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Computer Simulation of the Conformational Properties of Oligopeptides. Comparison of Theoretical Methods and Analysis of Experimental Results

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Abstract: A theoretical analysis of the conformational and statistical thermodynamic properties of series of oligopeptides containing alanine, methionine, valine, and glycine was carried out. A novel feature of these calculations is the determination of the relative vibrational *free energy* of different minimum-energy conformations. It was shown that in many of the peptides considered the entropic contribution to the relative stabilities of different conformations is comparable to the energy difference (~4–5 kcal/hexapeptide). In one case, Met_3-Gly-Met_2, the entropic contribution dominated, causing a reversal in stability of the energetically favored α-helical conformation to a more extended form. Monte Carlo simulations of several hexapeptides were carried out in order to better simulate the ensemble of conformations present in solution. Average energies, end-to-end distances, and probability maps of the conformational states of a given residue as a function of its position in the chain were generated. The effect of the insertions of a glycine in alanine host peptides was studied by this technique. It was found that, when glycine is present in the middle of a hexamer of alanine (Ac-Ala_3-Gly-Ala_2-NMe), a class of folded structures characterized by low entropy, low energy, and a small end-to-end distance dominates the conformations generated. This result permits an alternative interpretation of the CD spectra for Boc-Met_3-Gly-Met_2-OMe.

I. Introduction

A great deal of effort has been expended in recent years on the determination of the solution conformation of oligopeptides.[2,3] The impetus for these studies arises from the intrinsic interest in understanding the factors which influence molecular conformation in solution, from the fact that these compounds are composed of the same structural units as proteins and peptides, and with the firm conviction that biological activity is intimately related to the accessible, low-energy conformations of these molecules.[2a,4,5] The continuing discovery of short, biologically active peptides, perhaps the most well-known recent examples being the pentapeptide enkephalins,[6] serves to maintain interest and active research in this area.

Various techniques have been applied to the study of the conformation of peptides, including spectroscopic methods (IR, NMR, CD), X-ray crystallography, and theoretical conformational analysis.[2,3] Each of these methods has its limitations; spectroscopy, the indirect nature of the results from which only aspects of the structure can be deduced, and those only by implication; X-ray crystallography, the need for a crystal and the effect of crystal forces on the molecular conformation;[7] and finally theoretical analysis, where solvent effects, local minima, and the adequacy of the potential functions are the outstanding problems. It would seem clear that a combination of the three techniques, each contributing and providing feedback for the other, would be the desired approach to understanding conformational behavior at the molecular level.[8]

In this paper we treat a series of alanine and methionine oligopeptides as well as several host-guest peptides, all of which have been well characterized by experimental spectroscopic techniques, by a variety of theoretical methods. We have a twofold objective. The first is to investigate the effect of several common approximations and assumptions, such as rigid geometry and neglect of vibrational free energy, on the conclusions drawn from such calculations. The second objective is to determine to what extent the combination of various theoretical techniques such as energy minimization, Monte Carlo chain simulation, and the inclusion of the vibrational effects can help to extend the understanding of the conformational behavior of these molecules, as deduced from experiment, to the energetic and molecular level.

Secondary Structure. The study of the critical chain length for the onset of secondary structure in oligopeptides has been conducted using a number of physicochemical techniques including polarimetry, ultraviolet spectroscopy, circular dichroism (CD), and nuclear magnetic resonance spectroscopy.[2b,9-15] Peptides composed of amino acids such as γ-ethyl-L-glutamic acid and L-alanine were observed to begin forming helices in organic solvents at a chain length of six or seven residues. In additional studies, oligomers composed of hydrophobic amino acids were found to form β structures in pentamers and hexamers.[12-15] Although much information has been gained from these studies, there still remain many unanswered questions concerning the exact structure of these oligopeptides in solution. This is especially true in the case of peptides undergoing transition as they are undoubtedly in dynamic equilibrium between a number of energetically preferred conformations. Thus, for example, the CD patterns of hexamers, heptamers, and octamers of L-alanine or γ-ethyl-L-glutamic acid in organic solvents do not conform to

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those found for α-helical polypeptides. Rather, they are intermediate between those observed for polypeptides in the "random coil" and helical state.

Host–Guest Peptides. In an attempt to gain further insights into factors affecting oligopeptide stereochemistry, studies have been carried out on peptides in which one amino acid (host residue) has been replaced by a second amino acid (guest residue). Thus, for example, peptides composed primarily of methionine were modified by the substitution of either one glycyl or one valyl residue.[16,17] Using CD, it was found that methionine oligopeptides (Boc-Met_n-OMe) form helices when $n = 7$ in trifluoroethanol,[18] while valine oligopeptides assume β structures under the same conditions,[13] and glycine is known to strongly destabilize helical conformations.[19,20] Insertion of one valyl residue at the amino terminus or in the center of a heptamer of methionine did not prevent the formation of a partially helical structure as judged using CD.[21] Furthermore, although it is difficult to definitely assign the conformation, in a solution of Boc-Met_6-OMe in trifluoroethanol, substitution of one valyl residue in this hexamer does not cause significant changes in the CD patterns and presumably does not result in a marked change in the ϕ, ψ angles of the oligopeptide. In contrast, insertion of one glycyl residue in the center of hexa- or heptamethionine results in a compound which exhibits little, if any, ordered secondary structure in solution.[21] Glycine at the amino or carboxyl terminus of a hexamer, however, causes only small changes as shown by comparison of its CD spectrum with that of Boc-Met_6-OMe.

Theoretical Treatments. Conformational energy calculations on oligopeptides are a complementary tool to spectroscopic investigations and provide additional information about the energetics of interactions which determine the observed conformation. Many calculations have been carried out to determine the minimum-energy conformation of oligopeptides and these are referred to in the recent reviews by Ingwall and Goodman[2b] and Nemethy and Scheraga.[3] In particular, studies of different members of homologous series of oligopeptides have been carried out by Lewis et al.[22] and Ralston and De Coen.[23] In both studies it was found that the preferred conformations of short peptides were repeating seven-membered hydrogen-bonded rings (each residue in the C_7 conformation). Ralston and De Coen also studied the effect of side chains on the adoption of folded conformations and on the stability of the α helix. They predicted a transition to α-helical conformation at around 12 residues for the oligoalanines.[23]

In the present study, we attempt to apply conformational energy calculations to the problem of the structure of oligomers and cooligomers containing methionine, alanine, valine, and glycine. In particular, we have applied three approaches to the theoretical prediction of oligopeptide conformation. The first of these was minimization of the molecular energy of the chain by variation of the ϕ, ψ, and χ angles, assuming a rigid standard geometry[24] for the remainder of the oligopeptide. Using this method, we also studied the effects of chain length, residue substitution, blocking groups, assumptions concerning 1–4 (vicinal) nonbonded interactions, and dielectric constant on the conformation of oligopeptides of alanine and methionine. As a check on this classical "rigid geometry" procedure, we have also minimized the energy with respect to an unconstrained molecular geometry.[25] This is the first time to our knowledge that peptide conformation has been extensively studied using a valence force field "flexible geometry" method which also includes a calculation of the *vibrational free energy (and the entropy)*. This method enables one to assess the importance of vibrational entropic effects which have been neglected heretofore. Finally, since the oligopeptide in solution represents an ensemble of systems, the third approach we apply is the Monte Carlo method to calculate statistical thermodynamic averages of the oligopeptide chains. Previous applications utilizing this technique for calculation of the end-to-end distance and energy distributions for short polypeptide chains have been carried out by Premilat and Hermans[26] and Hesselink.[27] Premilat and Maigret[28] considered the effect of long-range interactions on the conformational statistics of oligoglycine and oligo-L-alanine chains (<20 peptide units) generated by the Monte Carlo method by including interactions between all atoms in the chain. They also studied the effect of using different potential functions on the mean square end-to-end distance of these chains. Warvari, Knaell, and Scott[29] used the Monte Carlo method to study models of polyglycine, poly-L-alanine, and copolymers of the two peptide units. They permitted only a limited number of allowed residue conformations and used a hard sphere model potential.

Table I. Geometry of Peptide Residues[a]

bond	Å	angle	deg
C′-N	1.32	C′NH	123
N-H	1.00	C′NC	121 (123)[b]
N-C	1.47	NCH	110
C-H	1.08	NCC	110
C-C	1.53	NCC′	110 (111)[c]
C-C′	1.53	CC′O′	121
C′=O′	1.24	CC′N	114 (115)[b]
CH_2-S	1.83	CCC	110
S-CH_3	1.785	CCS	110
C-O	1.45	CSC	100
O-C′	1.36	CCO	110 (111)[b]
C^{Ph}-C^{Ph}	1.40	COC′	112
C^{Ph}-C	1.53	OC′O′	123
		$C^{Ph}C^{Ph}C^{Ph}$	120
		$C^{Ph}CO$	111
		CC′O	114

[a] C′ and O′ are carbonyl carbon and oxygen, respectively; C^{Ph} is an aromatic carbon. [b] The number in parentheses refers to the case when C is CH_3 or C^{Ph}. [c] The number in parentheses is used in the case of Met and Val.

II. Methods

A. Rigid Geometry Minimization. The oligopeptides were constructed by linking together blocking groups and transplanar peptide units with standard bond length and bond angles as given in Table I.[30,31] Methyl and methylene groups were considered as united atoms,[32] except for the case of the C_α atom in glycine. The energy of the molecule is taken as the pairwise summation over all atom–atom van der Waals and Coulomb interactions for atoms separated by three or more bonds. (The distances between atoms separated by fewer bonds remain constant.)

$$E = \sum_{i=1}^{N-1} \sum_{j>i}^{N} (A_{ij}/r_{ij}^{9} - B_{ij}/r_{ij}^{6} + q_i q_j/\epsilon r_{ij}) \qquad (1)$$

where A_{ij} and B_{ij} are the repulsive and attractive terms for a particular atom pair i and j, q_i and q_j are the partial charges of atoms i and j, r_{ij} is their interatomic distance, ϵ is the dielectric constant, and N is the number of atoms in the molecule. The values of A, B, and q were taken from Hagler, Huler, and Lifson[33] and ϵ was taken as equal to one. The energy for a particular oligopeptide was calculated by initially setting all side-chain torsion angles χ equal to 180° and setting all ϕ, ψ pairs initially equal, their values being one of the five points in the Ramachandran map given in Table II.

The energy of the molecule was minimized with respect to the ϕ, ψ, and χ angles using a quasi-Newton minimization technique.[34] This results in a minimum-energy conformation for each of the five starting pairs of ϕ, ψ angles, each of which corresponds to a local minimum. In practice, C_7 and β starting conformations often lead to identical minimum-energy conformations.

Table II. Initial Values of ϕ and ψ for Rigid Geometry Minimization

conformation	ϕ	ψ
α helix	−60	−60
β (extended)	−160	160
α' helix	−160	−60
C_7^{ax} (axial)	80	−80
C_7 (equatorial)	−80	80

B. Flexible Geometry Minimization. The energy of an oligopeptide was calculated using a preliminary amide valence force field. The general expression for this potential is

$$E = \sum\{D_b[1 - e^{-\alpha(b-b_0)}]^2 - D_b\} + \frac{1}{2}\sum H_\theta(\theta - \theta_0)^2 + \frac{1}{2}\sum H_\chi\chi^2 + \frac{1}{2}\sum H_\phi(1 + s\cos n\phi) + \sum\sum F_{bb'}(b - b_0)(b' - b_0') + \sum\sum F_{\theta\theta'}(\theta - \theta_0)(\theta' - \theta_0') + \sum\sum F_{b\theta}(b - b_0)(\theta - \theta_0) + \sum\sum F_{\phi\theta\theta'}\cos\phi(\theta - \theta_0)(\theta' - \theta_0') + \sum\sum F_{\chi\chi'}\chi\chi' + \sum\epsilon[2(r^*/r)^9 - 3(r^*/r)^6] + \sum q^2/r \quad (2)$$

where b, θ, ϕ, and χ are the bond angles, torsion angles, and out-of-plane bending angles, respectively; b_0 and θ_0 are parameters representing the corresponding reference values. Further explanations of the analytical form are given in ref 35. The potential parameters used in the force field were those obtained by optimization of the force field to give the best agreement with the experimental structure and the vibrational frequencies of *N*-methylacetamide.[36] The energy of a particular oligopeptide was calculated by minimizing this potential with respect to the Cartesian coordinates of all atoms in the molecule, thereby achieving the conformation corresponding to the minimum energy. In order to compare the flexible geometry calculations with the rigid geometry calculations, the flexible geometry calculations were applied to the α and C_7 conformation obtained by using the rigid geometry method. (These were, in general, the two conformations of lowest energy). This yields α and C_7 conformations with ϕ, ψ, and χ angles similar to those obtained by the rigid geometry method, but with bond lengths and bond angles relaxed to reduce unfavorable interatomic interactions.

Since using this method allows one to readily calculate the vibrational frequencies of the molecule, the energy and free energy of vibration were also calculated using the Einstein equations:[37]

$$E_{vib} = \sum_i [h\nu_i/2 + h\nu_i/(e^{h\nu_i/kT} - 1)] \quad (3)$$

$$A_{vib} = \sum_i [h\nu_i/2 + kT\ln(1 - e^{-h\nu_i/kT})] \quad (4)$$

where E_{vib} is the vibrational energy, A_{vib} is the vibrational free energy, and the sum is over all vibrational frequencies ν_i of the molecule, T is the temperature, and k and h are the Boltzmann and Planck constants, respectively. The vibrational entropy is given by

$$S_{vib} = (E_{vib} - A_{vib})/T \quad (5)$$

and the total free energy of the molecule by

$$A = E_{conf} + A_{vib} \quad (6)$$

Thus, the differences in free energy, energy, and entropy of the α and C_7 conformations are readily calculated.

C. Monte Carlo Method. The Monte Carlo method is a stochastic method in which enough configurational states of the system are generated at random to allow calculation of the desired statistical thermodynamic averages[38]

$$\langle\chi\rangle = \sum\chi_i\exp(-E_i/RT)/Z \quad (7a)$$

$$Z = \sum\exp(-E_i/RT) \quad (7b)$$

where $\langle\chi\rangle$ is the desired thermodynamic average of property χ, χ_i is the value of this property in configuration i which has energy E_i, Z is the partition function, the temperature is denoted by T, while R is the Boltzmann constant. In the particular case of a polypeptide chain the configurational variables are ϕ and ψ and in the classical limit the partition function becomes[39a]

$$Z = \int\exp(-E\{\phi,\psi\}/RT)\,d\{\phi,\psi\} \quad (8)$$

where the integral extends over all the torsional degrees of freedom, $\{\phi,\psi\}$, in the molecule, and it is this integral which is approximated by the sum over N random chains:[26]

$$Z \simeq [(2\pi)^{2n}/N]\sum_{i=1}^{N}\exp(-E_i\{\phi,\psi\}/RT) \quad (9)$$

If the chain configuration were generated by a truly random procedure, most would be sterically forbidden and thus would not contribute significantly to the average properties. (The problem of convergence will be discussed further in the Discussion section.) Instead, the individual residue conformations in the chain are generated with a probability proportional to the statistical weight of an isolated residue, $\exp[-\epsilon_j(\phi,\psi)/RT]$ (where ϵ_j is the energy of residue j), so that the probability P_i of generating the ith configuration is given by

$$P_i = \prod_j\exp[-\epsilon_j(\phi_j,\psi_j)/RT] \quad (10)$$

the product of the individual a priori probabilities by which the isolated residues are chosen. This method has been described elsewhere.[26,38] The partition function for the chain then becomes in this approximation

$$Z \simeq [(2\pi)^{2n}/N]\sum_{i=1}^{N} W_i\{\phi,\psi\} \quad (11)$$

where W_i is the statistical weight for the ith configuration with the bias removed by dividing the Boltzmann factor by P_i:

$$W_i \equiv \exp(-E_i\{\phi,\psi\}/RT)/P_i \quad (12)$$

The average conformational energy is given by

$$\langle E\rangle \simeq [(2\pi)^{2n}/NZ]\sum_{i=1}^{N} E_i\{\phi,\psi\}W_i \quad (13)$$

and the entropy may be obtained from the free energy A

$$A = \langle E\rangle - TS \quad (14)$$

by substituting $-RT\ln Z = A$:

$$S = R\ln Z + \langle E\rangle/T \quad (15)$$

Other configurational averages such as the end-to-end distance may be obtained in a similar way to the average energy as illustrated in eq 7 and 13.

III. Results

The results of the rigid geometry minimization for several pairs of oligopeptides of chain length three and seven are summarized in Table III.

Blocking Group. The results in Table IIIA indicate that the end group can significantly affect the relative stability of different conformations, especially for shorter peptides. Replacement of the *N*-acetyl group by the benzyloxycarbonyl group results in a stabilization of 2.7 kcal/residue for the α-helical conformation of the trimer relative to the distorted

Table III. Energy Difference[a] per Residue as a Function of Chain Length

	A. Effect of Blocking Group, oligopeptide							
	Ac-Ala$_n$-NMe		Z-Ala$_n$-OEt		Ac-Met$_n$-NMe		Boc-Met$_n$-OMe	
no. of residues, n	3	7	3	7	3	7	3	7
E_α[b]	14.21	21.31	−49.18	−36.54	−0.43	−24.07	−30.97	−53.02
E_{C_7}[b]	1.17	15.89	−54.18	−37.51	−15.36	−27.23	−42.69	−54.99
$(E_\alpha - E_{C_7})/n$	4.35	0.77	1.67	0.14	4.98	0.45	3.91	0.28

	B. Effect of Neglecting 1-4 Interactions, oligopeptide			
	Ac-Ala$_n$-NMe		Ac-Met$_n$-NMe	
no. of residues, n	3	7	3	7
E_α	−15.75	−31.97	−34.37	−82.70
E_{C_7}	−16.36	−23.26	−33.78	−69.43
$(E_\alpha - E_{C_7})/n$	0.22	−1.24	−0.20	−1.90

[a] Energy in kcal/mol. [b] These correspond to the minimum-energy conformations which are distorted α and C_7 helices, respectively.

Table IV. Energy (kcal/mol) of Alanine and Methionine Oligomers as a Function of the Number of Residues

	A. Comparison of Rigid vs. Flexible Geometry Calculations					
	rigid geometry			flexible geometry		
oligomer	E_α	E_{C_7}	$E_\alpha - E_{C_7}$	E_α	E_{C_7}	$E_\alpha - E_{C_7}$
Ac-Ala$_3$-NMe	14.21	1.17	13.04	3.83	0.27	3.55
Ac-Ala$_6$-NMe	20.17	12.22	7.95	2.22	8.90	−6.68
Ac-Met$_3$-NMe	−0.43	−15.36	14.93	−1.84	−9.85	8.01
Ac-Met$_7$-NMe	−24.07	−27.23	3.16	−18.08	−13.84	−4.23
Boc-Met$_3$-OMe	−30.97	−42.69	11.72	−36.97	−40.84	3.87
Boc-Met$_4$-OMe	−36.75	−44.15	7.40	−41.63	−41.65	0.02
Boc-Met$_5$-OMe	−42.10	−49.12	7.02	−45.95	−42.56	−3.38
Boc-Met$_6$-OMe	−49.39	−52.14	2.75	−48.79	−43.70	−5.09

	B. Comparison of Energy, Entropy, and Free Energy							
oligomer	$E_\alpha - E_{C_7}$	$E_\alpha{}^v$	$E_{C_7}{}^v$	$E_\alpha{}^v - E_{C_7}{}^v$	TS_α	TS_{C_7}	$T(S_\alpha - S_{C_7})$	$A_\alpha - A_{C_7}$
Ac-Ala$_3$-NMe	3.55	123.71	124.05	−0.34	18.77	20.22	−1.45	4.67
Ac-Ala$_6$-NMe	−6.68	224.82	225.19	−0.37	36.08	41.68	−5.60	−1.45
Ac-Met$_3$-NMe	8.01	137.71	137.90	−0.19	43.71	44.32	−0.61	8.42
Ac-Met$_7$-NMe	−4.23	297.01	297.01	0.00	100.81	104.52	−3.71	−0.52
Boc-Met$_3$-OMe	3.87	142.44	142.50	−0.05	48.06	50.31	−2.24	6.06
Boc-Met$_4$-OMe	0.02	182.42	182.49	−0.07	63.00	65.10	−2.10	2.05
Boc-Met$_5$-OMe	−3.38	222.48	222.48	0.00	77.37	80.36	−2.98	−0.40
Boc-Met$_6$-OMe	−5.09	262.52	262.47	0.05	91.10	95.09	−3.99	−1.05

C_7 helix. On the other hand, by the time we get to the heptamer the effects are smaller, although still not insignificant. In no case is there a change in the predicted most stable conformation for the peptides considered here, although the results indicate that the α helix will become more stable at shorter chain lengths with an N-terminal benzyloxycarbonyl blocking group than with an acetyl group. The results also suggest that the relative stabilities of the distorted C_7 helix and the α helix are quite similar for alanine and methionine oligopeptides. In agreement with previous studies[22,23] our results indicate that a distorted repeating C_7 structure is most stable for short oligopeptides.[39b]

Vicinal Interactions. There has been some question as to whether the usual nonbonded interatomic functions are valid at the short distances characteristic of vicinal (1···4) interactions.[24,40] In order to see how deficiencies in the repulsive potential at short distances would be reflected in the relative conformational energies, calculations were carried out neglecting these interactions altogether. This yields a lower bound while the previous calculation should provide an upper bound since the usual interatomic interactions are too steeply repulsive for these short distances.[40] The calculations indicate that softening the 1-4 repulsion reduces the calculated stability of the "C_7" structure relative to the α helix. As shown elsewhere,[41] and seen in part below, the *apparent* inapplicability of the nonbonded parameters to these vicinal interactions appears to arise from the constraint of rigid geometry rather than any inherent defect in the functions themselves.

Valence Force Field Calculations. The results of relaxing all degrees of freedom of the molecule (flexible geometry) are presented in Table IVA, where they are compared with those obtained with rigid peptide units. This approach permits calculation of the free energy A, the vibrational energy E, and the vibrational entropy S, quantities derived from the calculated vibrational frequencies.[35,42] For all the homogeneous oligopeptides studied, $E_\alpha - E_{C_7}$ goes from positive to negative as n increases, analogous to the behavior observed upon omitting 1-4 interactions. As shown in Table IVB, $A_\alpha - A_{C_7}$ also becomes negative at about $n > 5$ (indicating that α-helix formation is favored), and $T(S_\alpha - S_{C_7})$ per residue decreases as n increases because the low-frequency vibrational modes for the longer oligopeptides in the more extended C_7 conformation have lower frequencies than in the α helix.

Host–Guest Peptides. Effect of Introducing a Glycine. The results of substituting a glycyl residue for alanine on the relative stabilities of C_7 and helical oligoalanines are given in Table V, while those of substituting a glycyl or a valyl residue in methionyl oligopeptides are given in Table VIA.

The results from the rigid geometry calculation are not in agreement with experimental observations. Specifically, CD

Table V. Energy of Cooligopeptides of Alanine and Glycine Calculated Using Rigid Geometry

oligopeptide	E_α	E_{C_7}	$E_\alpha - E_{C_7}$
Ac-Ala_6-NMe	20.17	12.22	7.95
Ac-Gly-Ala_5-NMe	20.89	11.65	9.24
Ac-Ala_5-Gly-NMe	22.13	11.38	10.75
Ac-Ala_3-Gly-Ala_2-NMe	22.25	11.72	10.53

studies in trifluoroethanol indicate that the α-helical content of hexamers and heptamers of methionine and one glycyl residue depends on the position of the glycine in the chain.[21] In contrast, the calculations for hexamers of alanine or methionine containing one glycyl residue give essentially the same relative stabilities, independent of the position of the glycine (although it might be argued that a glycyl residue at the beginning of the hexamer disrupts the α helix less than in the middle or at the end).

The results of replacing a methionyl residue with valine or glycine, as calculated in the flexible geometry approach, are compared with the results of the rigid geometry approach in Table VIA. The calculated differences between valine and glycine as guest residues in a methionine host hexamer are consistent with the CD results for solutions of these peptides in trifluoroethanol.[21] In all these hexamers, $E_\alpha - E_{C_7}$ is negative, indicating that the α helix is favored over the C_7 conformation. Putting glycine in the center of the hexamer significantly increases the energy of the α helix relative to the C_7 conformation whereas valine in the middle has only a small effect on E_α. In fact, as shown in Table VIB, the difference in free energy between α helix and C_7 conformations is *positive* for Boc-Met_3-Gly-Met_2-OMe, indicating that the C_7 is more stable, whereas, for all the other heterogeneous hexamers, $A_\alpha - A_{C_7}$ remains negative. Inspection of Table VIB reveals that this result stems from the fact that, in the case of Boc-Met_3-Gly-Met_2-OMe, the smaller negative value of $E_\alpha - E_{C_7}$ is overwhelmed by the larger entropic contribution. In most cases the entropic contribution is within 1–2 kcal of the energetic contribution. Thus it would seem that the usual procedure of relying upon the relative potential energies of two molecular conformations to determine which one is favored is not universally valid, and that indeed a correct evaluation of the relative stabilities of two conformations may require calculation of the free energy.

Monte Carlo Simulation. The results of the Monte Carlo chain simulation of alanine oligopeptides and Ala peptides with glycine as a "guest" are given in Table VII. Comparison with the energy of the minimized hexamer conformations of alanine (cf. Table V) show an average energy for Ac-Ala_6-NMe, Ac-Gly-Ala_5-NMe, and Ac-Ala_5-Gly-NMe somewhere intermediate between the E_α and E_{C_7} minimum energies. The unsubstituted alanine hexamer lies somewhat closer to the energy of the α helix while the hexamers with one glycine at either the amino or carboxyl terminus have average energies closer to the energy of the C_7 conformation.

The behavior of Ac-Ala_3-Gly-Ala_2-NMe is markedly different than that of the other hexamers. The average energy is 0.2 kcal/mol *lower* than that of the C_7 conformation and *TS* is about 1.8 kcal/mol lower than that of the other hexamers. Also, the average end-to-end distance of 7.2 Å is much less than the 20.8 Å of the homogeneous alanine hexamers, or the 19.1 Å of the other two hexamers containing one glycine. Analysis of the results for this hexamer with a glycyl residue in the center shows that a relatively small number of conformations account for most of the energy contribution since they have high statistical weights W_i (the Boltzmann factor of a given chain divided by the a priori probability of generating that conformation). Table VIII shows the breakdown of the distribution of statistical weights for the 588 167 conformations generated for this hexamer. In contrast, all the other hexamers have a more evenly distributed set of conformations none of which has a statistical weight exceeding ~500, a fact reflected in the higher entropy of these hexamers.

In an attempt to better understand the reason underlying the behavior in the hexamer with a glycyl residue in the fourth position, we examined a stereographic view of the conformation corresponding to the highest statistical weight (Figure 1). For comparison, stereographic views are also given for a "typical" conformation of Ac-Gly-Ala_5-NMe (Figure 2) and for the rigid geometry minimized C_7 conformation of Ac-Ala_6-NMe (Figure 3). In addition, the ϕ, ψ angles of the three conformations with the highest statistical weights are given in Table IX. In all three conformations, the glycyl residue has ϕ, ψ angles of 90, $-80 \pm 10°$ which leads to a reversal in the chain direction of the hexamer, allowing for attractive electrostatic interactions between residues. In particular, two of the conformations allow formation of a hydrogen bond between the carboxylic oxygen of the acetyl group and the amino hydrogen of the *N*-methyl group.

Statistical Distribution of Residue Conformation. In order to better represent the distribution of ϕ, ψ angles in the four hexamers studied, contour maps of the probability of occurrence of a pair of ϕ, ψ angles, $p(\phi,\psi)$, were plotted for each residue in a hexamer, as well as for the probability of a pair of

Table VI. Energy[a] of Cooligopeptides of Methionine with Valine or Glycine as a Function of Position of Guest Residue

A. Comparison of Rigid vs. Flexible Geometry Calculations

oligopeptide[b]	rigid geometry			flexible geometry		
	E_α	E_{C_7}	$E_\alpha - E_{C_7}$	E_α	E_{C_7}	$E_\alpha - E_{C_7}$
-Met_6-	−49.39	−52.14	2.75	−48.79	−43.70	−5.09
-Val-Met_5-	−39.68	−44.27	4.59	−46.03	−37.24	−8.79
-Met_3-Val-Met_2-	−41.48	−44.21	2.73	−46.23	−39.64	−6.59
-Gly-Met_5-	−39.65	−45.83	6.18	−48.21	−40.65	−7.56
-Met_3-Gly-Met_2-	−37.68	−44.21	6.53	−43.57	−41.22	−2.35
-Met_5-Gly-	−41.35	−47.65	6.30	−49.01	−42.79	−6.22

B. Comparison of Energy, Entropy, and Free Energy

oligopeptide[b]	$E_\alpha - E_{C_7}$	$E_\alpha{}^\nu$	$E_{C_7}{}^\nu$	$E_\alpha{}^\nu - E_{C_7}{}^\nu$	TS_α	TS_{C_7}	$T(S_\alpha - S_{C_7})$	$A_\alpha - A_{C_7}$
-Met_6-	−5.09	262.52	262.47	0.05	91.10	95.09	−3.99	−1.05
-Val-Met_5-	−8.79	260.14	260.19	−0.05	87.68	92.21	−4.53	−4.30
-Met_3-Val-Met_2-	−6.59	260.09	260.02	0.07	86.76	91.83	−5.08	−1.44
-Gly-Met_5-	−7.56	258.73	258.77	−0.04	82.47	87.63	−5.16	−2.45
-Met_3-Gly-Met_2-	−2.35	258.77	258.72	0.05	83.12	87.25	−4.13	1.83
-Met_5-Gly-	−6.22	258.65	258.75	−0.09	83.67	87.46	−3.79	−2.53

[a] Units: kcal/mol. [b] N-Terminal blocking group, Boc; C-terminal blocking group, OMe.

Table VII. Results[a] of Monte Carlo Calculations on Hexamers of Alanine

oligopeptide[b]	no. of chains	$\langle E \rangle$	$\langle r \rangle$	Z	$\langle A \rangle$	$\langle TS \rangle$
-Ala_6-	513 228	16.68	20.84	2.62×10^7	−10.12	26.79
-Gly-Ala_5-	503 540	15.44	19.15	3.31×10^8	−11.62	27.06
-Ala_5-Gly-	501 267	15.31	19.12	3.77×10^8	−11.69	27.00
-Ala_3-Gly-Ala_2-	588 167	11.50	7.20	10.48×10^9	−13.66	25.17

[a] Units: E, A, and TS in kcal/mol; r in Å. T = 298 K. [b] N-Terminal blocking group, Ac; C-terminal blocking group, NMe.

Table VIII. Distribution of Statistical Weights for Ac-Ala_3-Gly-Ala_2-NMe

stat wt (W_i)	% $\Sigma_i W_i$	no. of chains	% of total
$W_i \geq 10^5$	13.1	1	$<10^{-3}$
$10^5 > W_i \geq 10^4$	40.8	26	$<10^{-2}$
$10^4 > W_i \geq 10^3$	23.7	149	0.03
$10^3 > W_i \geq 10^2$	14.0	768	0.13
$W_i \geq 10^2$	91.6	944	0.16

ϕ, ψ angles occurring anywhere in the molecule. These four sets of seven contour maps per hexamer are presented in Figures 4–7. The probability density of the contours is indicated on representative maps.

Figure 4 shows that all the residues in Ac-Ala_6-NMe have very similar distributions of ϕ, ψ angles, independent of their position in the hexapeptide. Figures 5–7 show the difference in distribution of ϕ, ψ angles upon substituting a glycyl residue at the beginning, end, or middle of the alanine hexapeptide, as well as the slight change in distribution of ϕ, ψ angles in the alanines neighboring the glycine. Figure 7 shows that placing a glycine in the middle of the chain seriously distorts all the residues from the usual probability density of an alanyl residue; however, the jagged contours which result indicate that this conclusion should be taken with some caution as they arise from the fact that very few of the conformations sampled for this molecule account for so large a percentage of the total statistical weight (cf. Tables VIII and IX). Thus, it should be noted that, although the average energies and end-to-end distances for the hexapeptides studied appear to converge, the irregularity of the contours for all hexapeptides indicates some lack of convergence in the distribution of ϕ, ψ angles.

IV. Discussion

Conformational energy calculations have been extensively applied to the prediction of the secondary and tertiary structure of polypeptides and proteins.[2b,3,23] Previous attempts have also been made to utilize theoretical calculations to examine the onset of ordered secondary structures in homologous series of oligopeptides.[2b,23] In agreement with the results of many of these studies, we have shown that the most stable conformation of homooligopeptides of alanine and methionine, from trimer to heptamer, as predicted using a rigid geometry approach is a repeating C_7-type conformation. Furthermore, the calculations indicate that, as expected, $(E_\alpha - E_{C_7})$ per residue becomes smaller with increasing chain length indicating an impending change to an α helix at chain lengths greater than seven. Of greater significance is our comparison of different calculation procedures which shows that the predicted stabilities of various conformations of an oligopeptide depend to some extent on the method of calculation. Thus, in contrast to the rigid geometry calculations, the valence force field calculations yield a transition from C_7 to α helix somewhere between a trimer and a hexamer for oligomers of alanine or methionine.

It is difficult to comment on which predictive scheme better represents the experimental observation of the critical chain length for α-helix formation since solvent greatly influences this latter parameter. For example, CD studies in trifluoroethanol show that alanine oligomers favor β-associated structures in this solvent.[11] In contrast alanine oligomers in trifluoroethanol–1% sulfuric acid mixtures[11] and methionine oligomers in trifluoroethanol[18] exist in partially helical structures at the heptapeptide. Recently a more detailed analysis of CD curves of pentamers and hexamers of methionine or of methionine and one valine indicates that small amounts of some ordered structure exist at these chain lengths.[21] Finally, although the C_7 structure has been reported for a number of dipeptides[2b] in inert solvents such as $CDCl_3$, there are few reports of the presence of this conformation in higher oligomers of alanine or methionine. A recent NMR investigation, however, concluded that C_7 structures contribute to the conformation of the tetramer, pentamer, hexamer, and heptamer for the Boc-Met_n-OMe series in $CDCl_3$.[43] The conformations assumed by these oligomers are stabilized by intermolecular peptide–peptide interactions and it is not clear how this association influences the secondary structure of the isolated peptide chain. Since all of the calculation procedures are based solely on intramolecular interactions it is difficult to include the NMR results in our comparison of prediction with experiment. To summarize, the valence force field calculations predict the onset of helicity in oligopeptides of alanine and methionine at significantly shorter chain lengths than does the rigid geometry approach. Based on results in solvents where alanine and methionine oligopeptides assume helical structures it would thus appear that the valence force field findings are in better agreement with experiment.

Vibrational Entropy. The most significant feature of the valence force field calculations, aside from the insight they provide on the effect of geometry relaxation, is the information obtained about the vibrational free energy and the entropy of the oligopeptides. In the present study it was shown that the entropy contribution to the relative stability of different conformations was as large as the energetic contribution, and in some cases dominated. The entropy difference between the α-helix and C_7 conformations became more negative with increasing chain length. This arises because the low-frequency modes which increase in number and decrease in frequency

Table IX. ϕ, ψ Angles of the Three Conformations with the Largest Statistical Weight

ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_4	ϕ_5	ψ_5	ϕ_6	ψ_6	E	r	$W_i \times 10^{-5}$
−140	140	−160	−160	−70	120	90	−80	−80	110	−130	120	9.71	5.48	2.13
−70	120	−150	150	−70	130	90	−90	−80	100	−90	110	8.71	4.90	0.73
−70	120	−70	150	−70	130	90	−70	−90	80	−100	80	11.20	10.03	0.66

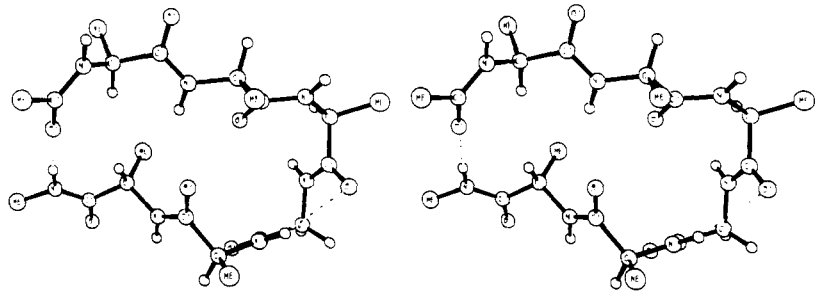

Figure 1. Stereographic view of the conformation of Ac-Ala$_3$-Gly-Ala$_2$-NMe corresponding to the highest statistical weight as generated in the Monte Carlo simulation. Hydrogen bonds are indicated by broken lines.

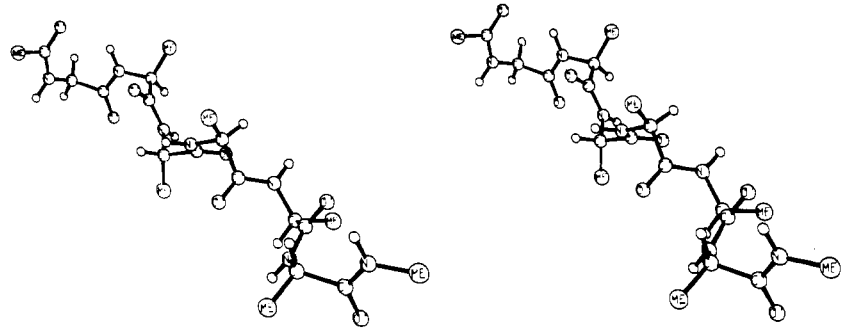

Figure 2. Stereographic view of a "typical" conformation of Ac-Gly-Ala$_5$-NMe generated in the Monte Carlo simulation.

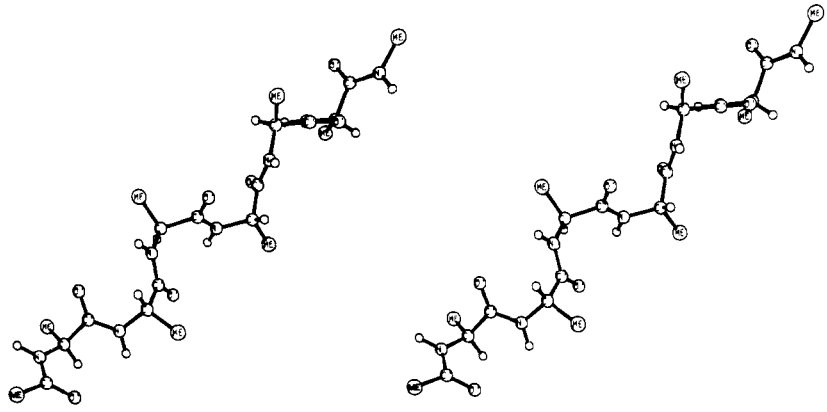

Figure 3. Stereographic view of the rigid geometry minimized C_7 conformation of Ac-Ala$_6$-NMe. The average values of the ϕ, ψ angles are -81, 108°.

with increasing chain length are lower in the more extended C_7 conformation than in the α helix.

It should be pointed out that in the case of the methionine oligopeptides these calculations have been carried out for initial conformations in which the side chains are in the fully extended conformation, i.e., all χ_i equal to 180°. It is conceivable that other starting conformations of the side chains might yield structures of similar free energy or might even change the relative stabilities of the α-helix vs. the C_7 conformation. Several additional calculations with all χ_i set initially to ±60° have been performed. Although the absolute energies and free energies of the molecule differed in the different local minima found, all were of higher free energy and the relative stabilities were not altered. This was by no means an exhaustive study of the effect of side-chain conformation on free energy, which remains a subject for future investigation.

Monte Carlo Simulation of Host–Guest Oligopeptides. Although both the rigid geometry and valence force field calculations were able to yield insight into the relative stabilities of regular structures, their ability to account for the structural consequences of introducing a guest glycyl residue in the oligopeptide was only partially successful. To a large extent this is due to the number of local conformational energy minima in the multidimensional energy surface of even small peptides,[2b,3] and the limited number of conformations sampled by these techniques. Several procedures have been developed to treat this problem within the context of energy minimization.[2b,3,44,45] We felt that the Monte Carlo method, which yields a statistical thermodynamic average over the configurational space of the oligopeptide, would better simulate the properties of these short oligopeptides in solution. The results of the Monte Carlo simulation account remarkably well for the experimentally observed behavior of the analogous Met oligopeptides in which the position of the Gly guest is varied. They also yield a rather unexpected prediction for the behavior of the oligopeptide in which Gly is substituted in the fourth position, which should provoke further experimental work.

The statistical properties of the oligopeptides with Gly

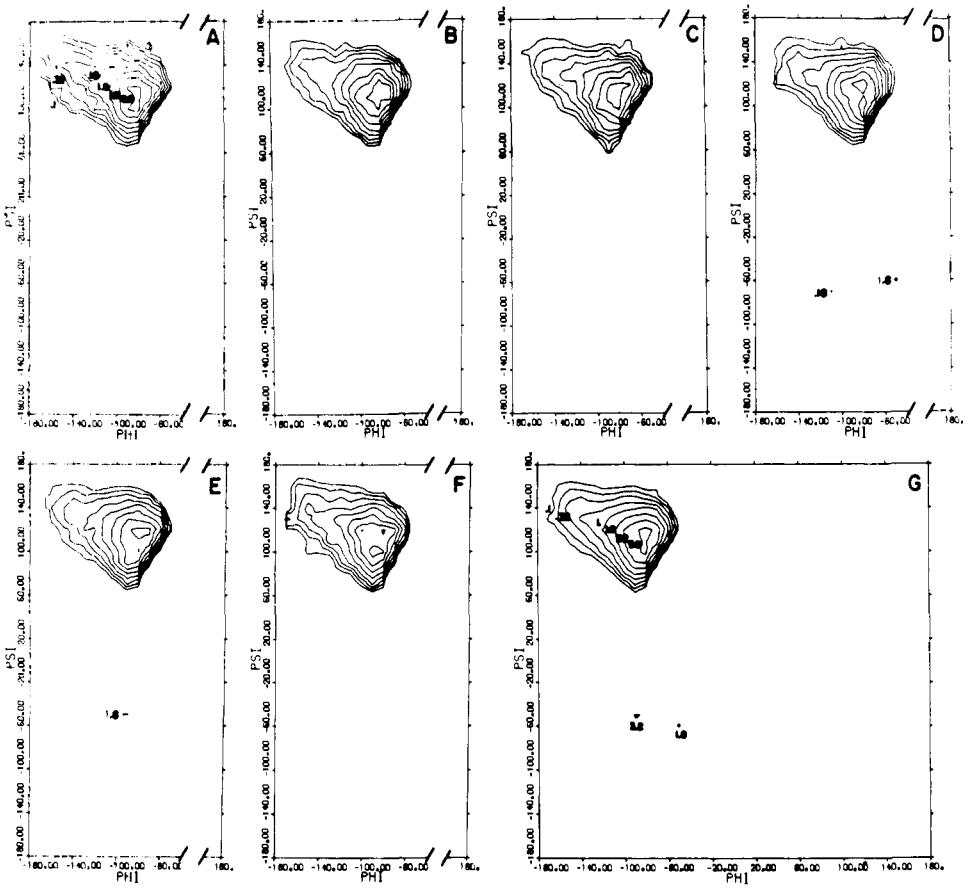

Figure 4. Contour map of the probability density of finding a given residue in Ac-Ala$_6$-NMe in a given region of ϕ–ψ space, derived from the Monte Carlo simulation. The first six maps correspond to the individual residues while the last map is cumulative density giving the probability independent of position in the chain. The values of the contours (×100) are indicated on the figure.

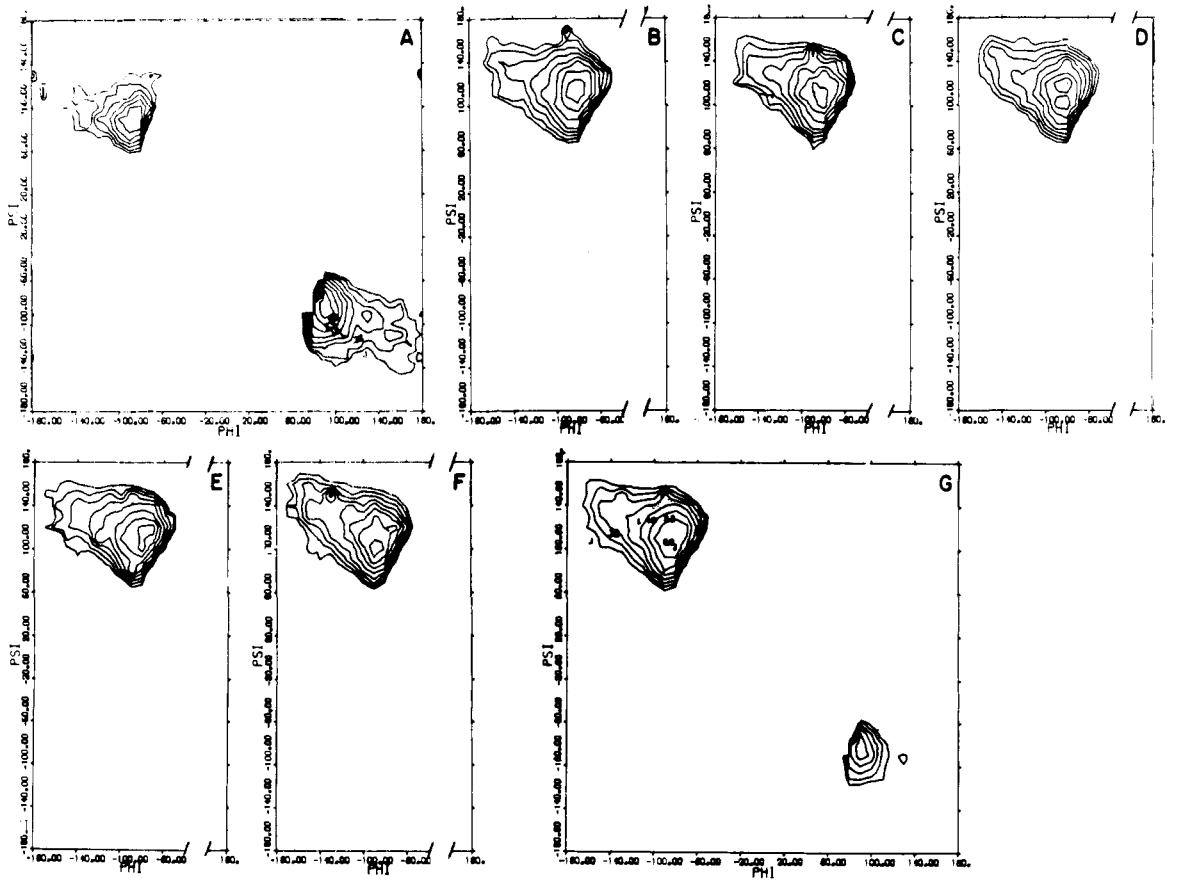

Figure 5. A 4 for Ac-Gly-Ala$_5$-NMe. Note the population of conformations for glycine in first position in lower right-hand corner of map.

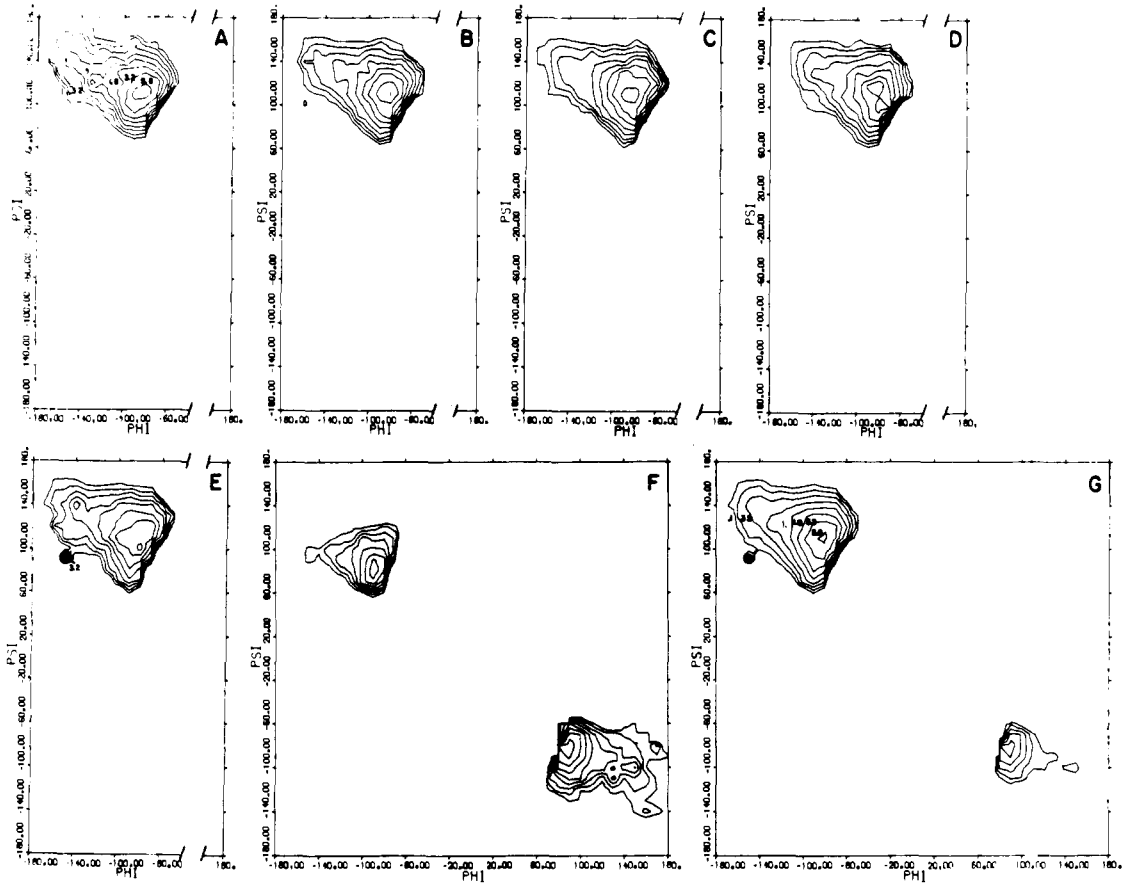

Figure 6. As Figure 4 for Ac-Ala$_5$-Gly-NMe.

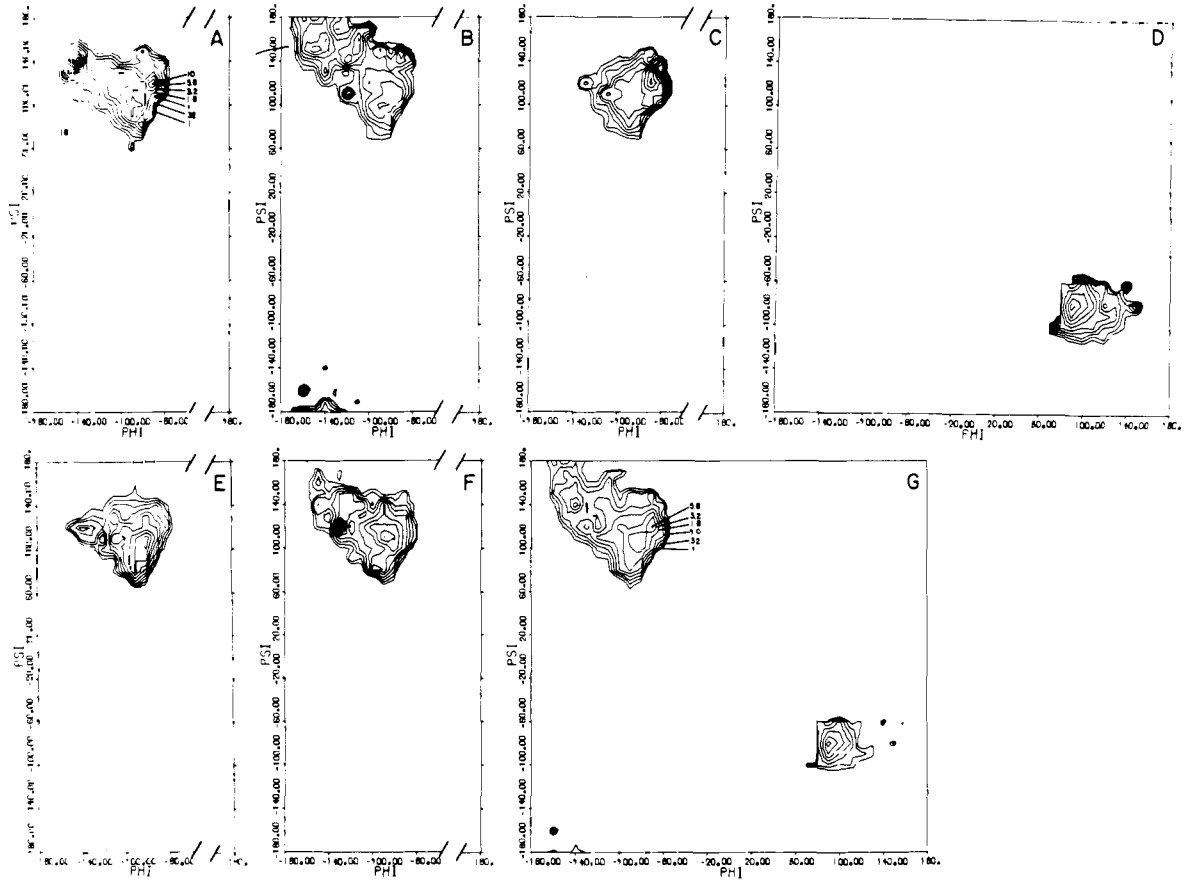

Figure 7. As Figure 4 for Ac-Ala$_3$-Gly-Ala$_2$-NMe. Note that glycine in third position occupies only one of the two regions available to it in the lower right-hand corner of map. Compare with Figures 5 and 6.

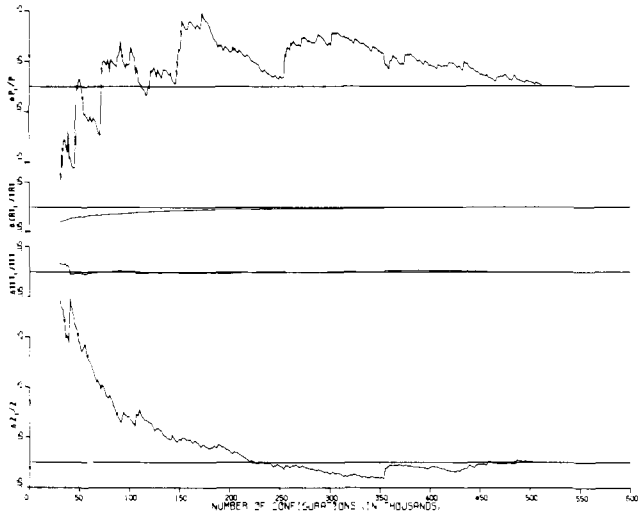

Figure 8. The convergence of a quantity X is plotted as $\Delta X_i/X$ vs. the number of configurations generated, i. X is the final value of the quantity and ΔX_i is the difference between the final value, X, and its value after i configurations. The plots are given for Ac-Ala$_6$-NMe and begin at 30 000 configurations. The following quantities are compared: (a) the partition function, Z; (b) the average energy, $\langle E \rangle$; (c) the end-to-end distance, $\langle R \rangle$; (d) the probability of finding the third residue in the conformation $\phi = -80°$, $\psi = 100°$. It is seen that there is a marked difference in the rates of convergence for different quantities. While the average energy and end-to-end distance converge after 30 000 configurations, the partition function requires about 200 000 configurations to converge and the probability of finding a residue in a given conformation requires at least 450 000.

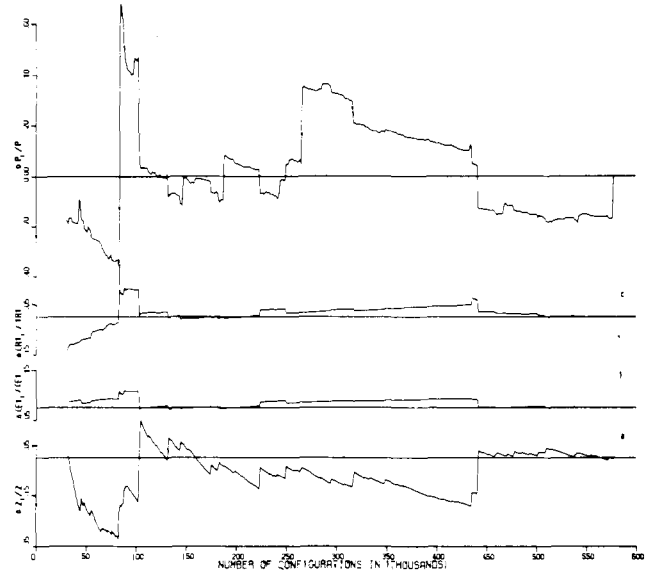

Figure 9. As Figure 8 for Ac-Ala$_3$-Gly-Ala$_2$-NMe. Note that the scale differs from Figure 8 by a factor of 2 and that the rate of convergence is much slower.

substituted in the first and sixth positions, as obtained from the Monte Carlo simulation, are very similar to those obtained from the unsubstituted hexapeptide. As noted above, the average energies obtained from the simulation consisting of the generation of ~500 000 chains fell between the energies of the minimized α-helical and C_7 conformations. The average end-to-end distance was somewhat shorter (1.7 Å) for the peptides with Gly, but all three are large, 19–21 Å, indicating an ensemble of extended structures for each peptide. Finally, the probability maps of the distribution of conformational states show a significant difference between the host hexamer and the hexamers with glycine at either terminus (e.g., Figures 4 and 5), namely, the accessibility of the C_7^{ax} region around $\phi, \psi = (+80, -80)$ to the glycyl residues, which according to these maps have roughly equal probabilities of being in the C_7^{ax} region or the region in the upper left-hand corner of the map. (This additional freedom arises from the absence of the C_β carbon in glycine and is also reflected in the usual energy map.) The only significant probability density for the remaining alanine residues is found in the upper left-hand corner of the map (Figures 5 and 6), as in hexaalanine (Figure 4), which results in the similarity of end-to-end distances.

The ensemble averages obtained from the simulation of the hexapeptide with a glycyl residue in the fourth position differ dramatically from the other three hexapeptides. The average energy is 3.9 kcal lower than the other two hexapeptides containing a glycine (one cannot compare it against the energy of hexaalanine, as the compositions differ). The statistically averaged end-to-end distance is only 7.2 or ~12–14 Å shorter than for the other three peptide chains, while the entropy is lower by ~6 eu. The probability maps indicate that, unlike the glycyl residues in the first and last positions, the glycyl guest in the fourth position only adopts conformations in *one* of the two regions accessible to it (the C_7^{ax} region).

The results of the Monte Carlo simulation are in qualitative agreement with those of CD studies on methionine-glycine "host-guest" peptides. These studies showed that the CD patterns for a methionine hexapeptide containing one glycyl residue in the center of the peptide were drastically altered as compared to those of the analogous hexamethionine or hexamers containing one glycyl residue at either the N or C terminus.[21] In fact the CD pattern for Boc-Met$_3$-Gly-Met$_2$-OMe resembled that of the trimer Boc-Met$_3$-OMe. These findings were interpreted as due to the introduction of increased flexibility by the glycyl residue causing a more disordered peptide chain.[21] It is clear that the results of the Monte Carlo simulations would predict major differences for the CD patterns of the host-guest peptides. In contrast to the conclusions of the CD studies, however, the statistical analysis concludes that the perturbation caused by glycine in position 4 is due to a *more ordered* and less flexible hexamer. The class of folded conformations adopted by this hexamer is characterized by low entropy, low energy, and a small end-to-end distance and is accomplished by the glycyl residue adopting the C_7^{ax} conformation. The possibilities of either a more flexible hexapeptide or of a folded structure stabilized by specific hydrogen bonding can be distinguished using NMR and IR techniques. Such investigations are being conducted in our laboratories.

Finally, it is worth mentioning one additional problem encountered with the biased sampling approach used here and in previous works.[26,46,47] We refer to the problem of convergence. With the more than 500 000 configurations used here (as much as two to three orders of magnitude more than considered typically), it was found that, although the average energy and end-to-end distance had converged, satisfactorily, the probability distribution had not fully converged, while for Ala$_3$-Gly-Ala$_2$ it is probable that none of the properties was fully converged. This is demonstrated in Figures 8 and 9. The fact that the convergence of a given property depends on the property itself as well as the sampling procedure used is well known.[48] The difference in the rates of convergence as a function of the guest position, or therefore the primary structure, to our knowledge has not been generally recognized. It would appear from these results that the use of the Metropolis method,[49] which has been applied to a wide variety of problems in statistical mechanics,[38] including the treatment of solvent effects around biological macromolecules[50] and the properties of oligopeptide chains,[28] might be a more efficient procedure for these oligopeptides. Further improvement in efficiency may also be obtained by the recently suggested "force bias" method proposed by Berne.[51] Studies of the relative merits of these

alternative procedures for peptide chains are underway in our laboratory.

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A Theoretical Study of the Disproportionation Reactions of N_2H_2 Species

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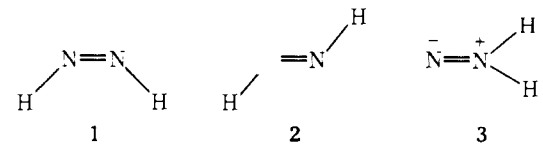
Abstract: Ab initio SCF calculations have been carried out on the disproportionation reactions of N_2H_2 (diimide) species to form dinitrogen and hydrazine. Concerted hydrogen transfer pathways for the reactions of *cis*- with *cis*- and *cis*- with *trans*-diimide possess lower energy barriers than does the reduction of ethene by *cis*-diimide, consistent with experimental observations. A two-step, hydrogen atom transfer process proceeding via N_2H and N_2H_3 radicals is also discussed and is considered to be an energetically feasible process for the disproportionation reaction. Calculations have also been carried out on the concerted transfer of hydrogen from 1,1-diimide (aminonitrene) to *cis*- and *trans*-diimide, these reactions having energy barriers substantially higher than for the transfer process involving *cis*-diimide.

Introduction

In a recent article we described the results of a theoretical study on the reactions of the various diimide species **1–3** with ethene.[1] The mechanistic implications derived from our study were in distinct contrast with those derived from earlier theoretical[2a] and gas-phase kinetic[3] studies in which the transfer of the hydrogens from **1** to ethene was indicated to be the rate-determining step instead of the isomerization of **2** to **1**. Our calculations indicated that the reaction of **1** with ethene



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