogen bonds, the Orn...Phe side chain-backbone hydrogen bond is seen in both structures. Only polar hydrogen atoms are shown in part A; no hydrogens are shown in part B.

Table I
Dihedral angles of the lowest-energy computed conformation of gramicidin S

Residue	ϕ	ψ	χ^1 (degrees)	χ^2	χ^3	χ^4
Val	-90	131	-178	60	60	
Orn	-147	126	166	72	166	60
Leu	-154	114	-175	75	60	60
D-Phe	59	-137	172	-89		
Pro	-75	8				

NH...OC-Leu and the Leu-NH...OC-Val hydrogen bonds, respectively, in the computed conformation (Fig. 1A), while the corresponding distances are 3.15 and 2.82 Å, respectively, in the X-ray structure (Fig. 1B). The Orn-NH...OC-Phe side chain-backbone hydrogen bond distance is 2.96 and 2.82 Å in the computed and the X-ray structures, respectively, indicating the close correspondence of the structures. The dihedral angles of the computed structure are listed in Table I. Except for the changed orientation of the Orn side chain, the conformation of the structure reported here is very close to the lowest-energy conformation computed earlier (structure M1 in Table V of ref. 3). The backbone and side-chain dihedral angles agree to within less than 12°, except for larger changes of ψ_{Val} and ϕ_{Orn} which reflect a small tilting of the plane of the Val-Orn peptide group that does not affect the overall conformation of the molecule. The energy of the conformation reported here is 1.4 kcal/mol lower than that of conformation M1 so that this structure is somewhat more favorable than the one reported previously (3).

Comparison of the theoretically-predicted conformation with the X-ray crystallographic structure serves as a confirmation of the validity of the theoretical approach and of the parameter set used in the computation.

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