Intrachain loops in polymers: Effects of excluded volume

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(Received 20 June 1988; accepted 13 September 1988)

We develop a theory for the formation of loops and intrachain contacts in polymer molecules which are subject to excluded volume. We use two methods: (i) exhaustive simulations of chain conformations on two-dimensional square lattices, and (ii) the Edwards path integral approach. The predictions are compared to those of the Jacobson–Stockmayer theory, which neglects excluded volume. Our results show that the cyclization probability in two dimensions depends on loop length to a power between $-1.6$ to $-2.4$, in contrast to the prediction of Jacobson–Stockmayer of a power of $-1$. In addition, the cyclization probability depends on the position in the chain, and end effects are significant. A principal result of the present work is the development of "topological" correlation functions among multiple loops in a chain. If two loops are far apart along the chain, they act independently, but as they approach each other, or if they are interlinked, then one can strongly hinder or enhance the likelihood of another. For these situations, the path integral theory is an improvement over Jacobson–Stockmayer, but misses some important features of short-ranged packing effects. A particularly striking conclusion is that in the presence of the most probable first loop, the formation of a second contact is strongly preferred to be in either of only two possible conformations: a helix or an antiparallel sheet. This suggests that the basis for secondary structure formation in globular proteins may be packing and conformational freedom, rather than hydrogen bonding or other specific interactions.

I. INTRODUCTION

Chain molecules have access to a large number of conformations. Some conformations involve intrachain loops which result in self-contacts of the chain. Loop formation is important insofar as it serves as the basis for intramolecular cyclization reactions,1–3 for the stabilization of globular proteins by cross links and disulfide bonds,4–10 for DNA double-strand separation processes involved in replication,4 and possibly for "action-at-a-distance" genetic regulatory processes which arise from the coupling of interactions among enzymes bound to DNA.11 In addition, compact chains such as proteins are subject to many self-contacts, often hydrogen bonded in the form of "secondary structures." Secondary structures include helices, sheets, and turns; these are specific molecular conformations involving single or multiple loops (self-contacts). Hence intrachain loops are of broad interest in polymer science and biology.

The principal theory for intrachain cyclization has been the simple and elegant treatment of Jacobson and Stockmayer (JS), wherein the chain is assumed to obey random flight statistics and effects of excluded volume are neglected. Our purpose here is to develop a better approximation by explicit accounting for the effects of excluded volume on conformations of cyclized chains. We use two methods: (i) exhaustive simulations of chain conformations on the two-dimensional square lattice, and (ii) the Edwards path-integral approach. The combination of methods permits us to determine the generality of the predictions, with respect to two considerations: (i) what features are lattice dependent and (ii) what is the length dependence for longer chains. Two-dimensional systems explored here offer computational advantages, but most of the conclusions presented here are readily generalized to three-dimensional systems.12

This work goes beyond earlier efforts in the following respects. First, excluded volume is shown to strongly affect the equilibrium properties of loop formation, and leads to two features not predicted by simpler methods: (i) that loop formation depends on the position along the chain sequence: there are large end effects, and (ii) when there is more than one loop in a chain, there are strong effects of interactions among loops; the probability of one loop may be independent of, enhanced by, or diminished by the presence of another loop. The implications for cross-link stabilization in proteins and the formation of secondary structures are discussed.

II. METHODS

We first define some terminology. We define "connected" neighbors as pairs of monomers which are adjacent along the sequence of the chain, residues $i$ and $i+1$. We define topological neighbors as pairs of monomers which are not adjacent in the sequence: $(i,j)$, $j \neq i-1$, $i$, $i+1$, but which are adjacent in space. We refer to a "contact" as a pair of topological neighbors, indicated as a dot on the "contact map," shown in Fig. 1. The contact map is a two-dimensional matrix that indicates which monomers along the chain are neighbors. Monomers are numbered sequentially from one end of the chain; a dot at the $ith$ row and $jth$ column represents the situation that the $ith$ and $jth$ monomers are in contact. Since the contact map is obviously symmetric under row–column interchange, only the upper triangular half is shown (Fig. 1). The connected-neighbor contacts between
map description is "topological" in the sense that one particular contact map may be realizable by many different geometries of the chain, including different spatial dimensionalities. Contact maps have long provided convenient representations of secondary structures in proteins, which are represented as simple patterns, as depicted in Fig. 1(c).

For the simulations to be presented below, chains are configured on two-dimensional square lattices. The lattice representation permits discretization of the conformational space, through limitation of the possible bond angles to ±90° and 180°. Excluded volume is taken into account by the requirement that no two chain segments occupy the same lattice site.

The correspondence between the lattice geometry and the contact map is illustrated by a simple example in Fig. 1(a). Bond lengths are normalized to unity, such that the lattice sites are located at \( r = (n,m) \), where \( n \) and \( m \) are integers. The \( n \)th monomer is located at the lattice site \( r_n \), and two monomers \( i \) and \( j \) are considered to be in contact if \( |r_i - r_j| = 1 \). The contact order \( k \equiv |i - j| \) is the number of bonds between the two contacting monomers. As shown in Fig. 1(b), contacts of the same order lie along the same diagonal on the contact map.

The present problem requires that we distinguish between two types of intrachain contact: (i) those which are premises, i.e., constraints which are specified \textit{a priori}, and (ii) those which follow as inferences, contacts whose probabilities depend on which constraints are given. A specific loop \((i,j)\) is an \textit{a priori} constraint imposed by the specification of the problem; we refer to these as "presumed contacts." Other contacts, not specified in advance, \textit{a posteriori} in nature, will arise from exploration of the remaining conformational degrees of freedom; the excluded volume interactions are of this type. Presumed contacts are represented here as dots on the contact map.

Before describing the theory, it is useful to give an example of this inference process. In general, many inferences can be drawn from any given set of presumed contacts. For example, some chain contacts are absolutely prohibited by the limitations on chain conformational freedom due to presumed contacts; we refer to these as \textit{inferred blocks}. (See Fig. 2.) In two dimensions, several blocks are implied by each presumed contact. The same is true in three dimensions, but there are fewer blocks for a given set of presumed contacts in three dimensions.\textsuperscript{12}

The inferred blocks circumscribe a sort of "sphere of influence" around a presumed contact. In this regard, each dot acts like a "particle" surrounded by a potential field. As two loops (presumed contacts) approach each other along the chain, their spheres of influence overlap, and the two contacts "interact"; see Fig. 3. As constraints are brought closer along the chain, the number of implied blocks must always increase. For a given set of presumed contacts, many other types of inference are also possible. Not only can some given loops cause other to be impossible, but also certain loops can simply enhance or diminish the likelihood of others. The nature of these inferences will be investigated systematically in the following sections.

Note that only contacts between even- and odd-num-

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monomers \( i \) and \( i + 1 \) are not explicitly indicated on our contact maps herein because they are assumed to be always present and would only serve to complicate these illustrations. Only topological neighbors are shown. The contact

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FIG. 1. (a) Contact Map Description: The contact map on the right corresponds to the conformation on the left, which has topological contacts: \{ (1,4) (3,6) (3,8) (2,9) \}. (b) Contact Order: The order \( k \) of a contact is the chain length between the two monomers in contact. Note that contacts of the same order lie along the same diagonal in the contact map. (c) Contact Maps For Secondary Structures: (i) All contacts for helices are along the order 3 diagonal. (ii) and (iii) Contacts for antiparallel sheets and beta turns form strings that are perpendicular to the main diagonal. (iv) Contacts for parallel sheets form strings that are parallel to the main diagonal.
bered monomers are possible on the square lattice. Even-even and odd-odd contacts are forbidden a priori, and need not be explicitly represented as blocks; they are implicit in the diagrams. These contacts which are forbidden in the lattice model capture the essential feature that real chains are not infinitely flexible. The minimum local curvature of real chains is dependent upon the chemical nature of the monomer, which could be explored approximately through use of models of different lattice geometries. Because these effects should be of relatively minor importance, we simply consider only two-dimensional square lattices here.

In order to explore the effects of intrachain steric constraint on the conformations of chains containing loops, we have used two approaches: (i) exhaustive enumeration of all the conformations of short chains on two-dimensional square lattices, and (ii) application of the Edwards path integral approach. These two approaches are complementary. The latter is not restricted to short chains, to two dimensions, or to a specific lattice, but it is not exact. It has been shown by Freed and his colleagues that the path-integral approach gives excellent predictions of long-wavelength properties, but it is not as clearly well suited for short-ranged effects. Because they are complementary however, the combination of these methods is useful for demonstrating which predictions are universal, and which are artifacts of the path integral approximations or are dependent upon lattice details.

The conformational freedom of a chain will be limited by the presence of presumed constraints. Consequently, the total number of accessible conformations \( \Omega_0(N) \) for a chain with \( N \) bonds \((N+1)\) monomers tends to decrease with increasing numbers of presumed contacts. The precise value of \( \Omega_0(N) \) depends on the exact locations of all the presumed contacts along the chain. For free chains without any presumed contacts (but including effects of excluded volume), the number of conformations \( \Omega_0(N) \) is expected to increase approximately exponentially with \( N \). Indeed, from our exact simulation of all chains with \( N < 18 \), the limiting behavior

\[
\Omega_0(N) \sim N^{\gamma \mu^N}
\]

is obtained, where

\[
0.206 < \gamma < 0.353
\]

and \( \mu = 2.64 \) for the square lattice. In our enumeration, the two ends of a chain are considered to be distinct, and only conformations that are not related by translation, rigid rotation or reflection are counted by \( \Omega_0(N) \). The scaling \( (2.1) \) is consistent with previous simulations by other authors and with renormalization group analysis. In the present context, this formula is useful for extrapolation of \( \Omega_0(N) \) to longer chains.

III. CHAINS WITH ONE PRESUMED SELF-CONTACT

Our aim here is to understand the general principles of interaction among any set of presumed contacts. A systematic approach to this problem is to first begin at the simplest level and consider contact maps with one contact, then two, etc., as one would do in the development of an understanding of gases and liquids from the virial expansion as a function of increasing particle density.

The effect of a single presumed self-contact on chain conformational freedom may be measured by the reduction factor \( R(N;i,j) \), also referred to as the cyclization probability, which is defined below for chains with \( N \) bonds. If \( \Omega(N;i,j) \) is the number of conformations which satisfy the condition that the monomer pair \((i,j)\) forms a contact, and \( \Omega_0(N) \) is the total number of accessible conformations, the reduction factor due to a single presumed constraint is
Hence, the reduction factor may be understood as the fractional restriction of conformational space for chains which are constrained to have contact \((i, j)\). Note that the conformations in the restricted set [corresponding to \(\Omega(N; i, j)\)] subject to one constraint only—that they must all possess the presumed contact. They are otherwise restricted only by excluded volume, and the formation of any number of additional contacts is allowed.

The simplest theory to account for this reduction in conformational space is due to Jacobson and Stockmayer (JS).

They assumed random-flight statistics for the chains and neglected excluded volume. In that model, the reduction factor is predicted by

\[
R(N; i, j) = \frac{\Omega(N; i, j)}{\Omega_0(N)}, \quad N \gg k. \tag{3.1}
\]

where \(d\) is the spatial dimensionality in which the chains are configured. The tolerance \(\Delta V\) is an empirical parameter that corresponds to the volume (area in the case of two dimensions) around a monomer within which another monomer must reside to be qualified as contacting the first.

According to JS, Eq. (3.2), the reduction factor is independent of the chain length \(N\) and independent of the location of the loop along the chain. The JS theory predicts that the reduction depends only on the contact order \(k \equiv |i - j|\) and varies as \(k^{-\frac{d}{2}}\).

The effects of excluded volume significantly alter these conclusions. Using exhaustive lattice simulations, we have computed exact reduction factors for various contact orders at different locations along chains of different lengths. Of particular relevance to the end-effect phenomenon is a class of simulations in which we embedded low-order contacts in relatively long chains, corresponding to the condition \(N \gg k\). Reduction factors \(R(N; j, j)\) in the \(N \to \infty\) limit are obtained by deducing limiting values of the ratio \(\Omega(N; i, j) / \Omega_0(N)\) from simulations of successively longer chains. In this limiting process, values for all \(\Omega(N; i, j)\) are enumerated exactly; values for \(\Omega_0(N)\) are enumerated exactly for \(N < 18\), whereas values for \(\Omega_0(N)\) with \(N > 18\) are estimated by Eq. (2.1).

Figure 4 shows that end effects are large; long-chain reduction factors \(R(N; j, j)\) of a single presumed contact of order \(|j - j| = 3, 5, 7, 9, 11\) decrease significantly as the loop moves from the end \(l_0 = 0\); \(l_0\) is defined by Fig. 4(a) towards the middle of the chain. Loop formation is more strongly preferred at the chain ends than in the middle, because excluded volume is a smaller hindrance at the ends. This follows from the simple observation that the formation of a contact along a chain with excluded volume has at least two effects. First, it restricts the conformational freedom of the \(k\) segments within the loop. Second, it restricts the conformational freedom of the two tail chains as well; see Fig. 4(a). In contrast, only the first effect applies to random-flight chains. As a consequence of the second effect, the reduction factor \(R(N; i, j)\) for chains with excluded volume should exhibit dependence on the two tail lengths \(l_0 = i - 1\) and \(l_1 = N - j - 1\).

Next we consider the power-law dependence of the reduction \(R(N; i, j)\) on the contact order \(k \equiv |i - j|\); it varies approximately as \(k^{-\nu}\) for large \(k\). For chains subject to excluded volume, it is well known that the exponent \(\nu\) is somewhat larger than the value \(d/2\) (\(d/2 = 1, 3/2\) in two and three dimensions, respectively) predicted by JS [Eq. (3.2)]. For example, earlier simulations by Hiley and Sykes\(^{16}\) and Martin, Sykes, and Hioe\(^{20}\) established that for \(k = N\), \(\nu = 11/6\) in two dimensions and \(\nu = 23/12\) in three dimensions.

Our simulations confirm this power-law dependence of the cyclization probability, but our data also indicates that the exponent \(\nu\) is strongly dependent upon end effects. Figure 5 shows that for loops of order \(k\), the cyclization probability varies as \(k^{-\nu}\) with \(\nu\) ranging from 1.6 to 2.4, depending on the precise location of the cycle within the chain. In general, \(\nu\) increases with the tail length \(l_0\) for cycles in long chains. The minimum value of \(\nu\) (\(\approx 1.63\)) corresponds to the case when the contact order and the chain length are equal, \(N = k\), such that the polymer forms a ring and thus has no tails.\(^{21}\) At the other extreme, the maximum value of \(\nu\) (\(\approx 2.40\)) occurs when the loop is in the middle of a very long chain, with two tails of infinite length.

All of the results above can be collected into a more convenient representation: a topological contact free energy surface, constructed as follows. The reduction factor \(R(N; i, j)\) is computed for every \((i, j)\) contact. The contours for the quantity \(\ln R(N; i, j) + \text{const}\) are then plotted on the contact map. This quantity is a dimensionless free energy (in units of \(kT\)), due only to the chain conformational entropy, for relative formation probabilities of the different contacts.

Figures 6 and 7 show contour plots\(^{22}\) in which the principal predictions of the excluded volume simulations and the
random-flight theory are compared for chains with 14 monomers. First, given that there is one loop in the chain, both treatments predict that the smallest loops are the most strongly favored (i.e., the deepest free energy trough is adjacent to the main diagonal and is shown as the darkest region on the diagram). This arises because the smallest loops least restrict the conformations of the rest of the chain. The two principal effects of excluded volume are: (i) to change the slope of the free energy well normal to the main diagonal, i.e., the dependence on the loop length, and (ii) to cause loop formation to be most strongly favored near the ends of the chain, rather than at middle segments, since there is less hindrance due to excluded volume at the ends. The least-favored loops, shown as the lightest regions in these contour plots, are also of interest, because they indicate, for example, the contacts whose formation would lead to the maximum stabilization of globular proteins through intrachain cross linking. Unfolded proteins are in equilibrium with the globular molecules. Hence factors which most restrict the number of conformations accessible to unfolded molecules cause the greatest enhancement in relative stability of the folded molecules. Thus, the highest (lightest colored) regions in these diagrams indicate contacts which, if formed, would most stabilize the folded state. Random-flight theory predicts that the greatest conformational restriction of unfolded molecules occurs when the two ends are linked together. The principal effect of excluded volume is apparent from Fig. 6: the greatest conformational restriction occurs if the segments chosen for cross linking are a few units in from either end, in this case at (3,12) rather than at (1,14).

IV. CORRELATION AMONG CHAIN CONTACTS

In the section above, we have considered chains which contain only a single presumed contact (one loop). In this section we study the correlation between two loops. When two presumed contacts (loops) are far enough apart along the chain, they act independently, but if they are sufficiently close, their effects are correlated. If contact \((i_1, j_1)\) is of order \(k_1 = j_1 - i_1, j_1 > i_1\) and contact \((i_2, j_2)\) is of order \(k_2 = j_2 - i_2, j_2 > i_2\), we can define a topological correlation function

\[
g_{k_1, k_2}(L) = \frac{R(N_{i_1, j_1}; i_2, j_2)}{R(N_{i_1, j_1})R(N_{i_2, j_2})},
\]

where \(L = j_2 - j_1\) is the separation (along the chain) between the two contacts. This separation is the number of bonds from the starting point on the larger loop to the corresponding point on the smaller loop. Without loss of generality, we let \(k_1 > k_2\) in the above definitions. \(R(N_{i_1, j_1})\) and \(R(N_{i_2, j_2})\) are the reduction factors defined in Sec. III for chains of length \(N\) with a single presumed contact, and \(R(N_{i_1, j_1}; i_2, j_2)\) is the reduction factor for the pair of presumed contacts. Graphical definitions for \(L\) are given in Fig. 8, and the three possible topologies for chains with two contacts of order \(k_1\) and \(k_2\) are shown in Figs. 8(b) and 8(c).

The correlation function (4.1) has the following physical meaning. It is the ratio of the actual number of conformations which satisfy the two presumed contacts to the number of conformations if the two contacts were indepen-
dent. Hence, it is a measure of the degree to which one loop hinders or enhances the formation of another. This correlation function is topological insofar as it is a function of position along the chain, and is averaged over all the spatial degrees of freedom. Hence it is not a function of spatial coordinates, as are the correlation functions commonly associated with potentials of mean force which describe molecular distributions in condensed phases. In principle, \( g_{k_2,k_1} \) need not be a function only of \( L \); however, as we shall show below, \( g_{k_2,k_1} \) depends only on \( L \) for both the random-flight model, and for chains with excluded volume when both of the contacts are far from the ends of a very long chain. (Infinite tail chain limit, i.e. \( l_0 = \infty \)).

To establish a perspective for the two-contact problem, we first consider the extension of the JS theory to the prediction of these topological correlation functions. Using Eqs. (3.2) and (4.1), for a pair of contacts of different orders \( (k_2 > k_1) \) and positive separation (\( L > 0 \)), we find

\[
g_{k_2,k_1}^{(O)}(L) = \begin{cases} 
  \left[ \frac{k_2}{(k_2 - k_1)} \right]^{d/2}, & \text{for } 0 < L < k_2 - k_1 \quad \text{(region I)} \\
  \left[ 1 - \left( \frac{k_2 - L}{k_2 - k_1} \right)^2 \right]^{d/2}, & \text{for } k_2 - k_1 < L < k_2 \quad \text{(region II)} \\
  1, & \text{for } L > k_2 \quad \text{(region III)} 
\end{cases}
\]

(4.2)

Here the labels I, II, and III represent different intervals of \( L \), corresponding to the three different topologies shown in Figs. 8(b) and 8(c). The superscript 0 in \( g_{k_2,k_1}^{(O)} \) indicates that this is the random-flight result. Correlation functions for negative \( L \) as readily obtained by symmetry:

\[
g_{k_2,k_1}^{(O)}(L) = g_{k_2,k_1}^{(O)}(k_2 - k_1 + |L|), \quad L < 0,
\]

(4.3)

and intervals for negative \( L \) are labeled by applying the definition (4.2) to the quantity \( k_2 - k_1 + |L| \). When the two contact orders are equal \( (k_1 = k_2 = k) \), region I is absent, and

\[
g_{k,k}^{(O)}(L) = \begin{cases} 
  \left[ 1 - \left( \frac{|L|}{k} \right)^2 \right]^{d/2}, & \text{for } 0 < |L| < k \quad \text{(region II)} \\
  1, & \text{for } |L| > k \quad \text{(region III)} 
\end{cases}
\]

(4.4)

Note that these random-flight predictions for \( g_{k_2,k_1}^{(O)} \) are independent of the chain length \( N \). Since \( R^{(0)}(i_1,j_1;i_2,j_2) \) in Eq. (3.2) is also independent of \( N \) in the JS treatment, the random flight two-contact reduction factor \( R^{(0)}(i_1,j_1;i_2,j_2) \propto g_{k_2,k_1}^{(O)}(L) \) is also independent of \( N \). While the reduction factor \( R^{(0)}(i_1,j_1;i_2,j_2) \) depends on the empirical parameter of tolerance \( \Delta V \) introduced in Eq. (3.2), the advantage of the correlation function \( g_{k_2,k_1}^{(O)}(L) \) is that it is independent of this parameter, since the factor of \( \Delta V \) is \( R^{(0)}(N\bar{n}_i,j_1;i_2,j_2) \) in the numerator of Eq. (4.1) cancels with two factors of \( \Delta V \) in \( R(N\bar{n}_i,j_1;i_2,j_2) \) in the denominator.

Similar random-flight predictions for the two-contact probability density, which equals \( R^{(0)}(i_1,j_1;i_2,j_2) \) divided by \( \Delta V \), have been obtained previously by Yamakawa\textsuperscript{23} as an application of the Wang–Uhlenbeck–Fixman\textsuperscript{24,25} method. Poland and Scheraga\textsuperscript{26} have also considered overlapping loops (region II) in random-flight chains, but their derivation is complicated due to the choice of the volume element in their analysis. Instead of using a volume element (\( \Delta V \)) as an empirical parameter of closest approach between two contacting monomers in the three-dimensional volume of integration \( d\tau \), they introduced two parameters of tolerance—\( \Delta \) for the radial \( d\tau \) integration and \( 4\pi \delta \) for the angular integration \( \sin \theta \, d\theta \, d\phi \). Although it was not stated explicitly in their paper, the choice of fixed \( \Delta \) and \( \delta \) for a chain is equivalent to choosing a variable volume element of closest approach \( \Delta V \approx 4\pi \delta^2 \Delta \), \( \delta \) being the radial distance of the contact from the origin, since the differential volume element in spherical polar coordinates is \( r^2 \sin \theta \, d\theta \, d\phi \). Their method therefore amounts to using a volume element that depends on \( r \) and averaging it over all possible values of \( r \). This is inappropriate, however, since it does not correspond to the physical picture of chemical bonds with approximately fixed bond lengths. Clearly the allowed separation between two contacting monomers cannot depend on the distance of the contact from some arbi-

FIG. 8. Separation \( L \) between Two Contacts: (a) Contact map definition. (b) \( L \) is defined to be the separation from the starting point of the larger loop to the corresponding point of the smaller loop. (c) Chain topologies for different separations between loops.
trarily chosen origin, but the Poland–Scheraga model with fixed \( \delta \) would give such dependence, since the acceptable separation between the two monomers on the plane normal to the radial vector is of order \( \sim |k| \delta \). Models such as ours with a fixed volume of tolerance \( (\Delta V) \) for all contacts are better approximations. Since Poland and Scheraga fixed \( \delta \) by requiring that the one-contact reduction factor calculated by their method equal that calculated by fixed \( (\Delta V) \) methods, then by definition the two different methods are equivalent in the one-contact case, and \( (\Delta V) \) in Eq. (3.2) is identified with the quantity \( 4\pi r^2\Delta \) of the Poland–Scheraga method. However, their method gives different predictions for chains with more than one contact. For example, for two contacts of order \( 2k \) separated by \( L = k \), the Poland–Scheraga method gives a reduction factor \( R(i,k) = 1 + 2k_1 + k + 3k \) which is \( 80/27 \) times that of ours. The multiplying factor \( 80/27 \) is a geometrical factor caused by the averaging of the volume element \( 4\pi r^2\Delta \delta \) in their method. The corresponding multiplying factors for chains with different numbers of contacts will be different. In contrast to previous implications, we note that the Poland–Scheraga method also differs from any application of the Wang–Uhlenbeck–Fixman method which utilizes a fixed volume of tolerance.

The general behavior of the random-flight correlation functions \( g^{(0)} \) are shown in Figs. 9(a) and 9(b). The contact map for a single presumed contact in the JS approximation is shown in Fig. 9(c), which is divided into three regions according to Eqs. (4.2)–(4.4), as in the plots in Figs. 9(a) and 9(b). The salient features are: (i) The loops are independent \( (g^{(0)} = 1) \) when the separation \( L > k_2 \) (region III), where \( k_2 \) is the largest of the two contact orders. (ii) When the loops are closer than that separation, the correlation rises above unity \( (g^{(0)} > 1) \), implying the two loops favor each other. (iii) The JS correlation \( g^{(0)} \) is never less than unity, predicting that the formation of one loop never hinders the formation of another loop. In short, the random-flight theory predicts that if two loops are sufficiently close along the chain, then one loop always enhances the probability of formation of the second, because it helps restrict the remaining conformational space within which the second loop will form.

However, when excluded volume is taken into account, the predictions are significantly different. Typical results of the exhaustive lattice simulations are given in Fig. 10. Some loops are enhanced by others \( (g > 1) \), but some loops are inhibited or prevented by others \( (g < 1) \); \( g = 0 \) corresponds to implied blocks. All the correlation functions in Fig. 10 exhibit strong fluctuation at small \( L \), not predicted by JS, and two loops are independent \( (g = 1) \) only when the separation \( L \) is substantially larger than the value \( k_2 \) predicted by the random-flight model. Nevertheless, the JS model is successful in providing a qualitatively correct prediction of enhancement when one loop is completely within another (region I). A most striking conclusion from the excluded-volume simulations is that certain secondary structures are favored conformations. For example, the peak in \( g_{33} (L) \) in Fig. 10 shows that a two-dimensional lattice helix [see Fig. 1(c)] is the most favored of all conformations that have two loops of order 3.

The relative probabilities of secondary structures can be tested more broadly through use of a modification of the contour free energy plots introduced earlier. In this case one contact is taken as given, shown as a dot in Figs. 11(a) and 11(b). Subject to this presumed contact \( (i', j') \), the conditional conformational reduction factor \( R[(i,j)|(i',j')] \), which equals \( R(Ni', j'; i, j) \) in Eq. (4.1) with fixed \( (i', j') \), is computed for a second contact \( (i, j) \). The logarithm of this quantity is plotted as contours for all positions \( (i, j) \) on the contact map. We refer to this as the inferred potential surface. It shows the relative driving force for formation of all the possible second loops, for a given presumed first loop.

The inferred potential surface for a presumed contact of order 3 is shown in Fig. 11. The presumed contact \( (6,9) \) is shown as a hollow circle in these plots. Figure 11(a) shows the excluded-volume simulations and Fig. 11(b) shows the random-flight approximation. The contours of the lowest potential show the most probable inferred contact (second dot), given one presumed contact (first dot). The excluded-volume simulations show only two very strongly favored conformations: helices and antiparallel sheets; all other conformations are less favored. Thus, for a molecule proceeding to increase density from one contact to two, the favored directions are to “zip up” the helix or antiparallel sheet. The free energy difference from the least favored to the most favored second loop is quite large in this case: about 2–2.5 \( kT \). This value will be even higher for longer chains. Our cubic lattice simulation shows that these two structures are also...
FIG. 10. Correlations of Two Presumed Contacts: The first four plots are correlations between contacts of the same order, the last four plots are correlations between contacts of different orders. Square dots represent lattice simulations, while the continuous line without square dots gives random-flight predictions $g^{(2)}$. 
favored in three dimensions, but that the driving potential is smaller, since excluded volume weakens with increasing dimensionality. The random-flight theory gives a poor prediction here; the most favored conformations are the tightest loops in a parallel sheet [see Fig. 11(b)]. Those, however, are physically impossible, because they are prohibited by excluded volume.

Thus from the simulations it is predicted that helices and antiparallel sheets are the favored two-loop conformations relative to all others. These secondary structures are favored simply by excluded volume and conformational freedom, and not by any specific interactions. This prediction bears on a puzzling observation in the problem of protein structure and stability. It has long been assumed that secondary structure formation in proteins is driven by hydrogen bonding, but paradoxically it has also been widely assumed that hydrogen bonds provide little driving force for folding, since the amino acid monomers also form hydrogen bonds to water in the unfolded state. Hence the question: Why is there so much secondary structure in proteins? Clearly, the absence of intrachain hydrogen bonding would prevent protein folding, hence hydrogen bonds must be expected to be present in the folded molecule. Thus the question is: Of all the possible hydrogen-bonded conformations of the folded molecule, why do proteins adopt so few motifs, namely the regular secondary structures? The conclusion from these simulations is that secondary structures are simply favored by packing efficiency. They are the conformations which permit multiple intrachain contacts with the minimum possible loss of conformational freedom of the rest of the molecule.

V. THE PATH-INTEGRAL APPROACH: A SINGLE PRESUMED CONTACT

The simulations described above were performed for short chains on two-dimensional square lattices. The obvious limitations of that approach can be circumvented in part through adoption of the analytical path-integral approach, described below.

The method is based on the standard path-integral representation of the chain partition function $Q_0(N)$. For chains of total length $N$ and unit segment length,

$$ Q_0(N) = \int [De] e^{-\theta(N,v_0)}, \quad (5.1) $$

where the Boltzmann factor

$$ H(N,v_0) = \frac{1}{2} \int_0^N d\tau \left( \frac{dc(\tau)}{d\tau} \right)^2 $$

$$ + v_0 \int_0^N d\tau \int_0^N d\tau' \delta[c(\tau) - c(\tau')] \quad (5.2) $$

consists of two terms: The first accounts for the random-flight statistics, while the second is a simple delta-function two-body repulsion that approximates the intrachain excluded volume effects. The quantity $v_0$ in the second term is an empirical parameter that gives the strength of excluded volume interaction, and permits continuous variation from random-flight ($v_0 = 0$) to chains with excluded volume ($v_0 > 0$).

In the equations, $\tau$ and $\tau'$ are contour lengths along the chain. The vector $c(\tau)$ specifies the chain conformation through the relation

$$ c(\tau) = v_0 r(\tau). \quad (5.3) $$

The vector $r(\tau)$ describes the spatial position of the chain at the contour distance $\tau$ from one end. Here we adopt the scaled variable $c$ instead of $r$ for specification of conformational geometry because $c$ gives a more convenient formulation in which the spatial dimension $d$ does not appear explicitly. Finally, the calculation of the partition function $Q_0(N)$ is completed by integrating $e^{-\theta}$ using $[De]$ as the functional integral measure over all possible conformations.

We first apply the path-integral method to chains with a single presumed contact. More specifically, we are interested in those conformations that are consistent with a presumed contact of order $k$, that starts at a contour distance $l_0$ from one end of the chain. The spatial separation $r = r(l_0) - r(l_0 + k)$ between the two contacting points $l_0$ and $l_0 + k$ must therefore lie within a volume of tolerance $(\Delta V)$, as in the JS theory (3.2). Consequently, the total number of such conformations is given by the restricted partition function,
The second line in Eq. (5.4) results from an approximate integration over \( r_{z} \), under the assumption that the integrand does not vary significantly within the small volume \( \Delta V \), and the third line follows from the relation (5.3) above and the identity \( \delta(ax) = \delta(x)/|a| \).

A minor notational consideration arises from the changeover from a discrete model to a continuum model. Throughout this paper, contacts are identified by pairs of integers \((i, j)\) that label the two monomers in contact. We have also conformed to the conventional practice of labeling monomers sequentially starting with \( i = 1 \) from one end of the chain. As a result, the residue labeled “1” is actually situated at one end of the chain, which corresponds to zero contour length \( l_0 = 0 \). Therefore, the present situation of having two points of contour lengths \( l_0 \) and \( l_0 + k \) in contact is equivalent to the contact pair \( l_0 + 1, l_0 + k + 1 \).

As in the lattice simulations, we are primarily interested in reduction factors of presumed contacts. By our earlier definition (3.1) and Eq. (5.4), we now obtain

\[
R(N; l_0 + 1, l_0 + k + 1) = \frac{Q_r(N; l_0, l_0 + k)}{Q_0(N)}
\]  

(5.5)

as the path-integral prediction for the reduction factor of a single presumed contact. It is useful to express this as

\[
R(N; l_0 + 1, l_0 + k + 1) = (\Delta V) d^{d/2} \frac{\overline{Q}_r(N; l_0, l_0 + k)}{Q_0(N)},
\]

(5.6)

where we have defined

\[
\overline{Q}_r(N; l_0, l_0 + k) = \int \left[ \Delta e \right] \delta [c(l_0) - c(l_0 + k)] e^{-\beta H(N_{\text{iso}})}
\]

(5.7)

as the restricted partition function corresponding to the number of conformations consistent with the condition that \( l_0 \) and \( l_0 + k \) are precisely in contact. In Eq. (5.6), both \( \overline{Q}_r \) and \( Q_0 \) are functions of the excluded volume interaction strength \( v_0 \). Insofar as perturbation theory is applicable, \( \overline{Q}_r \) and \( Q_0 \) may be expanded as a power series in \( v_0 \),

\[
\overline{Q}_r = Q^{(0)}_r + v_0 Q^{(1)}_r + v_0^2 Q^{(2)}_r + \cdots, \\
Q_0 = Q^{(0)}_0 + v_0 Q^{(1)}_0 + v_0^2 Q^{(2)}_0 + \cdots,
\]

(5.8)

where \( Q^{(m)} \) is the \( m \)th order contribution. Hence, the reduction factor may also be expressed as a similar expansion.

In principle, \( R(N; l_0 + 1, l_0 + k + 1) \) may be computed to all orders in \( v_0 \), but at present we have retained only the first order contribution, which we shall discuss below in more detail.

A convenient scheme for listing different contributions to various partition functions is in terms of Feynman-type diagrams that are similar to those commonly employed in quantum field theory. There are two main advantages in using these diagrams as calculational tools: (i) complicated algebraic expressions in perturbation theory can be concisely summarized by a set of diagrammatic rules; and (ii) the pictorial representation often leads to an intuitive understanding that would otherwise be more obscure in the formalism. In our present treatment of the reduction factor (5.6, 5.9), the diagrammatic representation of the first order contribution to the unrestricted partition function \( Q_0 \) is shown in Fig. 12, in which the dotted line connects the pair of points, of contour lengths \( \tau \) and \( \tau' \) along the chain, that engage in an excluded volume interaction contact. In general, an \( m \)th order contribution is represented by a diagram with \( m \) such contacts. The corresponding first order contribution to the restricted partition function \( \overline{Q}_r \) is more complex. Due to the presence of a single presumed contact of order \( k \), the basic structure of the chain consists of a loop and two tails of lengths \( l_0 \) and \( l_0' \), as in Fig. 4(a). Excluded volume interactions are then introduced into this basic structure. As indicated in Fig. 13 by the dotted lines, there are altogether six ways for different sections of the chain to interact.

Obviously, the restricted partition function \( \overline{Q}_r \) can be generalized to more than one presumed contact by simply including more factors of \( \delta [c(l_i) - c(l_j)] \) in the path integral (5.7). In the next section, we will extend the treatment

\[
(1) \quad (2) \quad (3) \\
(4) \quad (5) \quad (6)
\]

FIG. 13. Six Feynman-type diagrams that contribute to the first order correction to the JS reduction factor.
to the case with two contacts. Here we begin our analysis by stating the general rules for extracting the contribution to a partition function from a diagram with any number of presumed contacts and any number of excluded volume interactions. They are listed below in operational steps:

(1) Set up a coordinate system by fixing one end of the chain at contour length 0 and spatial position 0. The other end of the chain is at contour length \( N = \text{total chain length} \) and a spatial vector \( c(N) \) is assigned to describe its location.

(2) Identify the contour lengths \( l \)'s of all chain sections in the diagram.

(3) As in Fig. 13, a presumed contact always creates a vertex in the diagram. We assign a different spatial vector \( c \) to each such vertex. Excluded volume interaction contacts are connected by dotted lines, the same spatial vector is assigned to the pair of points at the two ends of a dotted line.

(4) A factor \(-v_0\) is associated with each excluded volume interaction contact. This accounts for the strength of the excluded volume repulsion.

(5) To each chain section in a diagram (see Fig. 14), we associate a propagator,

\[
G_0(c - c'; l) = G_0(c' - c) = \left( \frac{1}{2\pi l} \right)^{d/2} \exp \left[ -\frac{1}{2l} |c - c'|^2 \right],
\]

which accounts for the random-flight statistics of the chain section of length \( l \) that has an end-to-end separation \( c - c' \).

(6) To sum over all possible conformations, we integrate all spatial coordinate vectors \( c \) over all space. The integrand is the product of all the above factors.

(7) Finally, to sum over all possible intrachain excluded volume interactions, each pair of contour lengths \( (\tau', \tau) \) connected by dotted lines are integrated, subject to the condition that \( \tau - \tau' > a > 0 \), where \( a \) is a short-contour-length cutoff. The introduction of this cutoff is physically justified since it amounts to the removal of the interaction of a chain segment with itself.\(^{14}\) In fact, some diagrams will be formally divergent if \( a = 0 \). However, in all the applications in this paper, the \( a = 0 \) limit can always be taken in the final expressions without creating any unphysical singularity. In order to avoid the complications associated with an extra parameter, we will present all of our results in this limit.

As an explicit example of the method, we show the key steps in the evaluation of Fig. 15, which is diagram (2) in Fig. 13. According to the rules given above, the expression corresponding to this diagram is

\[
-\frac{v_0}{2\pi} \int_0^{l_0} d\tau \int_0^{l_0} d\tau' \int dc \int dc' \int dc_1 \int dc_2 \ G_0(c; \tau') \ G_0(c_1 - c; l_0 - \tau') \times G_0(c - c_1; \tau - l_0) \ G_0(c_1 - c; k - \tau + l_0) \times G_0(c_2 - c_1; l_0') \ .
\]

Substituting the explicit Gaussian factors in Eq. (5.10) for the propagators and integrating over the spatial vector \( c, c_1, \) and \( c_2 \) in two dimensions \((d = 2)\) yields

\[
-\frac{v_0}{2\pi} \int_0^{l_0} d\tau \int_0^{l_0} d\tau' \frac{1}{(\tau(2l_0 + k - \tau) - \tau'k - l_0^2)} .
\]

Final integration over \( \tau' \) and \( \tau \) results in

\[
-\frac{v_0}{2\pi} \left[ \ln \left( \frac{\sqrt{k(4l_0^2 + k)}}{k} \right) \right. \\
\times \ln \left[ \frac{\sqrt{k(4l_0^2 + k)}}{k} \right],
\]

which is the contribution of this diagram to the restricted partition function \( \bar{Q}_N(l_0, l_0' + l_0 + k) \) in Eq. (5.7).

Other diagrams in Fig. 13 are calculated by similar methods. In two dimensions, the reduction factor (5.5,5.6) may be expressed in the form

\[
R(N, l_0 + 1, l_0 + k + 1) = \left( \frac{\Delta P}{\pi k} \right) \left[ 1 - \frac{v_0}{2\pi} f(N, l_0 + 1, l_0 + k + 1) + O(v_0^2) \right],
\]

where we find that

\[
f(N, l_0 + 1, l_0 + k + 1) = k + (N - k)\ln(N - k) - N\ln N + k\ln(l_0' + k) + \sqrt{k(4l_0^2 + k)} \ln \left[ \frac{\sqrt{k(4l_0^2 + k)}}{\sqrt{k(4l_0^2 + k)}} \right] + k(4l_0^2 + k) \ln \left[ \frac{\sqrt{k(4l_0^2 + k)}}{\sqrt{k(4l_0^2 + k)}} \right] - k)
\]

and

\[
l_0' = N - l_0 - k.
\]

Not surprisingly, the zeroth-order contribution is the JS factor (3.2).

When the first-order correction factor, \( f(N, l_0 + 1, l_0 + k + 1) \), is taken into account, the theory predicts remarkably well the end effects, the power-law dependence of cyclization on contact order, and the free energy contour surface of contact formation. Figure 16 is the contour plot of
tour plot of \[ -\ln R(N; l_0 + 1, l_0 + k - 1) + \text{const} \] for \( N = 13, (\Delta V) = 1.6 \) and \( v_0 = 0.29 \). A comparison with Fig. 6 shows that the path-integral method reproduces well the lattice results. A more detailed analysis of the factor \( f(N;i,j) \) in Eq. (5.15) indicates that the two essential features, (i) enhanced probability of cyclization near the chain ends; and (ii) existence of a peak near, but not at, the upper right corner, always appear for chains of all lengths, provided there is excluded volume \( (v_0 > 0) \). The universality of these features leads us to conclude that the various end effects described in Sec. III are not artifacts of a lattice model; instead they follow from general principles of excluded volume interactions. Moreover, these deviations from the simple JSP prediction are not limited to two dimensions. Three-dimensional cubic lattice simulations show similar behavior. We present elsewhere the analytic calculation for the three-dimensional version of the above path-integral reduction factor, which also confirms the lattice results.\(^1\)

**VI. THE PATH-INTEGRAL APPROACH: CORRELATION BETWEEN TWO PRESUMED CONTACTS**

We now extend the path-integral method to the treatment of chains with two presumed contacts. As discussed in Sec. IV and illustrated in Fig. 8, there are three different ways in which two contacts can be formed along the chain. These three basic structures (labeled I, II, and III) for the pair of contacts \((i_1,j_1), (i_2,j_2)\), which are of order \(k_1 = j_1 - i_1, k_2 = j_2 - i_2\), are shown in Fig. 17, the corresponding definitions for the contour lengths of various chain sections and the separation \(L\) between the two contacts are given in Table I.

The main purpose of this section is to give the path-integral prediction for the two-contact reduction factor and the correlation \(g_{k_1,k_2}\) between any two contacts. Following the same procedure as in the one contact case, the two-contact reduction factor is defined to be

\[
R(N;i_1,j_1;i_2,j_2) = (\Delta V)^2d^4\bar{Q}_0(N;i_1,j_1;j_2-1)Q_0(N),
\]

where

\[
\bar{Q}_0(N;i_1,j_1;i_2,j_2) = \int [D\epsilon] \delta(\epsilon(l_1) - \epsilon(l'_1))
\times\delta(\epsilon(l_2) - \epsilon(l'_2))e^{-\beta H(N;\omega)}
\]

is a restricted partition function with two pairs of points of contour lengths \(l_1, l'_1\) and \(l_2, l'_2\) precisely in contact. According to definition (4.1), the correlation function \(g_{k_1,k_2}\) can now be calculated by dividing the two-contact reduction factor (6.1) by two one-contact reduction factors (5.14) from Sec. V.

The perturbation expansion of the two-contact \(\bar{Q}_0\) follows the general method outlined in Sec. V. In particular, the

---

**TABLE I.** Definitions of the separation \(L\) between two contacts and the contour lengths of various chain sections for the three basic structures in Fig. 17.

<table>
<thead>
<tr>
<th>Region I</th>
<th>Region II</th>
<th>Region III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 &lt; L &lt; k_1 - k_1</td>
<td>k_1 - k_2 &lt; L &lt; k_2</td>
<td>L &gt; k_2</td>
</tr>
<tr>
<td>i_1 &lt; i_2 &lt; j_1 &lt; j_2</td>
<td>i_1 &lt; i_2 &lt; j_1 &lt; j_2</td>
<td>i_1 &lt; j_1 &lt; i_2 &lt; j_2</td>
</tr>
<tr>
<td>l_0 = i_1 - 1</td>
<td>l_0 = i_1 - 1</td>
<td>l_0 = i_1 - 1</td>
</tr>
<tr>
<td>l_1 = i_2 - i_1 = k_2 - k_1 - L</td>
<td>l_1 = i_2 - i_1 = k_1 - k_2 + L</td>
<td>l_1 = i_2 - i_1 = k_1 - i_1</td>
</tr>
<tr>
<td>l_2 = j_2 - j_1 = L</td>
<td>l_2 = j_2 - j_1 = k_2 - L</td>
<td>l_2 = j_2 - j_1 = L</td>
</tr>
<tr>
<td>l_3 = j_2 - j_1 = L</td>
<td>l_3 = j_2 - j_1 = L</td>
<td>l_3 = j_2 - j_1 = L</td>
</tr>
</tbody>
</table>

---

first-order correction consists of taking account of one excluded-volume contact in the three basic structures in Fig. 17. There are, however, 15 ways for each of these three basic structures to interact, resulting in a total of 45 Feynman-type diagrams (Fig. 18) that contribute to the \( O(u_0) \) term in \( Q_i(N; i_1, 1; j_1, 1; i_2, 1; j_2, 1) \). The corresponding expressions for these diagrams are then extracted by the diagrammatic rules given in the last section. As an explicit example, consider diagram (45) in Fig. 18, which is redrawn in Fig. 19 with all the relevant quantities shown explicitly. The contribution from this diagram is

\[
- \frac{u_0}{2\pi} \int_0^{\tau_k} d\tau \int_0^{\tau_k} d\tau' \int d\mathbf{c}_1 \int d\mathbf{c}_2 \int d\mathbf{c}_3 \\
\times G_0(\mathbf{c}_1 - \mathbf{c}_1\tau) G_0(\mathbf{c}_2 - \mathbf{c}_2\tau) G_0(\mathbf{c}_3 - \mathbf{c}_3\tau)
\]

where

\[
q = l_1 + l_2 + l_1 l_2,
\]

follows from random-flight statistics, and is related to the random-flight correlation \( g^{(0)} \) in Eq. (4.2) by

\[
R^{(0)} = \langle \Delta V \rangle^2 \left( \frac{d}{2\pi k_1} \right)^{d/2} \left( \frac{d}{2\pi k_2} \right)^{d/2} g^{(0)}.
\]

The first order correction \( h(N; i_1, j_1, i_2, j_2) \) in Eq. (6.6) is calculated by collecting contributions from three separate groups of 15 diagrams in the three regions. The results in two dimensions \( (d = 2) \) are
h(N_i; i_1, i_2, i_3) = \begin{cases} h_1(N_i; i_1, i_2, i_3), & \text{in region I} \\ h_2(N_i; i_1, i_2, i_3), & \text{in region II} \\ h_3(N_i; i_1, i_2, i_3), & \text{in region III} \end{cases}

(6.10)

where

\begin{align*}
   h_1(N_i; i_1, i_2, i_3) &= l_1 + l_2 + l_3 + l_2 \ln l_2 - 3(l_1 + l_3) \ln (l_1 + l_3) \\
   & \quad + (l_0 + l_2) \ln (l_0 + l_0) - N \ln N + H(l_0, l_0, l_0) + H(l_1 + l_0, l_0, l_0) \\
   & \quad + \frac{H}{2} \left( l_2 + l_0 \right) \ln \left( l_2 + l_0 \right) + \frac{H}{2} \left( l_2 + l_0 \right) \ln \left( l_2 + l_0 \right) \\
   & \quad + 2 \int_0^{l_0} d\tau \sqrt{l_1 + l_3 + 4\tau(l_2 - \tau)}/l_2 \\
   & \quad \times \ln \frac{\sqrt{(l_1 + l_3) + 4\tau(l_2 - \tau)/l_2 + \sqrt{l_1 + l_3}}}{\sqrt{(l_1 + l_3) + 4\tau(l_2 - \tau)/l_2 - \sqrt{l_1 + l_3}}} \\

(6.11a)
\end{align*}

is the correction in region I.

\begin{align*}
   h_2(N_i; i_1, i_2, i_3) &= l_0 \ln l_0 + l_0 \ln l_0 - N \ln N \\
   & \quad + \left( l_0 + \frac{l_2 + l_0}{q} \right) \ln \left( l_0 + l_0 \right) + \left( l_0 + \frac{l_2 + l_0}{q} \right) \ln \left( l_0 + l_0 \right) \\
   & \quad - \left( l_0 + \frac{l_2 + l_0}{q} \right) \ln \left( l_0 + l_0 \right) - \left( l_0 + \frac{l_2 + l_0}{q} \right) \ln \left( l_0 + l_0 \right) \\
   & \quad + \left[ \frac{1}{2} \frac{H}{l_2 + l_3} + l_0 \right] + \frac{1}{2} \frac{H}{l_2 + l_3} \\
   & \quad + \frac{1}{2} \left( \frac{q}{l_2 + l_3} + l_0 \right) \ln \left( l_2 + l_3 \right) + \frac{1}{2} \left( \frac{q}{l_2 + l_3} + l_0 \right) \ln \left( l_2 + l_3 \right) \\
   & \quad + \frac{1}{l_2 + l_3} \left[ \ln \left( \frac{l_2 + l_3}{q} + 2 \ln \left( \frac{l_2 + l_3}{q} \right) \right) \right] \int_0^{l_0} d\tau \sqrt{q + 4l_2 \tau - 4\tau^2} \\
   & \quad \times \ln \left( \frac{2\tau(l_1 + l_2(l_3 + \tau) - \tau) + l_2 \left[ q + \sqrt{q(q + 4l_2 \tau - 4\tau^2)} \right]}{2\tau(l_2 + l_2(l_3 - \tau) + l_2 \left[ q - \sqrt{q(q + 4l_2 \tau - 4\tau^2)} \right]} \right) + \{l_1 \leftrightarrow l_2 \} + \{l_1 \leftrightarrow l_3 \} \\

(6.11b)
\end{align*}

is the correction in region II, and

\begin{align*}
   h_3(N_i; i_1, i_2, i_3) &= k_1 + k_2 - 3k_1 \ln k_1 - 3k_2 \ln k_2 - N \ln N + \left( l_0 + l_0 + l_0 \right) \ln \left( l_0 + l_0 + l_0 \right) \\
   & \quad + H(k_0, l_0) + H(k_0, l_0, l_0) + H(k_0, l_0, l_0) + H(k_0, l_0, l_0) \\
   & \quad + 2 \int_0^{k_0} d\tau \sqrt{k_1 + 4l + 4\tau(l_2 - \tau)/l_2} \\
   & \quad \times \ln \frac{\sqrt{k_1 + 4l + 4\tau(l_2 - \tau)/l_2 + \sqrt{k_1}}}{\sqrt{k_1 + 4l + 4\tau(l_2 - \tau)/l_2 - \sqrt{k_1}}} \\

(6.11c)
\end{align*}

is the correction in region III. In the above equations, the function \( H \) is defined to be

\[ H(k, l, m) \equiv \left[ k - 2m + \sqrt{k(k + 4l)} \right] \]

\[ \times \ln \left[ \frac{1}{2} k - 2m + \sqrt{k(k + 4l)} \right] \]

\[ + \left[ k - 2m - \sqrt{k(k + 4l)} \right] \]

\[ \times \ln \left[ \frac{1}{2} k - 2m - \sqrt{k(k + 4l)} \right] \]

(6.12)

and the notations \( \{l_1 \leftrightarrow l_2\} \) and \( \{l_1 \leftrightarrow l_3\} \) in \( h_3 \) denote terms that result from interchanging \( l_1, l_2 \) and \( l_1, l_3 \) in the preceding expressions enclosed in curly brackets. Although three different functions represent regions I–III in the path-integral prediction of the reduction factor (6.6), there is no discontinuity at any boundary among these three regions. In fact, by utilizing the identities

\[ H(k, l, k) = -H(k, l, 0), \]

\[ H(k, 0, 0) = 2k \ln k \]

(6.13)

for the function \( H \) defined above, it is straightforward to verify that the expression \( R(N_i; i_1, i_2, i_3) \) through first-order is continuous at the boundaries between regions I and II, II and III.

We now have all the ingredients for the path-integral prediction of the correlation between two presumed contacts through \( \mathcal{O}(i_0) \). Substituting the path-integral results (6.6) and (5.14) into definition (4.1), we obtain the correlation function for two contacts \( (i_1, j_1) \) and \( (i_2, j_2) \) along chains of total length \( N \):

\[ \text{J. Chem. Phys., Vol. 90, No. 1, 1 January 1989} \]
is the separation between the two contacts, and $g^{(0)}$ is the zeroth-order correlation given in Eq. (4.2). For chains with excluded volume ($\nu_0 > 0$), the correlation $g_{k, k_i}$ is not a function of $L$ alone—in general, it also depends on the two tail lengths $l_0$ and $l_0'$. However, as in the lattice simulations of Sec. IV, we are often interested in two contacts of low orders that are embedded in the middle of a very long chain. In this limit, end effects may be neglected and attention can be focused on the effect of the separation $L$ between the two contacts. Analytically, this corresponds to taking the limits $(k_{1,2}/l_0) \rightarrow 0$ and $(k_{1,2}/l_0') \rightarrow 0$ of the correlation function (6.14), which reduces to a quadratic on $L$ that depends on $L$ alone.

Figure 20 shows the correlation functions predicted by the path-integral theory compared with the lattice simulations. Representative results for low order contacts ($k_{1,2} < 5$) that are well embedded ($l_0, l_0' > 30$) in long chains ($N = 100$) are shown. In these figures, the results of the lattice simulations and the path-integral calculations for $\nu_0 = 0, 0.1, 0.2, 0.3$ are plotted. The case $\nu_0 = 0$ corresponds to random flights, which has also been shown in Fig. 10. It is immediately apparent that a value of $\nu_0 \approx 0.2-0.3$ gives reasonable agreement with lattice simulations in regions I and III. The path-integral correlation functions always reach a local maximum at $L = (k_2 - k_1)/2$ in region I and a local minimum at $L = k_2$ at the boundary between regions II and III, which are features that have also been observed in our lattice simulations. The agreement strongly suggests that these features are not limited to lattice chains, but are more universal in that they are consequences of general principles of excluded volume interactions. However, the path-integral model fails to predict the implied blocks ($g = 0$) and other large fluctuations in region II. The disagreement is not surprising, since the simple path-integral model is not accurate in accounting for local short-ranged excluded volume. Thus while the path-integral method satisfactorily describes effects of long-ranged excluded volume, the lattice is preferred to account for effects of short-ranged excluded volume.

We also compare the path-integral result (6.6) with the lattice simulations for prediction of the conditional probabilities of formation of a second contact after the first contact has been formed. This is accomplished by fixing $i$ and $j_1$ in $R(N_{i1}, i_2, j_2)$ to reduce it to a function of the second contact ($i_2, j_2$). In Fig. 21, we show four plots of this inferred potential (see Sec. IV) for a chain with 14 monomers ($N = 13$) and a presumed contact (6.9). The contours in these plots describe the relative entropic free energy $[\log R(13; 6, 9; i, j) + \text{const}]$ of formation of the second contact ($i_2, j_2$), in units of $kT$. The plots are given with $\nu_0 = 0.04, 0.12, 0.16$, and 0.18. The series of figures shows the continuous variation of the free energy surface with increasing strength of excluded volume interactions [see also Fig. 11(b)]. As was the case for the correlation functions (Fig. 20), the path-integral results for large $\nu_0$ are in reasonable agreement with the lattice simulations [Fig. 11(a) in regions I and III [see Fig. 9(c) for definition of these regions on the contact map], but fail to predict the implied blocks in region II.

An interesting feature of these plots is the local maxima of free energy around (2.6), (2.9), (6.13), and (9.13) when $\nu_0$ is sufficiently large, implying that these contacts are disfavored by excluded volume. These same four contacts are also disfavored according to the lattice simulations. As mentioned above, local maxima of this type are of interest because they correspond to the points at which intramolecular cross linking of proteins, for example, would lead to maximum stabilization of the folded state. These maxima result from a compromise between excluded-volume repulsion between two loops on the one hand, and end effects on the other. The repulsion between two loops is attributable to the interaction indicated by the dotted line in Fig. 19. It can be shown that the contribution (6.5) from this diagram decreases (i.e., is more negative) with decreasing $l$, and reaches its minimum at $l = 0$. This behavior is responsible for the minimum at $L = k_2$ in the correlation functions (Fig. 20).
and leads to the prediction that two contacts that share a common contacting monomer, such as the pair (2,6) and (6,9), are disfavored by excluded volume. Since the single presumed contact in Fig. 21 is (6,9), it follows that all contacts in the region that contains 6 or 9 are disfavored. Furthermore, in general, contacts of higher orders are intrinsically more disfavored; therefore within this region, we would expect the contacts that are most disfavored will be those with highest order, namely (1,6), (1,9), (6,14), and (9,14). However, the situation is partially modified by another consequence of excluded volume—end effects which favor contacts that contain a chain end. The four local maxima at (2,6), (2,9), (6,13), and (9,13) can thus be understood as a compromise of these two competing factors.

Finally, we comment on some issues pertinent to the application of the path-integral model to long chains. As is well known, there is a limitation to the applicability of perturbation theory because the effective dimensionless parameter of expansion is \( z \sim v_0 N^{(1 - \phi)/2} \), and the expansion is valid only when \( z < 1 \). Therefore, \( v_0 \) is restricted to small values for long chains (\( N \) large). A small \( v_0 \) is often adequate for describing polymers near the theta point, but it may not be sufficient to account for the full excluded volume effect in other situations. We were able to circumvent this restriction in our investigation of correlation functions because we limited ourselves to small contact orders; as a result, the large \( N \) dependence cancels among the \( h \) and \( f \) functions in Eq. (6.14). However, the restriction on \( v_0 \) is unavoidable if one needs to extend the study to contact orders that are comparable to the lengths of long chains. This limitation may be surmounted by renormalization group methods. To a certain degree, the limitation on chain length is also less severe in three dimensions \( (z \sim v_0 \sqrt{N}) \) than in two \( (z \sim v_0 N) \). Work in progress on the three-dimensional version of these path-integral calculations will be presented elsewhere.

Hence insofar as these limitations of the path integral theory can be circumvented, they permit generalization to longer chains than are accessible by exhaustive lattice simulations. Even small values of \( v_0 \) begin to show the general properties of systems subject to excluded volume. Figure 22 shows an example for a chain of length 100, for which the end effects and the peak in the upper right-hand corner are already obvious even with \( v_0 = 0.03 \).

**VII. EXPERIMENTS**

Experiments are not currently available for testing the present theory. Although we have considered two-dimensional chains here, our calculations for chains in three dimensions give similar results apart from the smaller end effects in three dimensions due to the less restrictive nature of excluded volume in higher dimensions. Although many experiments on loop formation have been performed, few
have yet been designed to directly address the issues raised here. Semlyen has reviewed experiments on ring-chain equilibria in different polymer systems in which rings of different lengths have been separated and analyzed by chromatography. Those experiments were performed in theta solvents and in bulk melts where Jacobson–Stockmayer theory is expected to hold, and is observed. The principal corrections which have been tested are those of rotational isomerism state theory to account for the chemical bond angles for cycles in short chains. Semlyen notes that in the few experiments available in good solvents, the exponent \( n \) is larger than that predicted by JS; this is consistent with the present predictions. Nevertheless, these systems involve only ring molecules, chains linked at the two ends. Therefore, the currently available data permit only limited tests of predictions on effects of excluded volume, on end effects of tails connected to loops, or of multiple loops.

In biological molecules such as proteins, these more complex loops occur naturally. Recently, many experiments have appeared in which a cross link such as disulfide bond is added or removed in a protein by chemical or genetic means. It is generally observed that the added cross link stabilizes the folded state, consistent with the view that it reduces the conformational entropy of the unfolded state. However, in many of these experiments, other uncontrolled factors are also changed by the residue replacement, including packing or specific interactions or factors which cause different susceptibilities to proteolysis. Nevertheless, Pace et al. have recently tested a data set of six such experiments against the JS theory. Although there is a considerable variation in the data, they assume the validity of the JS theory exponent of \( 3/2 \) for three-dimensional chains, and fit the data set to the equation

\[
\Delta S = -\frac{3}{2} R \ln k - 2.1. \tag{7.1}
\]

However, if their data is fitted instead to an equation which makes no assumption regarding the scaling exponent, JS or any other model, where both the slope and intercept are chosen as free parameters, then we find the best fit for five of the experiments that involve breaking only one cross link to be

\[
\Delta S = -2.41 R \ln k + 4.28. \tag{7.2}
\]

Thus, this data set shows significant deviation from the random-flight prediction, the steeper slope being consistent with our excluded volume treatment of chains in two or three dimensions. This particular data set is not yet large enough, however, to provide a quantitative test of theoretical models, for the following reasons: (i) The data set includes different proteins. Intermolecular comparison is difficult due to the different specific interactions in addition to entropic stabilization by cross links. (ii) The solvent conditions for testing stability are different, including different \( pH \) and denaturant concentrations. In view of the fundamental role of solvent in determining the importance of excluded volume on conformations of chains and loops, more quantitative tests will require control of the solvent conditions. (iii) The data set includes different types of cross links: disulfide bonds and ester bonds between glutamate and alanine, for example. The volume of tolerance (\( \Delta V \)) is likely to be different for each. (iv) Some of the proteins contain overlapping multiple cross links, the effects of which have not been fully taken into account in their analysis. As shown above, correlation effects for overlapping loops can be quite large, and are different when excluded volume is important than in theta solvents. In their experiment on Ribonuclease \( T_1 \), Pace et al. measured the stabilizing effect of two overlapping loops due to two disulfide bonds connecting residues 2 to 10, and residues 6 to 103. According to the JS result given above, these loops are essentially independent. In this case, we substitute \( k_1 = 10 - 2 = 8, k_2 = 103 - 6 = 97, L = 103 - 10 = 93, \) and \( d = 3 \) into the result for region II in Eq. (4.2) to obtain the random-flight correlation result \( g^{(0)} = 1.032. \) Hence these two loops in Ribonuclease \( T_1 \) are virtually independent, the correlation free energy being only 18 cal/mol. Nevertheless, proteins commonly contain strongly overlapping loops; experimental tests of correlations among multiple loops apparently do not yet exist.

**ACKNOWLEDGMENTS**

We thank K.F. Lau, J. Naghizadeh, and D. Yee for helpful discussions. We thank the NIH, the URI Program of DARPA, and the Pew Scholars Program in the Biomedical Sciences for support of this work.

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22The "initial ring closure probability" ($p(k)$) defined in Refs. 16 and 20 is proportional to our reduction factor $R(k - 1, k)$ for a contact of order $(k - 1)$. The power-law behavior $p(k) \sim k^{-2}$ established by these authors therefore relates the reduction factor for contact order $(k - 1)$ to $k^{-2}$, in contrast to relating it to $(k - 1)^{-2}$ as adopted in this paper. Although this discrepancy should be negligible in the asymptotic regime of large $k$, the difference in definition affects the estimation of $v$ from short-chain simulations. If their definition were adopted instead of ours, data from our simulation would have given an estimation of $v = 1.83$, identical to that given in the above references, instead of the value $v = 1.63$ reported in the text.
23Many of the figures in this paper are prepared with the assistance of the (plot) program, a computer graphics package. See the following reference for details: S. F. Beebe and R. P. C. Rodgers, (Plot 79): A Comprehensive Portable Fortran Scientific Line Graphics System Based Upon the SIGGRAPH CORE Proposal, as Applied to Biomedical Research (in press).
28We arrive at the factor 80/27 for this case through use of Eq. (36) of Poland and Scheraga (Ref. 26). In so doing, we also use the relation $\delta = (4/N)(\rho/r)$ in place of their Eq. (28), which contains a very small numerical error whereby 1.06 was erroneously substituted for 1.036 in the evaluation of the quantity $[27/(8\pi)]^{1/2}$ in their Eq. (27).
29The occurrence of implied blocks in two-dimensional square lattices is always confined to localized regions on the contact map. For a single presumed contact, the implied blocks can only occur in the two regions labeled II [see Fig. 9(c) ]. Within these regions, we can determine by explicit construction the smaller area which bounds the possible extent of implied blocks. For example, for a single presumed contact $(0, m + 1)$, implied blocks in the section of region II to the upper left of the presumed contact can only occur within the area on the contact map defined by the following list of contacts: $(-2m - 3 + r + n, 2m - r)$ where $0 < n < 2m - r + 2$, $0 < r < m - 2$, $(n, m + 1)$, $-(m + 2) < r < 0$ $(n, m - r)$, where $-m + r + 1 < n < 0$, $0 < r < m - 2$. The corresponding condition for the section of region II to the lower right of the presumed contact is obtained by symmetry. We should emphasize that the above condition is only necessary but not sufficient in that no implied blocks can be found outside the defined area, but not every contact within the area is necessarily an implied block. At present, the only method we have found to determine the exact locations of implied blocks is by exhaustive simulations.