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Influence of Ordered Backbone Structure on Protein Folding. A Study of Some Simple Models[1]

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ABSTRACT: Four series of model molecules, each of which contains a coil section and one or two sections of fixed ordered backbone structure, have been examined to locate their low-energy conformations in water. The four series are: helix–coil, helix–coil–helix, extended–coil, and extended–coil–extended. In each series, the length of the coil is allowed to vary from four to ten residues, while the nuclei (ordered backbone structures) are held fixed at six residues. By comparing these molecules, it is observed that the low-energy conformations of those containing two nuclei can be regarded as being derived from low-energy conformations of molecules containing one nucleus. This suggests that folding of proteins containing preformed nuclei proceeds through interactions between the nuclei and adjacent nonregular sections of the chain rather than between nuclei. It is also observed that helices are better promoters of globularity than extended strands. These results are compared with those from recent studies of various aspects of protein folding.

Several approaches are being used to compute the three-dimensional structure of a native protein in a given environment from a knowledge of its amino acid sequence. A useful (if somewhat simplistic) method for classifying these approaches is to divide them into mechanistically "directed" and "nondirected" folding procedures. In the nondirected procedures,[3–5] an attempt is made to incorporate features which will lead to a distribution of conformations which closely matches the native structure at the end point of the calculation. No effort is made, however, to ensure that the intermediate stages of the calculation resemble postulated stages in the folding of the molecule in solution. Indeed, in one procedure,[5] the intermediate stages do not necessarily possess the correct juxtaposition of neighboring residues.

The directed procedures[6–8] are based on the hope that the efficiency of the computational folding process can be increased by forcing it to model the actual folding mechanism as closely as possible. This should prevent the molecule from sampling regions of conformational space from whcih the native conformation is not likely to be attained. For example, in the three-step procedure of Tanaka and Scherage,[7] the first and second steps are characterized by the operation of short- and medium-range interactions, respectively. Their combined effect is to produce contact regions made up of residues which are proximal along the chain. These contact regions frequently consist (partially but not exclusively) of ordered backbone structures, such as helices, bends, or extended strands. In the third step, the long-range interactions between contact regions which are distant along the chain bring the molecule into the compact globular form which characterizes the native protein. Ptitsyn and Rashin,[8] who carried out model-building studies on a highly helical protein, were also concerned primarily with the packing of preformed helical nuclei; this corresponds essentially to the third step of the Tanaka–Scheraga model.

It is clear from the foregoing remarks that one of the central postulates of the directed methods of protein folding is that the preformed nuclei of ordered backbone structure interact with other parts of the chain in a manner which is crucial to the folding process. It remains to ask how this comes about. For example, is the folding due to the interaction between nuclei, or between each nucleus and nonregular parts of the chain? What are the roles of different types of ordered structure in the folding process? These questions are difficult to investigate by considering native proteins, where the number of interactions is large. It was felt, therefore, that a systematic study of simple molecules containing fixed nuclei of ordered structure would be a useful method of refining our understanding of the folding process. A number of studies have appeared, directed primarily toward the interaction of two helices.[9–12] While the percent work was in progress, the studies of Warshel and Levitt[13] on carp myogen appeared. They studied, among other things, the folding of two-helix fragments of the carp myogen sequence.

We shall consider both helices and extended structures, and will be interested in folding as a function of the separation of the ordered structures along the chain, in the presence of water. Our model is outlined more fully in the next section.

Methods

Four types of model molecules were considered here. Each contained one or two blocks of residues fixed in a regular structure and one nonregular block whose conformation was allowed to vary. For example, one type was the helix–coil–helix molecule, which we denote as $H_NC_MH_N$. This type consisted of two runs of N resides, each fixed in a helical conformation, separated by M residues in a nonregular coil. The remaining types were: helix–coil (H_NC_M), extended–coil–extended ($E_NC_ME_N$), and extended–coil (E_NC_M). In the present study, N was taken as 6, and M was allowed to vary from 4 to 10. The energy of each molecule (i.e., each value of M) was minimized with respect to the dihedral angles in the C_M block.

The H_N and E_N blocks consisted of alanine residues and the C_M block of glycine residues. Alanine was chosen because of the simplicity of its side chain and glycine because of the

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large volume of its conformational space. The dihedral angles chosen for the helical and extended regions were (ϕ,ψ) = (−58°,−47°) and (180°,180°), respectively. Although the intramolecular energy in the latter conformation is somewhat unfavorable for alanine,[14] we are interested primarily in the differing tendencies of the two backbone structures to form contact regions. We have chosen the dihedral angles (180°,180°) to emphasize the distinction between the extended and helical structures.

In order to reduce computational expense, while adequately sampling the conformational space of the molecule, the rotational isomeric state (RIS) approximation was used. The dihedral angles of the glycine residues were allowed to assume a discrete set of values at 30° intervals, viz., ϕ or ψ = −180°, −150°, ..., 150°. This provides points that are reasonably close in both energy and conformation to the principal minima of the glycine residue.

The minimization scheme consisted of two stages. The first was the random generation of conformations of each molecule under study. Each conformation was checked on an atom-by-atom basis for hard-sphere overlaps, using the hard-sphere radii of Tanaka and Scheraga.[15] Ten-thousand overlap-free conformations of each molecule were produced, and the conformational energy of each was calculated; the lowest-energy conformation in each set was identified.

In the second stage, this lowest energy conformation was used as the starting point for a step-wise energy minimization. The dihedral angles in the variable coil region were changed, one at a time, by one step in each direction (±30°) on the RIS lattice, and the energy of the resulting conformation was calculated. If the energy was higher than that of the original conformation (resulting from stage I), or if a hard-sphere overlap developed, the conformation was rejected. The lowest energy conformation of those remaining was then used as the starting point for another cycle of stage II minimization. This process was continued until the energy could not be lowered any further. The resulting conformation was the lowest energy conformation referred to in the remainder of this work.

The random number generator used in the first stage was RANDK, a subroutine written by the Cornell Office of Computer Services and stated by them to be superior to many of the commercially available generators with respect to sequence correlations. Each of the 28 generation sequences (seven values of M for each of four types of molecules) was started from a different initation point, in order to guarantee that the runs were independent.

The energy of an overlap-free conformation consists of a number of contributions. Since the H_N and E_N blocks were maintained fixed, the energies of these blocks were not computed. The only energies computed were those for the individual glycine residues, the interglycine residue interaction energies, and the energies of interaction between the various blocks. The energies of the glycine residues (short-range energy) were taken as those of N-acetyl-N'-methylglycineamide at the selected (RIS) values of ϕ and ψ.[14] Although these energies depend only on the dihedral angles of a single residue, the N-acetyl and N'-methyl blocking groups simulate parts of the backbone adjacent to the residue in question. These energies therefore account for intraresidue and part of the nearest neighbor (i to $i \pm 1$) interactions, as well as the C_7^{eq} hydrogen bond energy.[16] Since glycine has no side chain, it is expected that the interaction between residues i and $i \pm 2$ will be negligible and that those between i and $i \pm 3$ will take place over a very limited region of conformation space.[17] We, therefore, neglected them and assumed that the single residue and nearest neighbor interactions dominate on the scale $|i - j| \leq 3$. When $|i - j| = 4$, the possibility of hydrogen bonding leading to helix formation arises, provided that the intervening dihedral angles assume appropriate values. This is of particular interest in the present study, since the prefixed helices could serve as nuclei on which larger helices could grow. The i to $i \pm 4$ interaction, therefore, was modeled by a hard-sphere interaction together with a hydrogen-bond energy which was included if the correct dihedral angles were generated in the C_M sequence.

The above approximations mean that the medium-range ($2 \leq |i - j| \leq 4$) interactions were modeled by a hard-sphere potential plus an $|i - j| = 4$ hydrogen bond. It is possible that, by using this approximation, certain conformations which are stabilized by medium-range interactions, such as other types of hydrogen bonds, were ignored. In a glycine chain, however, the formation of such structures is entropically disadvantageous, and the approximation is probably not seriously in error.

The long-range ($|i - j| > 4$) interaction energies were calculated using the empirical free energies of Tanaka and Scheraga[15] for residue–residue contact. These parameters, which arise from a statistical analysis of residue pair contacts in 25 globular proteins, reflect the relative tendencies of various residues to associate with each other or to remain unpaired, in the presence of water.

Results

The four series of of low-energy conformations which resulted from the minimization procedure are shown as stereo ORTEP drawings in Figures 1–4. For simplicity, only the C^α and C^β atoms are shown, and the backbone is represented by virtual bonds connecting the C^α atoms.

In this section, the characteristics of the individual conformations are presented.

A. H_NC_M Series. M = 4–6. The conformations of these molecules are dominated by a tendency for the coil to fold efficiently against the fixed helix. There is no contribution to the stabilization energy from intracoil (IC) interactions (see Table I).

M = 7. The longer coil forms a somewhat wider loop, but the helix–coil (HC) interaction is still the dominant factor in folding, and there is no IC interaction.

M = 8. The coil is wrapped very tightly against the helix, and the IC interaction still plays no role in stabilizing the conformation.

M = 9. The coil is now long enough to form an open circular loop which lies against the helix. The stability of this conformation now has a significant contribution (18%) from the IC interaction.

M = 10. There is some indication of a circular loop (in the opposite sense to the M = 9 loop) forming in the coil as it folds against the helix. The less efficient formation of this loop is reflected in the smaller contribution (5%) of the IC interaction to the stability of the conformation.

B. $H_NC_MH_N$ Series. M = 4. The molecule assumes a simple U shape with nonparallel arms. The angle between helix axes is 112°, and the stability of the conformation is due priamarily (73%) to the interhelix (HH) interaction. The HC interaction is relatively small (see Table I), and there is no IC interaction.

M = 5. The presence of an additional residue in the coil greatly increases the flexibility of the molecule. Although the interhelix angle is the same as in the M = 4 case (112°), there is now a much more favorable orientation of the C-terminal helix relative to the coil. This is reflected in the much greater HC interaction (51%). The two types of long-range interaction (HC and HH) are sufficient to compensate for a slightly unfavorable short-range (SR) interaction (−2%). There is no IC interaction.

M = 6. As the flexibility of the coil increases with length, we begin to see a feature common to several of the longer HCH molecules. This is the insertion of one helix into a "pocket"

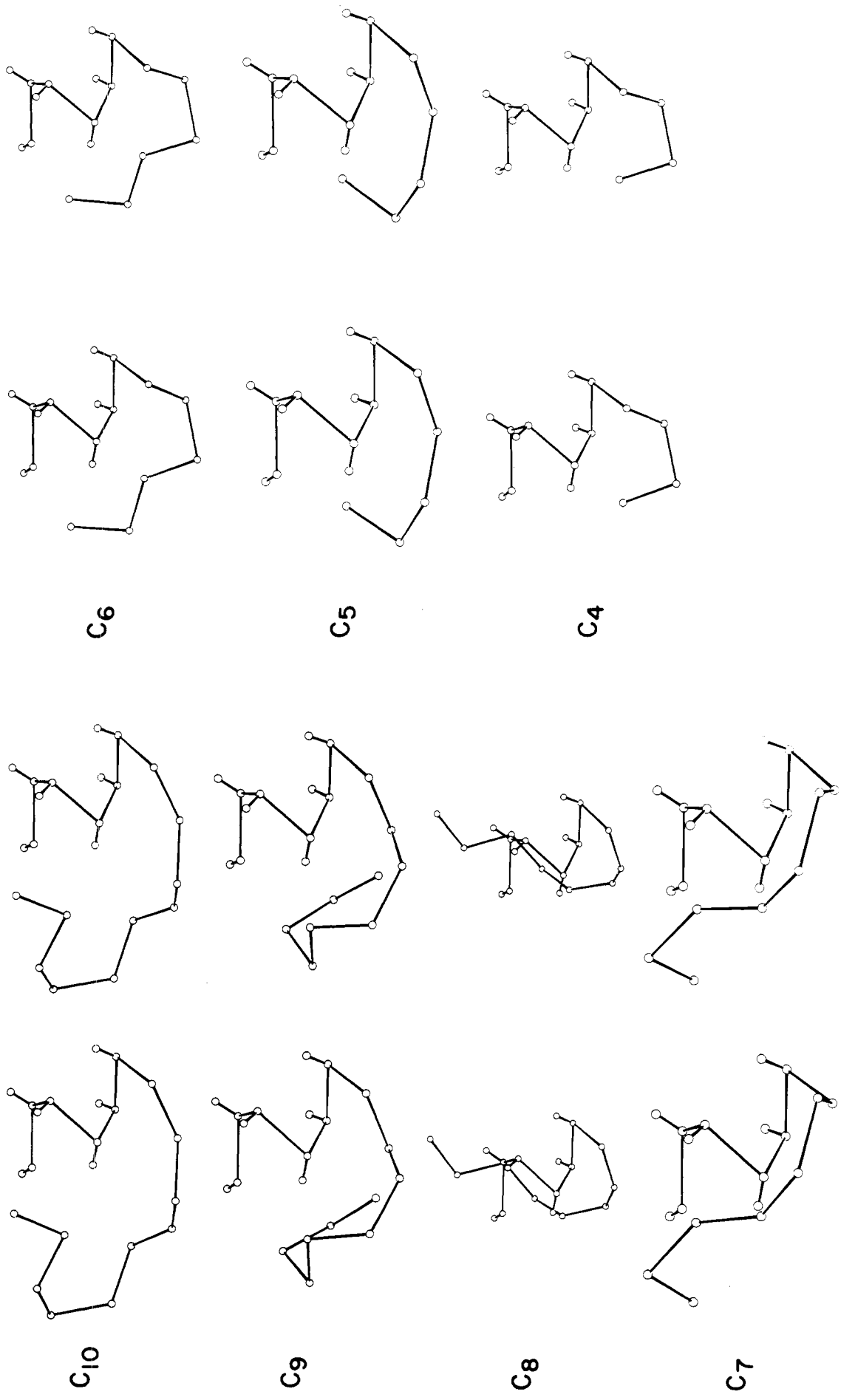

Figure 1. Stereoscopic views of low-energy conformations of the $H_N C_M$ series. Only the C^α and C^β atoms are shown, and the backbone is represented by virtual bonds of 3.8 Å length connecting the C^α atoms. The N-terminal fixed backbone structure is in the same orientation in each figure to facilitate comparison.

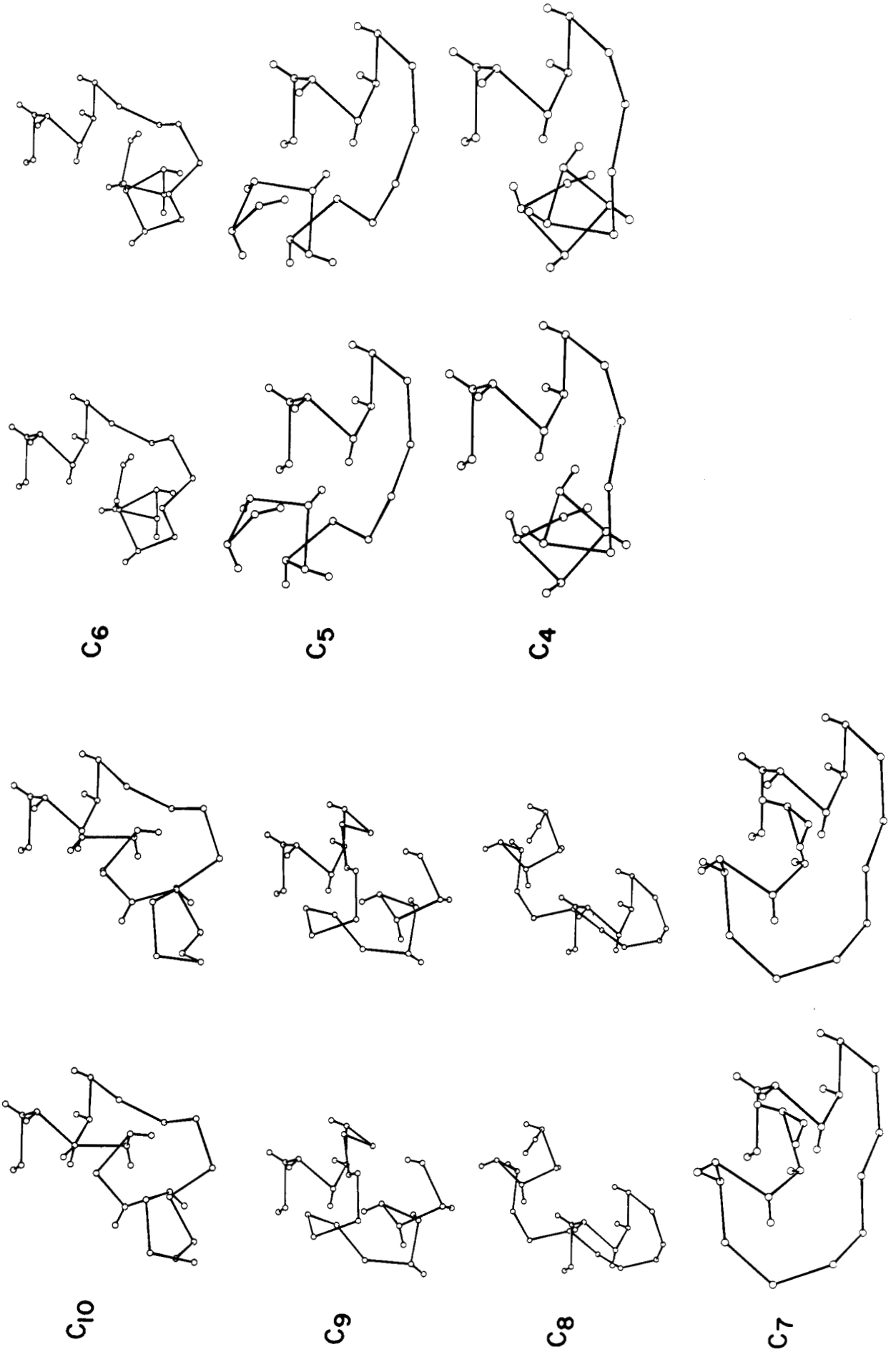

Figure 2. Same as Figure 1 but for the $H_N C_M H_N$ series.

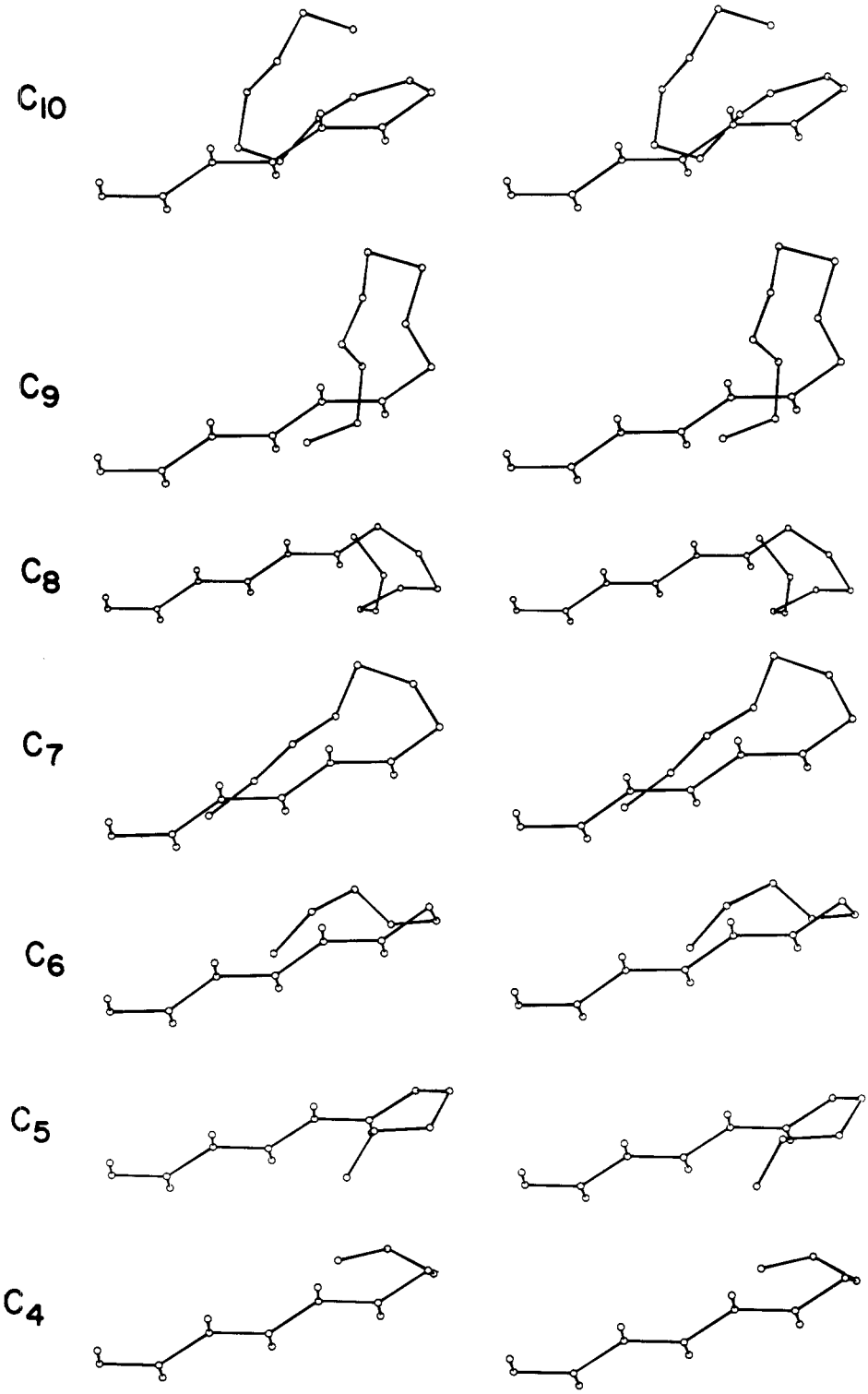

Figure 3. Same as Figure 1 but for the $E_N C_M$ series.

formed by the coil and the other helix. The two helices in this case are essentially perpendicular (89°), with the C-terminal helix fitting between the coil and the N-terminal helix. The HC interaction accounts for 67% of the total stabilization and the HH interaction for 32%. The SR interaction is very slightly favorable (1%).

***M* = 7.** Here, again, the C-terminal helix is in a pocket formed by the coil and N-terminal helix. In this case, however, the C-terminal helix protrudes from the pocket so that the two helices are nearly parallel (the interhelical angle being 16°). This leads to a favorable HH interaction; although the proportion (26%) is smaller than for $M = 6$, the numerical value is larger. The HC interaction is 58% of the total, and the SR energy is 15%.

***M* = 8.** The situation that we have encountered thus far is reversed here. We now find the *N-terminal* helix in a pocket formed by the *C-terminal* helix and the coil. The two helices are relatively closely aligned (37°). The proportional contributions by the various interactions to the stability are the same as in the $M = 7$ case.

***M* = 9.** This is an exception to the pocket-forming tendency that we have seen thus far. The coil loops around fairly tightly, so that a pocket is not formed. Rather, the coil folds efficiently against the C-terminal helix and (for the first time in this se-

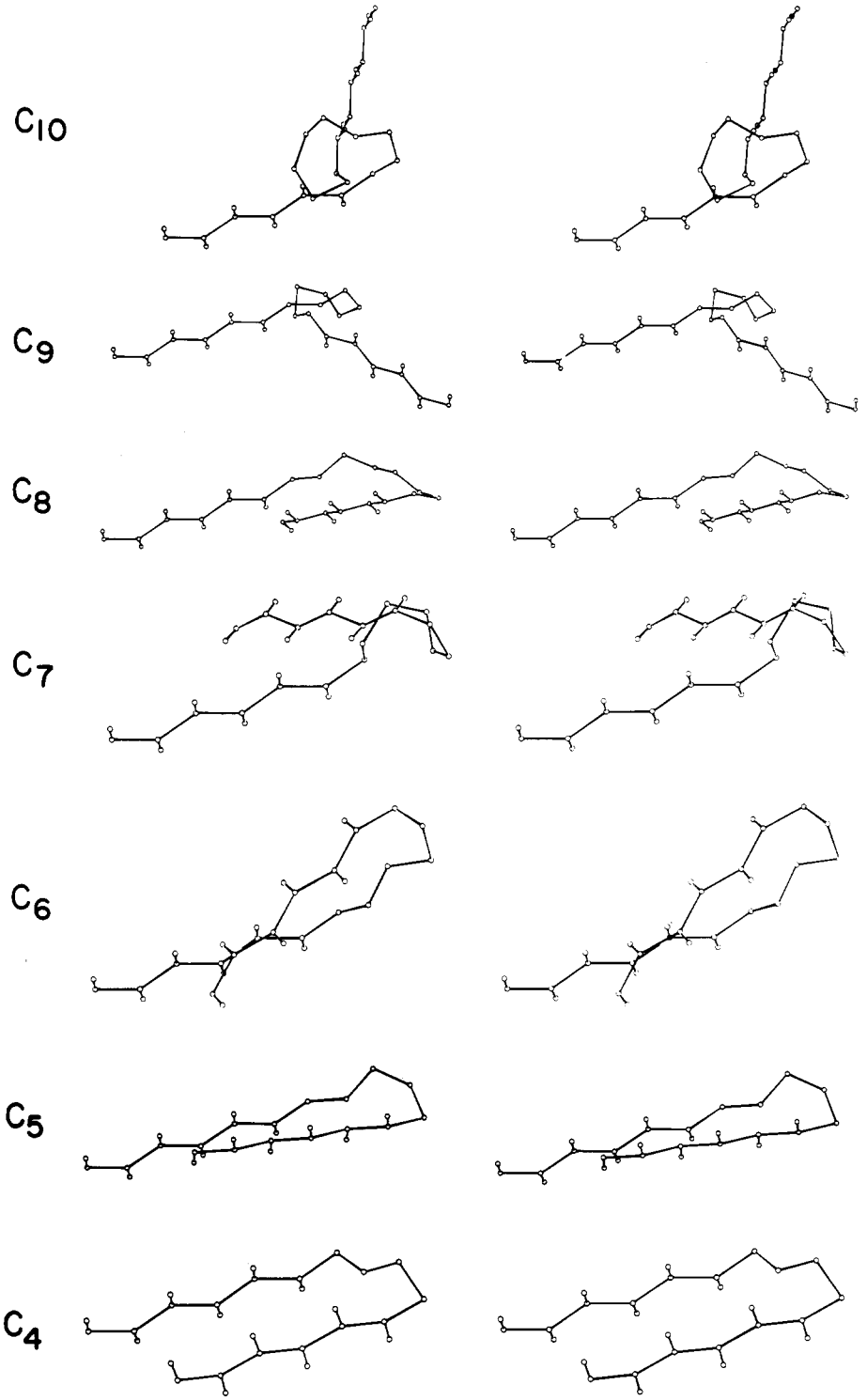

Figure 4. Same as Figure 1 but for the $E_NC_ME_N$ series.

ries) with itself. The HC interaction accounts for 93% and the IC interaction for 11% of the total stabilization. This is balanced by a rather unfavorable SR interaction (−6%). The interhelix angle is 55°, and the HH interaction is very small.

***M* = 10.** Once again, we find a "pocket" conformation, but, because of the length of the coil, there is significant stabilization due to IC interactions (5%). There is some HH interaction (9%), but the HC interaction dominates the stability of the molecule. This conformation is much more stable in terms of short-range energies (4%) than is the case for *M* = 9.

C. E_NC_M Series. *M* = 4–7. In this group, the coil folds against the strand to form β-hairpin-like structures. This is particularly evident in the *M* = 6 and 7 cases. There is no IC interaction in any of these conformation. The extended strand–coil (EC) interaction increases from 35% for *M* = 4 to 71% for *M* = 7 (see Table I).

***M* = 8.** The greater flexibility of the longer coil enables the structure to form a loop, with substantial IC interaction (30%). The EC interaction drops drastically, to 29%. The hairpin-like shape is no longer present.

***M* = 9.** The coil forms a loop, rather resembling that in $E_6C_9E_6$ (see below). The longer coil interacts efficiently with

Table I
Contribution of Various Types of Interactions to Stabilization of Model Molecules

	Fraction of total enegy of H_NC_M molecules[a,b]			Fraction of total energy of $H_NC_MH_N$ molecules[b,c]				Fraction of total energy of E_NC_M molecules[b,d]			Fraction of total energy of $E_NC_ME_N$ molecules[b,e]			
M	HC	IC	SR	HH	IC	HC	SR	EC	IC	SR	EE	IC	EC	SR
10	0.58	0.05	0.37	0.09	0.05	0.82	0.04	0.38	0.30	0.32	0.03	0.25	0.56	0.16
9	0.67	0.18	0.15	0.02	0.11	0.93	−0.06	0.50	0.18	0.32	0.0	0.21	0.56	0.23
8	0.74	0.0	0.26	0.26	0.0	0.58	0.16	0.29	0.30	0.41	0.09	0.0	0.86	0.05
7	0.71	0.0	0.29	0.26	0.0	0.58	0.15	0.71	0.0	0.29	0.21	0.0	0.57	0.22
6	0.70	0.0	0.30	0.32	0.0	0.67	0.01	0.65	0.0	0.35	0.42	0.0	0.41	0.18
5	0.66	0.0	0.34	0.51	0.0	0.51	−0.02	0.50	0.0	0.50	0.53	0.0	0.28	0.19
4	0.58	0.0	0.42	0.73	0.0	0.15	0.13	0.35	0.0	0.65	0.77	0.0	0.14	0.09

[a] For the conformations of Figure 1. [b] Abbreviations are: HC, helix–coil interaction; IC, intracoil interaction; SR, single-residue energy; HH, interhelix interaction; EC, extended strand–coil interaction; EE, extended strand–extended strand interaction. [c] For the conformations of Figure 2. [d] For the conformations of Figure 3. [e] For the conformations of Figure 4.

the extended strand. The EC interaction is responsible for 50% of the total stabilization, and the IC interaction contributes 18%.

***M* = 10.** The coil forms a loop which folds against the extended strand as well as against itself. The IC interaction is 30% and the EC interaction is 38% of the total stabilization.

D. $E_NC_ME_N$ Series. *M* = 4. Because of the short coil, the only efficient way for the molecule to fold is in a structure which closely resembles a β hairpin. The angle between strands is 177°, which means that they are essentially antiparallel. Most of the stabilization comes from the interstrand (EE) interaction (77%), with 14% due to the strand–coil (EC) interaction. There is no IC interaction.

***M* = 5.** Again we find a hairpin, with interstrand angle 172°. The EE interaction contributes 53% to the stabilization and the EC interaction 28%. There is, again, no IC interaction.

***M* = 6.** The coil becomes longer, and the hairpin is skewed so that a significant amount of the coil is packed against the C-terminal strand. The interstrand angle is 152°, and the EE (42%) and EC (41%) interactions are nearly equal.

***M* = 7.** The coil is now long enough so that EC interactions dominate the stabilization energy (57%). The angle between strands is 127°, and the EE interaction is only 21% of the total. There is no IC interaction. The hairpin shape is beginning to disappear.

***M* = 8.** The disposition of the extended strands is now essentially dictated by the coil, which loops around to maximize its interaction with the C-terminal strand. The EC interaction constitutes 86% of the total stabilization and the EE interaction only 9%. There is no IC interaction. The interstrand angle is 126°.

***M* = 9.** The coil completely dominates the conformation of the molecule. The EE angle is 57°, but there is no EE interaction. The coil loops back on itself so that, for the first time in this series, we find an IC interaction (21%). The EC interaction is 56% of the total.

***M* = 10.** Again the coil dominates the conformational preference. The loop is even sharper than in the *M* = 9 case, and the EE interaction is very small (3%). The EC interaction is 56%, and the IC interaction is 25% of the total stabilization. The interstrand angle is 63°.

Discussion

On the basis of the results presented above, we shall consider the questions about long-range interactions which were posed in the introductary section.

It is clear that the folding of the H_NC_M series is governed by the need to optimize the contact between the coil and helix sections. Significant intracoil interactions appear only when the coil becomes long enough to make contact with itself without sacrificing helix–coil contacts.

Comparison of this series with the $H_NC_MH_N$ series is instructive. As a point of departure, we focus our attention on the H_6C_8 and $H_6C_8H_6$ conformations. The similarity between these two is striking; in fact, they are identical through the fifth residue in the coil. (In this connection, it should be noted again that the random number and minimization sequences used to generate these conformations were completely independent.) This suggests that the folding of the $H_NC_MH_N$ molecules is governed essentially by the interaction of the coil with one of the fixed helices. In fact, the "pocket" conformation can be viewed as arising from this type of interaction. Careful inspection of the other long ($M > 5$) $H_6C_MH_6$ molecules reveals that most of them can be thought of as deriving from C_MH_6 molecules in which the coil is wrapped against the helix (which is now C-terminal). When the intervening coil drops below a certain length, the interference between the two bulky helices begins to govern the conformation. As was mentioned in the previous section, the $H_6C_9H_6$ conformation is something of an exception to the pocket-forming rule. Perhaps a lower energy conformation with a pocket-type structure might be found. Nevertheless, it is possible to view the conformation which was found as derived from an efficiently packed C_9H_6 conformation.

The conformational tendencies pointed out above are likewise evident in the E_NC_M and $E_NC_ME_N$ series. The shorter ($M < 8$) E_6C_M molecules form hairpins in which the coil arranges itself against the fixed strand. As the coil becomes sufficiently long and flexible, an energy advantage is gained by loop formation. Inspection of the $E_NC_ME_N$ series indicates that there is generally a much stronger interaction of the coil with the C-terminal extended strand than with the N-terminal strand. At $M = 7$, the tendency of the coil to loop begins to manifest itself and becomes increasingly significant toward the long end of the series. We suggest that all of the $E_6C_ME_6$ low-energy conformations found here can be derived from similar C_ME_6 conformations.

It is possible that the tendency, manifested in both series, to favor interaction of the coil with the C-terminal rather than the N-terminal fixed nucleus, is due to the basic asymmetry of the peptide chain. Conceivably, though, corresponding low-energy conformations exist in which the coil interacts preferentially with the N-terminal nucleus. In any case, limited evidence obtained in the course of this study indicates that conformations in which the interactions are more symmetrically distributed tend to be of higher energy than the asymmetric conformations; i.e., interactions between the fixed nuclei and the coil are favored over those between the fixed nuclei at both ends of the molecule.

The foregoing remarks suggest that the extent to which protein folding is influenced by interaction *between* preformed nuclei is governed by the length of the nonregular

section separating them. Inspection of Table I shows that, for the $H_NC_MH_N$ series, the HC interaction begins to dominate at $M = 5$, while the corresponding value for the $E_NC_ME_N$ series is $M = 7$. It would seem that the relevant parameter is the ratio between the length of the fixed nuclei and that of the adjacent nonregular section. If this parameter is sufficiently small, it may be an unwarranted simplification to consider only packing of preformed (helical or extended) nuclei.

The conclusion that the importance of the H···H or E···E interactions in folding is dependent on the length of the nonregular coil separating the nuclei is consistent with the observation of Levitt and Chothia[18] that, in native proteins, ordered nuclei which are adjacent along the chain are generally spatially close. As they remark, the nonregular portions in native proteins are frequently short. Even if the nonregular coil is of the same length as or longer than the nuclei that it connects, optimal contact between one nucleus and the coil will tend to bring the second nucleus into proximity with the first (although, from an energetic viewpoint, the two nuclei may not be in particularly advantageous contact). Our results are also consistent with the results of Warshel and Levitt[13] on the folding of carp myogen segments containing pairs of helices. They consider two fragments: helices C (10 residues) and D (9 residues) together with the intervening 10 residues, and E (10 residues) and F (7 residues) with the intervening 10 residues. They arrived at minimum-energy structures which are essentially variations on the nonparallel "U" forms that were observed here for the shorter $H_NC_MH_N$ molecules. Furthermore, the "idealized native" conformations for the myogen fragments which Warshel and Levitt took from the actual native structure seem to be similar to the twisted U shape which we found for $H_6C_5H_6$. Both in the myogen fragments and in the model molecules to which we have compared them, the number of residues in the fixed nuclei and in the intervening coil are approximately equal, although the number of residues in the two studies are significantly different (10 vs. 6). It should also be remarked that the energy functions used in the two studies are very different. These two observations imply that the conformations of these molecules are probably determined by considerations of packing efficiency and that in this connection the relation between the length of the fixed nuclei and that of the variable coil may indeed be an important parameter.

Another interesting observation arising from the low-energy conformations found here is the greater efficiency of the helix over the extended strand for promoting compact structures. For example, in the $E_6C_7E_6$ through $E_6C_{10}E_6$ conformations, the extended strands are unable to overcome the looping propensities of the coil. This is undoubltedly due to the slimness of the extended strand, which imposes stringent conformational requirements on a coil with which it is to fold compactly. One is led to suspect that there may be an advantage for folding in the fact, observed by Finkelstein and Ptitsyn,[19] that there is a higher probability for helices to preform in the denatured protein than for any other regular structure. The presence of preformed helices might enable the correct globular form to be produced quickly and directly.

In closing, we remark that our results are subject to the uncertainly inherent in all such conformational energy calculations due to the so-called local-minimum problem: the lack of assurance that the minimum found for the free energy of each molecule is the gobal one. Inspection of the low-energy conformations found here suggests that, in most cases, they are packed as efficiently as possible (with expections which can be understood in terms of the structure of the molecule), and that the global minimum cannot be radically different in its important features from those found here. Objective evidence for this belief comes from a consideration of the distribution of radii of gyration in the different series. In Table II we show the average and standard deviation for each series. It can be seen that the values are well separated and have reasonably small standard deviations (about 10%). More to the point, within a given series, the radii of gyration generally increase monotonically with M. The efficiency of packing is thus fairly constant within a given series, which is consistent with our belief that energies cannot be lowered significantly.

Table II
Average Radii of Gyration and Standard Deviation for the Four Series of Conformations

Series	Radius of gyration, Å	
	Av	Stand Dev
H_NC_M	5.28	0.56
$H_NC_MH_N$	6.64	0.66
E_NC_M	7.76	0.52
$E_NC_ME_N$	10.87	1.27

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References and Notes

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(2) (a) NIH Trainee, 1975–1976; NIH Postdoctoral Fellow, 1977–1978. (b) To whom requests for reprints should be addressed.
(3) M. Levitt and A. Warshel, *Nature (London)*, **253**, 694 (1975).
(4) M. Levitt, *J. Mol. Biol.*, **104**, 59 (1976).
(5) I. D. Kuntz, G. M. Crippen, P. A. Kollman, and D. Kimelman, *J. Mol. Biol.*, **106**, 983 (1976).
(6) A. W. Burgess and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1221 (1975).
(7) S. Tanaka and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 3802 (1975).
(8) O. B. Ptitsyn and A. A. Rashin, *Biophys. Chem.*, **3**, 1 (1975).
(9) D. A. Parry and E. Suzuki, *Biopolymers*, **7**, 189 (1969).
(10) D. A. D. Parry and E. Suzuki, *Biopolymers*, **7**, 199 (1969).
(11) D. N. Silverman and H. A. Scheraga, *Arch. Biochem. Biophys.*, **153**, 449 (1972).
(12) G. D. Rose, R. H. Winters, and D. B. Wetlaufer, *FEBS Lett.*, **63**, 10 (1976).
(13) A. Warshel and M. Levitt, *J. Mol. Biol.*, **106**, 421 (1976).
(14) S. S. Zimmerman, M. S. Pottle, G. Némethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).
(15) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 945 (1976).
(16) A. W. Burgess and H. A. Scheraga, *Biopolymers*, **12**, 2177 (1973).
(17) P. J. Flory, *"Statistical Mechanics of Chain Molecules,"* Wiley-Interscience, New York, N.Y., 1969, pp 251 and 252.
(18) M. Levitt and C. Chothia, *Nature (London)*, **261**, 552 (1976).
(19) A. V. Finkelstein and O. B. Ptitsyn, *J. Mol. Biol.*, **103**, 15 (1976).