Minimization of Polypeptide Energy. IV. Further Studies of Gramicidin S*

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ABSTRACT: Using energy minimization techniques previously described, the structure of gramicidin S proposed by Hodgkin and Oughton and by Schwyzer was taken as a starting point for the minimization procedure. After energy minimization, the resulting structure differed from the initial one, and had an energy which was considerably lower than that of any structure of gramicidin S heretofore reported. However, like the Hodgkin–Oughton–Schwyzer structure from which it was derived, it is consistent with the chemical evidence.

Using energy minimization techniques, we have previously (Scott *et al.*, 1967) evaluated the structures of gramicidin S proposed by Vanderkooi *et al.* (1966), designated as GS_I, and by Liquori *et al.* (1966), designated as GS_{II}; starting with these two conformations, the structures obtained *after energy minimization* were referred to as GS_{Ia} and GS_{III}, respectively. We report here similar calculations for the structure proposed by Hodgkin and Oughton (1957) and by Schwyzer (1958, 1959), designated as GS_{IV}, and supported by recently obtained chemical evidence (Schwyzer and Ludescher, 1968). After energy minimization, a structure, designated as GS_V, is obtained which is different from that proposed by Hodgkin and Oughton, and Schwyzer; further, the computed structure has a considerably lower energy than any of those examined heretofore.

On the basis of nuclear magnetic resonance studies, Schwyzer and Ludescher (1968) have shown that the aromatic parts of the D-phenylalanine groups must be near the ornithine groups. These interactions occur only in model GS_{IV} and not in the other models, GS_{Ia} and GS_{III}. Hence, model GS_{IV} was evaluated by taking it as a starting conformation for energy minimization.

Experimental Procedure

Model GS_{IV} was constructed from a set of nonspace-filling models, so as to have four intramolecular hydrogen bonds (see also Craig, 1968) and to conform as closely as possible to that proposed by Hodgkin and Oughton (1957) and by Schwyzer (1958, 1959). Two sets of backbone dihedral angles (shown in parentheses and in square brackets, respectively, in Table I) were measured from this model, these two sets representing the range of possibilities consistent with the given model and with the method of estimating the angles. After energy minimization, by the procedure described below, both starting conformations converted to the *same* low-energy structure.

All the energy parameters and the procedure for calculation were the same as reported by Scott *et al.* (1967), except that the program was converted from a CDC-1604 to an IBM 360–65 computer system. Also, in contrast to the previous calculations, the ornithine amine groups were not allowed to take part in hydrogen bonds; the energies of structures GS_{Ia} and GS_{III} were recomputed on this same basis.

Results and Discussion

The structure (GS_V) obtained after energy minimization, starting from structure GS_{IV}, is shown in Figures 1 and 2, and the energy and dihedral angles are given

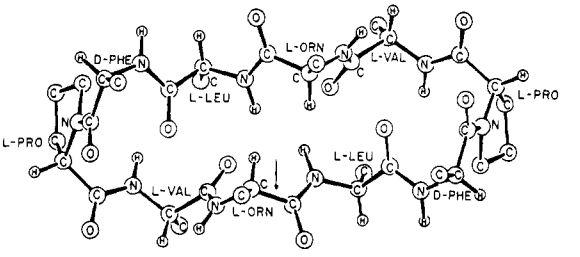

FIGURE 1: View down the twofold axis of symmetry of conformation GS_V, with most of the side chains omitted. The arrow indicates the position at which the ring was closed. The cartesian coordinates of structure GS_V have been deposited as document no. as yet unavailable with the ASIS National Auxiliary Publication Service, CCM Information Sciences, Inc., 22 West 34th St., New York, N. Y. 10001. A copy may be secured by citing the document number and by remitting $1.00 for microfilm or $3.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS. The coordinates are in a format compatible with the plotter program OR TEP (ORNL-3794, Revised, June 1965).

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TABLE I: Dihedral Angles (in degrees) of Structures[a] GS_V Obtained by Energy Minimization from Structure GS_{IV}.[b]

Residue	ϕ(N–C$^\alpha$)	ψ(C$^\alpha$–C′)	χ_1	χ_2	χ_3
L-Val	59.0 (15) [40]	283.0 (340) [320]	317.0		
L-Orn	103.0 (10) [50]	270.5 (300) [300]	297.1	88.2	40.9
L-Leu	81.6 (20) [40]	314.3 (325) [290]	331.5	299.4	
D-Phe	139.1 (75) [210]	120.3 (195) [90]	39.0	84.3	
L-Pro	123.0 (123) [123][c]	120.3 (160) [160]			
L-Val	62.3 (15) [40]	275.6 (340) [320]	321.7		
L-Orn	107.1 (10) [50]	287.1 (300) [300]	291.8	85.4	48.1
L-Leu	77.7 (20) [40]	304.2 (325) [290]	332.0	293.5	
D-Phe	147.1 (75) [210]	120.6 (195) [90]	39.6	90.8	
L-Pro	123.0 (123) [123][c]	120.7 (160) [160]			

[a] The energy of GS_V is −112 kcal/mole. [b] The backbone dihedral angles of the two starting structures are given in parentheses and in brackets, respectively. [c] Fixed angle of proline, *i.e.*, set at 123°.

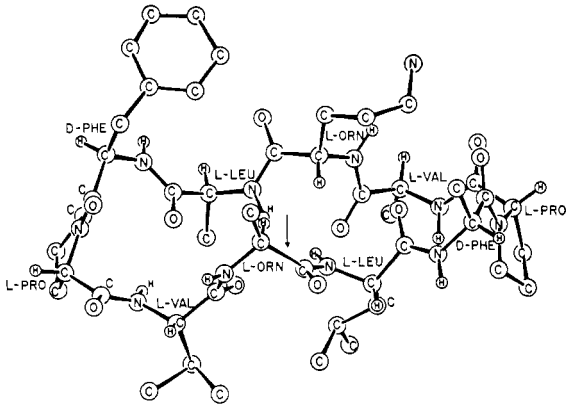

FIGURE 2: View of conformation GS_V, rotated 40° about the line joining the C$^\alpha$ atoms of the proline residues. For clarity, some of the side chains have been omitted. The apparent asymmetry of the molecule in this figure is due to the angle of observation.

in Table I. The loop was closed, during the calculation, by joining the C′ and C$^\alpha$ atoms of one of the ornithine residues, and the deviation from closure was 0.023 Å at the end of the calculation; the deviations of the bond angles at the gap were only several degrees.

The two starting conformations (GS_{IV}) had very high energies. However, after several computing cycles, the energies of both of these structures converged to −112 kcal/mole, and both structures converged to structure GS_V. The recomputed energies of structures GS_{Ia} and GS_{III} were −98 and −100 kcal/mole, respectively (previously reported as −96 and −98, respectively, the energy difference arising from the inclusion of a hydrogen-bonding function for the ornithine group in the earlier calculations). Thus, the energy of structure GS_V is considerably lower than those of any of the other structures of gramicidin S which have been considered. Its resulting twofold symmetry is nearly perfect, the average deviations in the backbone and side-chain dihedral angles both being ±3°.

Structure GS_V is different from the starting one (GS_{IV}), the major difference being that GS_V does not have any formal hydrogen bonds. Instead, its low energy arises from favorable nonbonded and electrostatic energy contributions.

A study of Figures 1 and 2 shows that GS_V is very compact. Also, it retains the vicinal relationship of the phenylalanine and ornithine groups, required by the nuclear magnetic resonance results of Schwyzer and Ludescher (1968). Further, there appear to be three conformationally different types of exchangeable hydrogen atoms. The first set includes two which would form relatively weak hydrogen bonds; these are the NH hydrogens of leucine which are 2.3 Å from the ornithine oxygens. These are rather long distances for hydrogen bonds, but the hydrogens are firmly held in the interior of the ring. The second set includes the two valine NH hydrogens which are 3.3 and 4 Å from the D-phenylalanine and proline oxygens, respectively. Even though these hydrogens are not hydrogen bonded, they are nevertheless buried inside the ring and surrounded by side-chain atoms. The third set includes the remaining four exchangeable hydrogens, and are outside the ring.

Although GS_V has a considerably lower energy than the structures previously examined, it cannot yet be regarded as the final computed "most stable" conformation, because we have not yet examined the complete energy surface nor the possibility that gramicidin S may contain *cis* amide bonds; such calculations are now in progress.

Acknowledgments

After this paper was submitted for publication, the paper of Stern *et al.* (1968) appeared. These workers carried out a nuclear magnetic resonance analysis of gramicidin S, and proposed a conformation based on their data. Taking account of two typographical errors in their Figure 5 (confirmed by subsequent correspondence with Dr. Gibbons), *viz.*, the Val-Pro amide bond is *trans*, even though it appears as *cis* in the drawing, and the value of ψ for Phe is about 130° instead of 330°, then their proposed structure is similar to that computed here for GS_V. However, there are some differences, *viz.*, Stern *et al.* propose that the values of ϕ for residues Val, Orn, and Leu are all equal, and they

TABLE II: Dihedral Angles (in degrees) of Structure[a] GS_{VI} Obtained from a Statistical Search Procedure.

Residue	ϕ(N–C$^\alpha$)	ψ(C$^\alpha$–C′)
L-Val	71.6	258.2
L-Orn	125.9	271.3
L-Leu	46.4	305.8
D-Phe	122.9	121.9
L-Pro	(123.0)	130.3
L-Val	75.7	258.1
L-Orn	127.4	271.3
L-Leu	43.3	304.8
D-Phe	126.9	122.1
L-Pro	(123.0)	126.2

[a] The energy of GS_{VI} is −114 kcal/mole.

propose the existence of four hydrogen bonds.

Also, since submitting this paper for publication, we have developed a method for statistically searching the energy hypersurface for low-energy minima. In contrast to the procedure of the present paper, in which we started with the proposed structure of Schwyzer, this new method identifies various starting structures (prior to energy minimization) on a statistical basis, without an *a priori* bias toward any given starting structure. By this procedure, we have found all the previous low-energy GS structures reported by Liquori *et al.* (1966) and by us (Vanderkooi *et al.*, 1966; Scott *et al.*, 1967). During the course of this work, another low-energy structure (of energy 2 kcal/mole lower than that in Table I, and with a deviation from closure of 0.0003 Å) was found; its dihedral angles (for structure GS_{VI}) are shown in Table II. The values of the χ's are the same as those of Table I, and are not shown in Table II. The "hydrogen bonds" of structure GS_{VI} are less favorable than "those" of structure GS_V; also, the twofold symmetry of GS_{VI} is better than that of GS_V. Since work on the statistical search procedure is continuing, structure GS_{VI} should not yet be regarded as that corresponding to the global minimum. When this work is completed, the details of the method, together with the computed structure, will be reported.

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