Initial Attempts at a Theoretical Calculation of the Conformation of Gramicidin-S*

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ABSTRACT: Computer methods for the generation and testing of polypeptide chains have been applied to the study of the cyclic decapeptide, gramicidin-S. A relatively small number of values for the backbone rotational angles were taken from the allowed regions of the steric dipeptide maps for the individual residues, and all of the sterically allowed backbone conformations were generated using these angles. Conformations (282) which could fit side chains resulted.

Calculations of the energies of each of these conformations were carried out in order to find the conformation of lowest energy. Torsional, electrostatic, nonbonded, and hydrogen-bonded energy contributions were included. It proved possible to select one of the conformations as being energetically the most favorable. This conformation seems to be at or near a potential energy minimum. Its geometrical properties agree well with the information available from X-ray diffraction studies on gramicidin-S derivatives.

In a previous publication (Némethy and Scheraga, 1965), a mathematical method was developed for the determination of sterically allowed conformations of a polypeptide chain of a given amino acid sequence. The restrictions on the conformations of a peptide unit, arising from steric overlaps, have been obtained from systematic studies of peptides of the various amino acids (Leach *et al.*, 1966a,b; Némethy *et al.*, 1966). These studies indicated the sterically allowed ranges of the angles of rotation around the N–C^{α} and C^{α}–C′ single bonds of the peptide unit (denoted by ϕ and ψ, respectively[1]); the information obtained was conveniently summarized on dipeptide maps. In the computation of allowed conformations of longer polypeptides, it is necessary to select a discrete number of conformations (*i.e.*, pairs of ϕ and ψ values) for each amino acid from the allowed regions of the dipeptide maps. Originally (Némethy and Scheraga, 1965), these points were chosen to correspond to the assumed minima of the potential functions for rotation around the single bonds, but this method of selection must be abandoned in favor of a procedure to be discussed below, since the energy barriers to rotation around the N–C^{α} and C^{α}–C′ bonds are now thought to be low (Brant and Flory, 1965; Scott and Scheraga, 1966b).

In the earlier work, conformations were accepted or rejected on the basis of the steric criterion; *i.e.*, the atoms were treated as hard spheres which could not overlap. Many sterically allowed conformations of apparently equal merit can be expected to result with that method. It would be desirable to replace the steric approach by one in which the conformation with minimum total free energy is found. In the present work, the conformation of minimum energy was sought. A combination of the steric and energy approach was used; many sterically allowed conformations were first found, and the conformational energy of each of them was calculated. This procedure is intermediate between the original hard sphere method and a complete calculation (now being developed) in which the energy is minimized as a function of the rotational angles.

After first testing the polypeptide-generating procedures on a peptide loop of ribonuclease (Némethy and Scheraga, 1965), it was desirable to apply the method to the computation of the conformation of a naturally occurring peptide, for which results could be compared with observed data. Gramicidin-S was chosen, since it has several advantages over other molecules of similar size. It is a cyclic decapeptide, consisting of two identical pentapeptides joined into a ring by peptide bonds

$$\text{-(L-Val-L-Orn-L-Leu-D-Phe-L-Pro)}_2\text{-}$$

The presence of the prolyl residues and of several residues with bulky side chains (especially valyl) aids in shortening the computation, since these residues have only a few allowed conformations (Leach *et al.*, 1966a). Also, an early, incomplete structural determina-

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[1] The proposed standard conventions and nomenclature (Edsall *et al.*, 1966) are used throughout this paper.

tion by Schmidt *et al.* (1957), using X-ray diffraction, showed that the molecule in the crystal has a twofold symmetry axis. By using this piece of information in the computation, the number of variables was cut in half.

A preliminary report on the theoretical investigation of gramicidin-S has appeared (Scheraga *et al.*, 1965). The result given in that paper was obtained by restricting all single-bond rotations to their assumed positions (Némethy and Scheraga, 1965) of minimum torsional energy. This restriction has now been removed; instead, the much weaker restriction that the backbone rotational angles must lie in the allowed regions of the steric dipeptide maps (Leach *et al.*, 1966a) was used. As a result, a different conformation was obtained in the present work than that given in the preliminary report (Scheraga *et al.*, 1965).

Methods

In general, the methods used in the present work were the same as have already been described (Némethy and Scheraga, 1965; Leach *et al.*, 1966a); only changes and additions will be explained here.

The steps in the calculations were as follows. For a given set of backbone rotational angles for each residue, all sterically acceptable backbone conformations which fulfilled the requirement of a twofold axis of symmetry were found. Side chains were then added to these backbones; backbone conformations which could not sterically accommodate side chains were discarded. The energies of the remaining backbone conformations were computed. The backbones with low energies were subjected to minor variations in ϕ and ψ in order to further lower their energies. All possible sterically allowed combinations of side-chain conformations were then generated on the backbones of low energy, and the energy contributions due to the side chains were calculated. The conformation with the lowest total energy could thus be chosen out of the entire list of sterically allowed conformations.

The backbone valence bond angles and lengths were held fixed at the Corey and Pauling (1953) values, as quoted by Leach *et al.* (1966a). The side-chain coordinates were as given by Leach *et al.* (1966a), except that the CCC bond angles at side-chain atoms which were not branch points were increased to 112.5°; the bond angles at branch points were retained at 109.5° (Scott and Scheraga, 1966a,b). The proline geometry was the same as obtained from the L-Leu-L-Pro-Gly structure (Leung and Marsh, 1958) by Leach *et al.* (1966a). The van der Waals contact distances used in the steric tests for atomic overlap were the same as given in Table VIII of Leach *et al.* (1966a).

ϕ and ψ values corresponding to rotations about the N–C^{α} and C^{α}–C′ bonds, respectively, were chosen from the sterically allowed regions of the appropriate dipeptide maps (Leach *et al.*, 1966a). Several points were taken from each map. The points were fairly uniformly distributed over all of the allowed regions; *no preference was given to the angles of minimum torsional energy*. The rotational angles of the side-chain single bonds were placed at the minima of threefold potentials, but if steric overlaps resulted, rotations out of the minima by $\pm 20°$ were allowed.

Generation of the Symmetrical Gramicidin-S Ring. The ϕ and ψ values for each new backbone[2] were chosen by systematically cycling through the lists of angles selected from the dipeptide maps. The backbone of the first six peptide units was generated in the manner described by Némethy and Scheraga (1965), using a coordinate system based on the first peptide unit (the unit bounded by the α carbons of the prolyl-10 and valyl-1 residues) to record the positions of the atoms. The coordinates of the remaining residues were found by symmetry, in the following manner. The twofold axis of symmetry was taken to be the vector perpendicular both to the vector joining the C^{α} atoms at the beginnings of peptide units one and six, and to the vector joining the C^{α} atoms at the ends of peptide units one and six. The C^{α} atoms at the beginnings of these peptide units belonged to the prolyl residues, and those at the ends to the valyl residues. The axis was positioned so as to bisect the vector joining the C^{α} atoms at the beginnings of these peptide units. An "axis of symmetry coordinate system" was thereby established, in which the z axis was the symmetry axis, the x axis was the line joining the C^{α} atoms at the beginnings of units one and six, and the y axis was chosen to make a right-handed Cartesian coordinate system. The coordinates of all of the atoms of peptide units one through six were transformed into the axis of symmetry coordinate system. The axis of symmetry coordinates of units seven through ten were then found by a symmetry operation (twofold rotation), since (x, y, z) of peptide unit i transforms to $(-x, -y, z)$ in peptide unit $i + 5$. Thus, the atoms within peptide units one and six only were not necessarily symmetrical (the distinction between unit and residue is given elsewhere[1]).

Steric overlap tests were performed among the atoms of the first six peptide units as they were being generated. The atoms of units seven through ten only had to be tested against those of units one through six, because of the symmetry property. Generation was immediately terminated if an overlap was found.

The eight side chains were added to the complete backbone. The first four side chains were generated in the conventional manner, but the remaining four were found from the first four by symmetry. Steric overlap tests were performed between the side chains and the backbone, and among the side chains.

A problem which always arises in generating closed ring polypeptides is how to get the correct orientation and length of the closing bond. The procedure used here was to simply calculate the value of an expression which indicates how well the ring closes, and to reject conformations which have an unsatisfactory value.

[2] The backbone, as defined here, includes the C^{β} atoms and the proline rings, since the positions of these atoms depend solely on the ϕ and ψ values.

The expression used was

$$C = (1/5)\sum_{i=1}^{5}[(x_{i1} + x_{i6})^2 + (y_{i1} + y_{i6})^2 + (z_{i1} - z_{i6})^2]^{1/2}$$

The subscripts 1 and 6 refer to peptide units one and six. The summation is taken over the five atoms of these units which were not forced into a symmetrical relationship by the generation procedure. Axis of symmetry coordinates were used. The resulting closing constant, C, is a measure of the average departure from symmetry of the five atoms, expressed in angstroms. A value of $C = 0.0$ A would mean that the peptide units one and six are perfectly symmetrical, and likewise that the closing orientation and distance is exactly correct. In initial surveys, conformations with $C > 2.0$ A were discarded.

Energy Functions. The conformational energy was calculated by summing over torsional, electrostatic, nonbonded, and hydrogen-bonded contributions. Solvent effects were not included, since the molecule was assumed to be in a crystal. However, the effect of packing the molecules in a crystal lattice has as yet not been considered.

The energy functions and parameters were the same as described in detail by Scott and Scheraga (1966b) except that the parameters for the nonbonded potential functions were those of Gibson and Scheraga (1966). The functions will be briefly described here.

A cosine function with threefold equivalent minima was used to calculate the torsional potential energy. The barrier heights were 0.6, 0.2, and 2.8 kcal/mole for the N–C^α, C^α–C′, and side-chain single bonds, respectively. However, the C^β–C^γ bond in phenylalanine was assigned a barrier height of zero. Variation of the N–C^α and C^α–C′ barrier heights between 0.2 and 0.6 kcal/mole did not significantly affect the results obtained.

The electrostatic energy was calculated by representing the dipole moments of the amide groups by partial charges, and summing over all the Coulomb interactions among these charges. The charge assignments, in terms of the electronic charge, were: O, −0.416; C′, +0.449; N, −0.305; H, +0.272. The dielectric constant was set at 4.0. No charge was placed on the ϵ-NH_2 group of ornithine.

Lennard-Jones 6–12 potentials were used to calculate the nonbonded energy. Contributions from all pairwise interactions of atoms or groups of atoms not rigidly joined to each other were included, with the exception of those atoms involved in a hydrogen bond. The individual atoms of the backbone skeleton were used, but the side chains were divided into spherical CH_2 and NH_2 groups.

The hydrogen-bonding energy was calculated with the equation of Schroeder and Lippincott (1957), as modified by Scott and Scheraga (1966b) to include angular dependence explicitly. The optimum N···O distance was taken to be 2.79 A. This equation consists of the sum of nonbonded attractive and repulsive terms, and a pair of angular-dependent "hydrogen-bonding" terms. It was used to calculate the interaction between NH and CO groups not on the same or adjacent peptide units, for which the H···O distance was less than 5.0 A. It was also used for the interactions of the NH_2 groups of the ornithyl residues with the backbone carbonyl groups.

Results

Two different sets of ϕ and ψ rotational angles were selected from the dipeptide maps of Leach *et al.* (1966a). In the first set, six, ten, ten, six, and two points were chosen from the maps for L-Val, L-Orn, L-Leu, D-Phe, and L-Pro, respectively. In the second set, six, eight, eight, five, and three points were chosen, in the same order. All of the sterically allowed conformations were found, using all combinations of the angles within each set. Backbone conformations (125) resulted from the first set, and 234 from the second set, giving a total of 359 backbones. Side chains could fit on only 282 of these backbones.

Energy calculations were performed for the 282 *backbones* which would sterically accommodate side chains. The distribution of energies for these 282 backbone conformations without side chains is shown in Figure 1. The energy distribution is sharply peaked in the −20 to −30 kcal/mole range, but falls off rapidly below that. Investigation of the ten backbone conformations with energies lower than −35 kcal/mole showed that only three of these were distinctly different acceptable conformations. As shown in the Methods section, peptide units one and six are not necessarily symmetrical. Three of the ten conformations were found to have the plane of peptide unit six rotated by 90–180° away from the position of symmetry with respect to peptide unit one; these conformations were therefore discarded. Of the remaining seven conformations, four differed in apparently insignificant ways from the three distinct acceptable conformations, and thus were not examined further.

The backbone energies and closing constants of the three lowest acceptable conformations were: I, −49.93 kcal/mole, and 0.279 A; II, −38.89 kcal/mole, and 0.507 A; III, −37.41 kcal/mole, and 0.086 A. The backbone rotational angles of these three conformations were then permuted through many combinations of 10° variations in the hope of lowering the energies and decreasing the closing constants. The best variants which resulted had the properties: I, −54.49 kcal/mole, and 0.005 A; II, −38.95 kcal/mole, and 0.049 A; III, no improvement over the original. Of course, it may be that different "best" conformations would have resulted if rotational increments of greater than or less than 10° were used, but it was not feasible to test this possibility with the present computer program.

All sterically allowed side-chain combinations were generated on the best variant of each of the three conformations, and the side-chain contribution to the total energy was calculated for each combination.

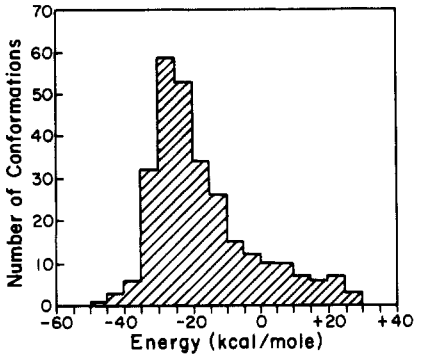

FIGURE 1: Distribution of the energies of the sterically acceptable backbones which will accommodate side chains.

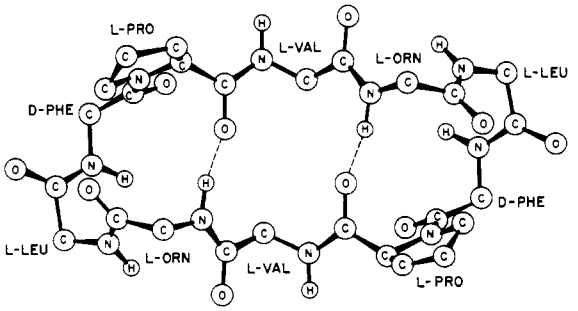

FIGURE 2: View down the axis of symmetry of conformation I, with most side-chain atoms omitted.

Several of the combinations had similar energies. The number of sterically allowed combinations and the minimum side-chain energy value found for each case were: I, 54 combinations, and −27.26 kcal/mole; II, 30 combinations, and −18.51 kcal/mole; III, 24 combinations, and −21.18 kcal/mole. Adding the backbone and side-chain contributions gives the total minimum energy for each conformation: I, −81.75 kcal/mole; II, −57.46 kcal/mole; III, −58.59 kcal/mole.

On the basis of these results, conformation I could reasonably be chosen as having the lowest energy and the best ring closure of the several hundred conformations examined. The assumption was made, of course, that the major differentiating contribution to the total energy would come from the backbone[2] and that the distribution of energies would not be significantly modified if the side chains were included. For this reason the side-chain contributions were determined only for the three backbones of lowest energy.

Properties of Conformation I. The energy of this conformation could not be lowered by the variational procedure used here. It therefore probably is near an energy minimum. This was demonstrated by performing another set of 10° variations on its backbone rotational angles. Out of the entire list of variants which resulted, two had slightly lower energies (<1.0 kcal/mole lower), but larger closing constants, than the starting conformation; all the rest (nearly 300) had higher energies and larger closing constants than the starting conformation.

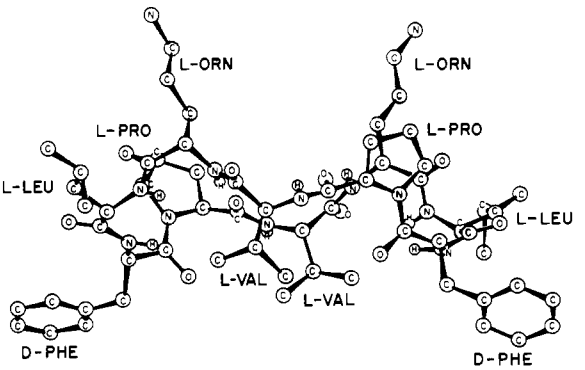

FIGURE 3: View of conformation I from 80° away from the axis of symmetry, with the side chains included. The apparent asymmetry of the molecule in this figure is due to the angle of observation.

Pertinent data are given in Tables I–III; Figures 2 and 3 illustrate the conformation. Two across-the-ring hydrogen bonds can be seen in Figure 2. The N···O distance is 2.84 A in each case. The ornithyl and prolyl residues provide the NH and CO groups, respectively. It is noteworthy that, out of all the 359 backbone conformations generated, only this conformation had intramolecular hydrogen bonds. The ornithine side chains are shown in the extended conformation in Figure 3, for clarity, but a *gauche* conformation gave a slightly lower energy. The conformation of lowest energy is given in Tables I and II. No intramolecular hydrogen bonding involving the ornithine NH_2 groups was found.

TABLE I: Dihedral Angles for Conformation I (in degrees).

Residue	ϕ(N–C$^\alpha$)	ψ(C$^\alpha$–C′)	χ_1	χ_2	χ_3
L-Val	60	310	180[a]		
L-Orn	70	240	300	300	180
L-Leu	230	230	300	300	
D-Phe	320	100	60	90	
L-Pro	120	320			

[a] In the case of the valyl side chain, this is the only allowed conformation.

Figure 4 shows the relationship of the ϕ and ψ values in conformation I to those of known structures. Most of the angles lie in the *larger* of the allowed regions of the dipeptide maps (Leach *et al.*, 1966a), but L-Leu,

TABLE II: Axis of Symmetry Coordinates for the Atoms of Conformation I.[a,b]

Residue	Atom	Coordinates (A)		
		x	y	z
L-Val-1	C^{α}	−1.084	−2.007	0.971
	C'	−0.709	−3.145	0.020
	N	−2.434	−1.524	0.649
	H	−3.146	−2.222	0.729
	O	−1.476	−4.101	−0.166
	H^{α}	−0.429	−1.259	0.868
	C^{β}	−1.056	−2.518	2.413
	C^{γ_1}	0.350	−3.021	2.747
	C^{γ_2}	−1.428	−1.380	3.365
L-Orn-2	C^{α}	1.027	−3.974	−1.502
	C'	2.206	−4.702	−0.853
	N	0.471	−2.998	−0.554
	H	1.067	−2.214	−0.382
	O	3.341	−4.648	−1.351
	H^{α}	0.322	−4.639	−1.748
	C^{β}	1.506	−3.247	−2.760
	C^{γ}	0.398	−2.505	−3.508
	C^{δ}	−0.752	−3.427	−3.916
	N^{ϵ}	−1.817	−2.714	−4.636
L-Leu-3	C^{α}	2.871	−6.130	1.026
	C'	4.099	−5.259	1.302
	N	1.891	−5.363	0.245
	H	0.965	−5.386	0.620
	O	5.243	−5.677	1.069
	H^{α}	2.461	−6.409	1.894
	C^{β}	3.293	−7.371	0.237
	C^{γ}	2.144	−8.331	−0.071
	C^{δ_1}	1.488	−8.724	1.254
	C^{δ_2}	2.615	−9.592	−0.799
D-Phe-4	C^{α}	4.842	−3.074	2.130
	C'	4.441	−1.686	1.727
	N	3.813	−4.068	1.793
	H	2.878	−3.762	1.970
	O	4.023	−0.883	2.617
	H^{α}	5.663	−3.342	1.626
	C^{β}	5.130	−3.105	3.632
	C^{γ}	5.643	−4.447	4.157
	C^{δ_1}	4.748	−5.388	4.636
	C^{δ_2}	7.000	−4.716	4.153
	C^{ϵ_1}	5.237	−6.669	5.137
	C^{ϵ_2}	7.490	−5.997	4.654
	C^{ζ}	6.592	−6.929	5.130
L-Pro-5	C^{α}	4.161	0.000	0.000
	C'	2.684	0.282	0.286
	N	4.536	−1.331	0.438
	O	1.831	−0.610	0.172
	H^{α}	4.724	0.698	0.442
	C^{β}	4.392	−0.018	−1.355
	C^{γ}	5.242	−1.164	−1.772
	C^{δ}	5.004	−2.180	−0.655
L-Val-6	C^{α}	1.087	2.012	0.971
	C'	0.709	3.145	0.020
	N	2.437	1.526	0.650
	H	3.150	2.222	0.732
	O	1.476	4.101	−0.166
	H^{α}	0.429	1.259	0.868
	C^{β}	1.056	2.518	2.413
	C^{γ_1}	−0.350	3.021	2.747
	C^{γ_2}	1.428	1.380	3.365
L-Pro-10	C^{α}	−4.161	0.000	0.000
	C'	−2.684	−0.280	0.287
	N	−4.536	1.331	0.438
	O	−1.833	0.614	0.176
	H^{α}	−4.724	−0.698	0.442
	C^{β}	−4.392	0.018	−1.355
	C^{γ}	−5.242	1.164	−1.772
	C^{δ}	−5.004	2.180	−0.655

[a] H and H^{α} are the hydrogen atoms on the nitrogen and C^{α} atoms, respectively. [b] The molecule was divided into peptide units for the calculations, but the coordinates are reported here in terms of amino acid residues, in keeping with the proposed standard conventions (Edsall *et al.*, 1966). As a result, the atoms of the unsymmetrical peptide units one and six are divided between residues ten and one, five and six. The coordinates of residues seven to nine have been omitted, as they are obtainable from the coordinates of residues two to four by the transformation $(x, y, z)_i \rightarrow (-x, -y, z)_{i+5}$.

which turns the corner in the model, is at the left-handed α-helix position. The point for D-Phe lies in the large allowed region of a dipeptide map for D residues. (Incidentally, substitution of L-Phe for D-Phe in gramicidin-S would result in a major alteration of the structure.)

Discussion

It was shown in the Results section that the use of energy calculations made it possible to select a single optimal conformation for gramicidin-S out of the several hundred sterically allowed conformations. It was also shown that this conformation is probably near an energy minimum. It was not shown, however, whether this is the only energy minimum, and if not, whether there may be other minima lower than the one found. To answer these questions would require an exhaustive search of the potential energy surface, and this would take a prohibitive amount of computer time, by the present methods. Energy minimization techniques are under development in this laboratory; they should greatly increase the efficiency of this search.

No detailed X-ray analysis of gramicidin-S or its derivatives has yet appeared, but the structure described above agrees well with the available preliminary X-ray data on gramicidin-S derivatives (Schmidt *et al.*, 1957;

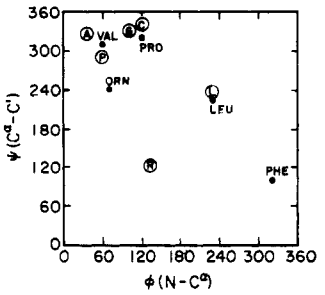

FIGURE 4: Dipeptide map showing the relationship of the dihedral angles in conformation I to the angles in various regular structures (Edsall *et al.*, 1966). All of the residues are in the L configuration except Phe, which is in the D configuration. While Phe falls in a disallowed region for an L configuration, it falls in the allowed region for a D configuration (Leach *et al.*, 1966a). R is the right-handed α helix; L, left-handed α helix; P, parallel chain pleated sheet; A, antiparallel chain pleated sheet; G, polyglycine II; C, collagen.

Hodgkin and Oughton, 1957; M. Harding, personal communication, 1965). These workers state that the atoms tend to be concentrated in layers 4.8 A apart along the *c* axis of the crystal, which is at right angles to the molecular symmetry axis; this figure is to be compared with a spacing of 4.5–5.0 A between the two sides of the ring in the model. According to M. Harding (unpublished data), the molecule tends to be turned down at the ends; this property can be seen in Figure 3. Our model is somewhat similar to the structure based on the β-pleated sheet suggested by Hodgkin and Oughton (1957). Infrared dichroism studies by Abbott and Ambrose (1953) on crystals of gramicidin-S derivatives have indicated that the NH and CO bonds principally lie parallel to the *c* axis, which is the direction of the 4.8-A spacing. It can be seen from Figure 2 that 4 NH and 4 CO bonds lie roughly in this direction in the model. The conclusion from the above is that, although all possible conformations were not surveyed in the calculations, it appears likely that the conformation described may be the same as or similar to the conformation found in crystalline material.

The results obtained in this work are of course valid only if the energy functions and parameters employed give a reasonable approximation to the real case. Studies on known crystal structures are in progress in order to check and perhaps improve these functions. The structure reported here should be regarded as an initial one, obtained by the method of calculation reported here. It will be tested by the energy minimization procedure now under development in our laboratory.

Added in Proof

A minor programming error, which caused the H^{α} atom of L-Pro-10 to be slightly misplaced, has been detected. The coordinates of this H^{α} atom have been corrected in Table II. However, all of the backbone energies reported in the paper should be corrected by the addition of approximately −3.6 kcal/mole; this correction will not change the shape of the distribution curve of Figure 1, or the choice of the best conformation, or any of the conclusions of this paper.

TABLE III: Contributions to the Energy of Conformation I.

Contribution	Energy (kcal/mole)
Backbone energy[a]	
Torsional	+3.50
Electrostatic	−6.28
Nonbonded	−38.88[b]
Hydrogen bonded[c]	
Nonbonded part	−6.72
Angular-dependent bonded part	−6.11
Total backbone energy	−54.49
Side-chain energy[d]	
Torsional	0.00
Nonbonded	−27.26[e]
Total side-chain energy	−27.26
Total backbone + total side-chain energy	−81.75

[a] "Backbone energy" includes all interactions among the atoms of the backbone, as defined in footnote 2. [b] Of this, −3.10 kcal arises from the nonbonded interactions between the nonhydrogen-bonded NH and CO groups for which the Lippincott–Schroeder equation was used. [c] This arises from equal contributions from the two hydrogen bonds. [d] "Side-chain energy" includes all interactions between the side chains and the backbone, and among the side chains. [e] As in footnote *b*, −1.13 kcal arise from the ϵ-NH_2 groups of ornithine.

Acknowledgments

The authors wish to thank Mrs. Judy Liebman for her excellent programming assistance.[3]

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[3] A listing of the computer program used in this work has been deposited with the American Documentation Institute, Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington, D. C. 20540, from which it may be obtained by ordering Document 9061, and remitting $3.00 for microfilms or $8.75 for photoprints. Make checks payable to Chief, Photoduplication Service, Library of Congress.

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Optical Rotatory Dispersion Studies of Yeast Alanine and Tyrosine Transfer Ribonucleic Acids. Evidence for Intramolecular Hydrogen Bonding and Discussion of Conformational Aspects*

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ABSTRACT: Optical rotatory dispersion measurements were made on solutions of yeast alanine and tyrosine transfer ribonucleic acids (t-RNA's) at neutral pH, in the presence and absence of Mg^{2+} ion, and over the temperature range 5–90°. The data were interpreted in terms of neighbor–neighbor base stacking and intramolecular hydrogen bonding. Comparison of t-RNA data with those for the poly-(G-C) and poly-(A-U) double helices shows that alanine and tyrosine t-RNA's have a significant amount of double-stranded structure (primarily G-C hydrogen bonding) at low temperatures. Upon heating, the hydrogen bonds and most of the stacking interactions are disrupted. The addition of Mg^{2+} ion stabilizes the low-temperature form, presumably by binding along the phosphate backbone, thus reducing electrostatic repulsions in the t-RNA molecules, so that the transition temperature is increased. Calculations of ORD curves are performed, assuming several conformations (*i.e.*, single-strand with no hydrogen bonding, a hydrogen-bonded conformation, a hypothetically hydrolyzed sample, etc.) including ones previously proposed by R. W. Holley and J. T. Madison for alanine and tyrosine t-RNA, respectively. These calculated curves are compared to experimental data; they agree quite well with the observed curves.

The use of optical rotatory dispersion as an experimental tool for the investigation of the conformation of polyribonucleotides has been well demonstrated (Fasman *et al.*, 1964; Holcomb and Tinoco, 1965; Lamborg *et al.*, 1965; Brahms *et al.*, 1966; Poland *et al.*, 1966; Vournakis *et al.*, 1966). It has been shown that neighbor–neighbor base stacking (without hydrogen bonding) exists in single-strand oligomers of adenylic acid, and that the state of stacking of a given base pair is essentially independent of the state of stacking of the rest of the chain (Brahms *et al.*, 1966; Poland *et al.*, 1966). An ORD study of the 16 dinucleoside phosphates corresponding to the 16 possible pairings of A, U, C, and G[1] with one another

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[1] Abbreviations used: G, guanosine; C, cytidine; U, uridine; A, adenosine.