

## Properties and origins of protein secondary structure

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Proteins contain a large fraction of regular, repeating conformations, called secondary structure. A simple, generic definition of secondary structure is presented which consists of measuring local correlations along the protein chain. Using this definition and a simple model for proteins, the forces driving the formation of secondary structure are explored. The relative role of energy and entropy are examined. Recent work has indicated that compaction is sufficient to create secondary structure. We test this hypothesis, using simple nonlattice protein models.

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Recently, there has been a great deal of interest in the study of proteins from a physical perspective [1–6]. Most of these works have focused on the folding problem; i.e., how does the sequence of amino acids encode the three-dimensional structure of the protein? Although progress has been made in this area, there is still a long way to go before there is a complete understanding of how proteins fold. However, proteins have many other interesting properties. While each protein has a specific structure determined by its sequence, all proteins share several common structural features. They are highly compact, with very little free internal space. More striking is the high degree of order found, which consists of regular periodic arrangements of the main chain into one of a few universal patterns (called *secondary structure*). Roughly 50% of the structure of all proteins is in some form of secondary structure [7]. In this paper we define in a simple, generic way precisely what secondary structure is. This definition will be valid not only for proteins but for simpler polymers and simple proteinlike models. We then use it to investigate what forces are responsible for the formation of secondary structure. Although this is not directly related to the folding problem, a thorough understanding of what factors are responsible for secondary structure may aid in the study of the folding problem.

There has been a great deal of past work attempting to understand the origins of secondary structure. At first it was believed that *local* interactions (local hydrogen bonds or dihedral angle potentials, for example) were responsible. Here, the term local means close with respect to the separation along the polymer chain. For example, a hydrogen bond between monomer  $i$  and  $i + 4$  would be a local interaction, as would an angle potential. Several recent studies indicate that local forces may not be the dominant effect, rather compaction of the chain may be the important factor. By examining exhaustive enumerations of short chains on a lattice, Chan and Dill [8–10] found that as the compactness of the chains increased so did the percentage of secondary structure present. They also found that the maximally compact chains had

roughly the same amount of secondary structure as real proteins and the proportions of helices to sheets was also approximately the same. Subsequently, Gregoret and Cohen [11] studied nonlattice models. Their results also suggest that compactness does influence the amount of secondary structure, but they indicate that the effect is most pronounced at densities 30% greater than that of real proteins. In both of these studies, however, local interactions were present. For example, a lattice has a specific set of allowed bond angles, which provides an effective bond angle potential. In the nonlattice work, compact chains were generated using a biased random walk in which the bond angles were chosen not from a uniform distribution but from the distribution observed in real proteins. This also provides an effective angle potential. Therefore, it is not clear from these works whether compaction is sufficient to generate secondary structure. We wish to determine whether compaction, without local interactions, is sufficient.

There are two distinct questions to keep in mind: (1) why do proteins (or other polymers) form regular structures and (2) why do proteins form particular types of secondary structure? Question (1) is equivalent to asking the following: why do proteins form helices and sheets? The second question asks the following: why are these helices  $\alpha$  helices and the sheets  $\beta$  sheets? The answer to the second question certainly involves local interactions. It is the specific hydrogen bonding patterns in proteins which favor the formation of  $\alpha$  helices. In other polymers, different local interactions would favor other forms. For example, the structures of 179 polymers have been solved and 79 are found to be in one of 22 different types of helices [9,12]. In each polymer the specific types of local interactions determine the preferred type of secondary structure. In this work we are interested in studying the first question: what forces are responsible for formation of regular structures. Specifically we will test the previous suggestions that compaction of the chain is the key driving force. To do so we will be using models without any local interactions. However, without local interactions there is no way of knowing beforehand what types of secondary structure will be formed. Most definitions of secondary structure are specific to a given type of structure (i.e.,  $\alpha$  helices); consequently one needs

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to know *a priori* what types of secondary structures will occur in order to detect their presence. To overcome this problem we developed a generic method of determining whether secondary structure is present without the need to know *a priori* what its specific form is.

A simple way of defining secondary structure is to realize that it consists of repeating patterns. Consequently the polymer chain should be correlated with itself along the chain. The correlation length should be related to the average size of secondary structures. To detect secondary structure we measure the correlations between different points along the protein chain. Specifically, let  $\theta_j$  represent the value of the dihedral angle associated with the  $j$ th  $\alpha$  carbon (see Fig. 1). We then calculate

$$C_\theta(\Delta) = \left\langle e^{i(\theta_j - \theta_{j+\Delta})} \right\rangle_C. \quad (1)$$

The average is over  $j$ ; that is, over all pairs of angles separated by a distance  $\Delta$  along the chain. The subscript  $C$  indicates that the mean,  $|\langle e^{i\theta_j} \rangle|$ , has been subtracted from  $\langle e^{i(\theta_j - \theta_{j+\Delta})} \rangle$ . If secondary structure is present then  $C_\theta(\Delta)$  will be nonzero for  $\Delta \lesssim l_{\text{avg}}$  where  $l_{\text{avg}}$  is related to the average length of secondary structure. Note, this definition makes no reference to any particular type of secondary structure; therefore, any form of regular structure will be detected. For example, if helices are present there will be a nonzero correlation length no matter what period the helices have. Equation (1) also has the advantage that it can be calculated analytically in a simple model.

To test our definition we examined the crystal structures from 112 proteins which have been recorded in the protein data bank [13]. The correlation function was calculated for each protein and normalized so  $C_\theta(0) = 1$ . Then an average correlation function was computed for all proteins. Examining this correlation function (shown in Fig. 2) we see that protein chains are positively correlated up to separations of approximately nine monomers. This is comparable to the average length of secondary structure (roughly ten monomers) measured by others [7]. At distances greater than nine monomers the chains become negatively correlated. This negative correlation may be partly due to *supersecondary* structure, which consists of combinations of secondary structural elements. For example,  $\beta$  sheets are usually followed by

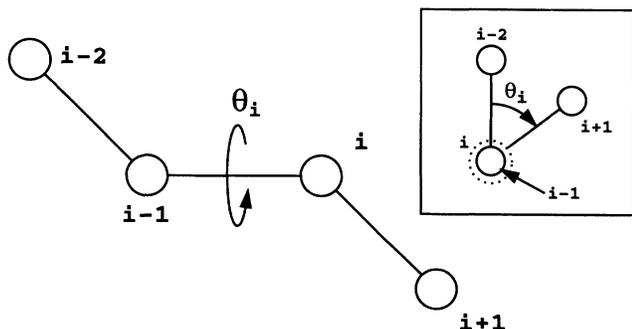


FIG. 1. The dihedral (also called torsion) angle,  $\Theta_i$ , associated with the  $i$ th monomer. The inset shows the view along the bond from monomer  $i-1$  to  $i$ . The angle shown is defined as positive by our sign convention.

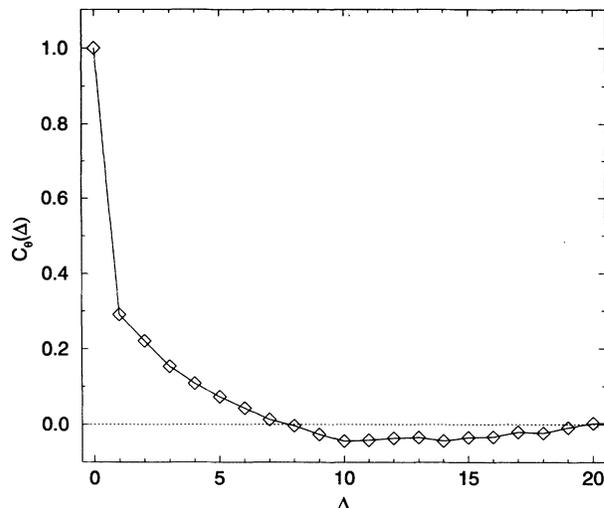


FIG. 2. Real part of the dihedral angle correlation function averaged over 112 proteins from the protein data bank. The distance,  $\Delta$ , is the number of monomers along the chain.  $C_\theta(0)$  has been normalized to one.

reverse turns. There is also the  $\beta\xi\beta$  unit where two parallel  $\beta$  sheets are separated by some piece  $\xi$  which can be a random coil, an  $\alpha$  helix, or another sheet [14]. Eventually the correlations fall off to zero (at around  $\Delta = 16$ ).

We now examine what forces drive the formation of secondary structure, specifically the question of whether the loss of entropy due to compaction is sufficient. To do this we need a model without any local interactions. Lattice models are not acceptable since the restricted degrees of freedom imply local bond angle potentials. An off-lattice model was used instead. As in lattice and other simple models we neglect the internal degrees of freedom of the amino acids and represent each as a single point in space. Monomers that are connected along the chain are constrained to be separated by a fixed distance. The next step is to fold the chains into compact conformations. The following procedure was used. Take a potential energy function whose minima are compact conformations. Then minimize this potential energy to fold the chain. Because the model we are using is a *homopolymer* there are many compact local minima (the number grows exponentially with chain length [10]). We will generate an ensemble of compact conformations, using chains of several different lengths. One can think of this ensemble of different compact structures as representing the collection of native structures of many different sequences of amino acids. We will calculate the average correlation function [Eq. (1)] of the ensemble of compact conformations we generate and look for long range correlations which will indicate the presence of secondary structure. It is important to note that the previous works showing the connection between compaction and secondary structure [8–11] also used a homopolymer model and many homopolymers show secondary structure in their compact states [12]. Therefore, it does not appear necessary to have a heteropolymer and a unique ground state to get secondary structure.

There are several different potentials that have compact minima. The dominant force for the folding of pro-

teins is the *hydrophobic effect* [15]. This is primarily a bulk, entropic effect caused by interactions of the polymer with the surrounding water. The protein collapses to create a hydrophobic core with polar groups on the surface. One could simulate a polymer in a solution of water, however, this is much more complex than necessary. Instead of doing a full water-polymer simulation we simply choose an effective potential which will also cause the polymer to collapse. The particular one used in this work was

$$V(\{\vec{r}_i\}) = \sum_{i=1}^{N-1} \frac{1}{2} k_c (|\vec{r}_i - \vec{r}_{i+1}| - l_c)^2 + \epsilon \left\{ \sum_{i < j}^N \left( \frac{\sigma_{ev}}{r_{ij}} \right)^{12} - \frac{1}{N} \sum_{i=1}^N |\vec{r}_i - \vec{r}_{com}|^2 \right\}, \quad (2)$$

where  $r_{ij} = |\vec{r}_i - \vec{r}_j|$ ,  $\vec{r}_i$  is the position of the  $i$ th monomer, and  $\vec{r}_{com} = \frac{1}{N} \sum \vec{r}_i$  is the position of the center of mass. The first term represents the covalent forces that bind the monomers along the chain. The constants  $k_c$  and  $l_c$  are both set equal to 1, determining the energy and length units. The middle term (which is the repulsive part of a Lennard-Jones potential) is the excluded volume term which prevents the chain from compacting to a single point. The last term is the radius of gyration of the chain. This term provides the compacting force. The two constants,  $\epsilon$  and  $\sigma_{ev}$ , are determined by examining real proteins. The difference in energy scales between covalent and noncovalent forces determines  $\epsilon$ . In proteins the typical noncovalent interaction is roughly one-hundredth the energy of a covalent bond, so  $\epsilon$  is set equal to 0.01 [16]. The compactness of the chains will be controlled by the value  $\sigma_{ev}$ . To determine the value of  $\sigma_{ev}$  and measure compactness we looked at two features of real protein structure: the pair-correlation function (also called the radial distribution function) and the radius of gyration. First, the pair-correlation function was measured for both real proteins and our chains. This function gives the probability that two  $\alpha$  carbons are separated by a given distance, indicating how closely the  $\alpha$  carbons are packed together. We adjusted  $\sigma_{ev}$  until the position of the nearest neighbor peak for our chains closely matched the one for real proteins [17]. Next, we measured the radius of gyration as a function of chain length for real proteins. Our chains had a slightly smaller radii of gyration as proteins the same length (see Fig. 3). This is not surprising since the potential we used will generate nearly spherical shapes while proteins are ellipsoidal with varying eccentricities. An ellipsoid will have a larger radius of gyration than a sphere of equal volume.

The chains were compacted by minimizing this potential energy [Eq. (2)]. The algorithm used was a conjugate-gradient descent minimizer [18]. At each iteration in this algorithm the energy is decreased, so it is somewhat analogous to a zero temperature Monte-Carlo simulation, in that only energy reducing steps are accepted. There is the possibility that for some potentials this type of algorithm will be trapped in local noncompact minima. However, for the potential used here, this

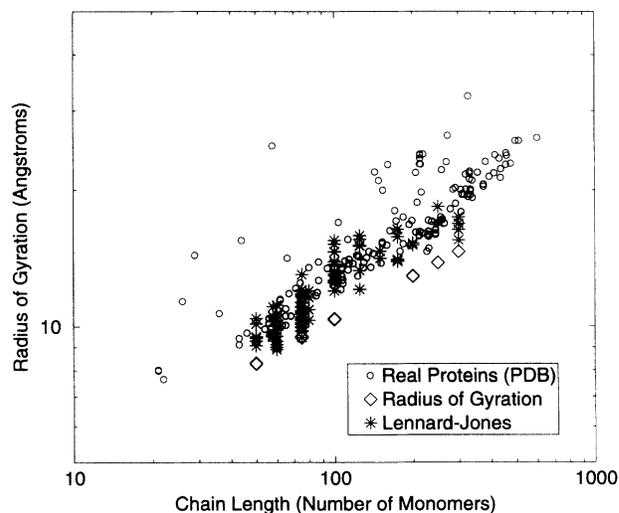


FIG. 3. The radius of gyration versus chain length (plotted on a log-log scale) for real proteins (small circles), chains compacted using the radius of gyration potential (diamonds), and the Lennard-Jones potential (stars). The radius of gyration for the three systems is very similar indicating that they all have the same level of compactness.

was not a problem. All minima that we generated were observed to be compact; i.e., their radius of gyration was roughly the same as those of proteins the same length (see Fig. 3). Starting from a random initial condition (which was taken to be a self-avoiding random walk) 200 chains, ranging in length from 50 to 450 monomers [19], were folded. The average dihedral angle correlation function was then calculated for these chains to determine if any secondary structure was present. Figure 4 shows the average for the compacted chains with the correlation function for real proteins superimposed. The compacted chains show no long range correlations. The plot falls almost immediately to zero, with a slight negative corre-

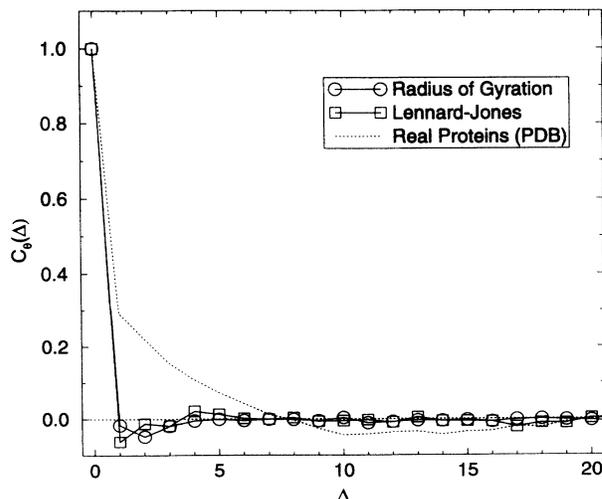


FIG. 4. The two solid lines show the correlation functions for the radius of gyration potential (circles) and Lennard-Jones potential (squares). The dotted line is the real protein correlations (from Fig. 2) for comparison.

lation at separations of roughly two monomers. This lack of any correlations indicates the absence of any secondary structure.

The potential [Eq. (2)] was chosen to have no local interactions other than the one term which bonds a monomer to its two neighbors along the chain. Again, local here means local (close) as measured along the chain, not through space. The excluded volume term is through space local, but in a folded structure any two monomers can interact via the excluded volume term regardless of their separation along the chain. In particular, there is no angle term in the potential (either implicit or explicit). The previous works which did find secondary structure with increasing compactness did have implicit angle potentials. It appears that compacting the chain is not enough to generate secondary structure. It is possible that the particular form of the compacting potential we used destroys secondary structure or was biased in favor of compact conformation without secondary structure.

To test this we tried a different compacting potential, the Lennard-Jones 6-12 potential. We replaced the radius of gyration term in Eq. (2) by a  $r^{-6}$  term to give

$$V(\{\vec{r}_i\}) = \sum_{i=1}^{N-1} \frac{1}{2} k_c (|\vec{r}_i - \vec{r}_{i+1}| - l_c)^2 + \epsilon \sum_{i < j}^N \left\{ \left( \frac{\sigma_{ev}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ev}}{r_{ij}} \right)^6 \right\}. \quad (3)$$

By itself the 6-12 potential is too short-ranged to compact an extended chain so we did a two stage minimization. At the first stage we added an additional  $1/r$  piece which is long ranged and will collapse an extended chain. Once the chain was semicompact, we finish the minimization without the  $1/r$  term. We generated an ensemble of compact chains and measured the average correlation function (see Figs. 3 and 4). Again there were no long range correlations, hence no secondary structure.

To explore the forces responsible for the formation of secondary structure in proteins we have defined a sim-

ple, generic method of measuring secondary structure in polymers. This method consists of calculating the angle correlation function along the chain and looking for long range correlations. If secondary structure is present there will be long range correlations with a length comparable to average size of the secondary structure. This method does not depend on the precise details of what type of structure is present and can be used when these details are not known. Real proteins whose structures have been solved were examined and long range correlations were found. This technique was then used to examine whether compaction leads to the formation of secondary structure. Simple models with no local interactions were used and two different compacting potentials were examined. There were no long range correlations indicating the absence of secondary structure. These results indicate that compaction by itself is not sufficient to generate secondary structure. In the previous studies demonstrating a connection between secondary structure and compaction there was always some form of local interactions present. It appears, however, that local interactions are not sufficient since compactness was also necessary to get structure. In proteins the formation of secondary structure appears to result from the combination of both the entropic effect of compaction and local energetic effects. The loss of entropy from compaction is not enough to force the chain into regular conformations. Using our definition of secondary structure further studies can be carried out to determine the relative importance of these two factors.

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