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On the Theory of Helix-Coil Transition in Polypeptides*

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The evaluation of the configurational partition function of a polypeptide molecule, with the internal rotation angles as variables, leads to an improved treatment of the phenomenon of helix-coil transition in polypeptide molecules. The conditional probabilities of occurrence of helical and coiled states of the peptide units are obtained in the form of a 3×3 matrix. The order of this matrix is the lowest possible for the model employed, and is derived by a logical procedure which serves to eliminate redundancies in the enumeration of states. The eigenvalues of this matrix yield the various molecular averages as functions of the degree of polymerization, temperature, and molecular constants. Explicit formulas are given for the degree of intramolecular hydrogen bonding, average number of helical sequences, and the distribution of their lengths, as well as the number average and the weight average of these lengths.

INTRODUCTION

THE statistical thermodynamics of the helix-coil transition in polypeptide chains has been studied extensively in recent years by a number of authors.[1–8] In the present paper we propose a solution of the same problem from a different point of view, by applying the internal rotation angles of the polypeptide chain molecule as the variables which determine the microscopic configurations of the molecule. A comparison of the other theories with the present treatment will show that the latter, being more rigorous, improves upon the former, and yet, in a sense, it is simpler. Like Zimm and Bragg,[4] we apply a matrix representation of the partition function, from which the degree of helicity and other related quantities are calculated. Our choice of the matrix elements is, however, different. Zimm and Bragg,[4] as well as Gibbs and DiMarzio[5] and Nagai,[8] consider the probability that a chain element is hydrogen bonded as being dependent on the state of the neighboring chain elements, but assume that the probability of a chain element being nonbonded is independent of the state of its neighbors. However, the probability of a chain element being nonbonded is actually smaller if its two neighbors are bonded than if they are not. Although the effect of that assumption on the final results might be small in many cases, it is not always negligible. Moreover, it appears that while eliminating this assumption, the order of the matrix of conditional probabilities can be reduced from eight in the theory of Zimm and Bragg, to three in our present treatment. This simplification makes it possible to calculate the various molecular averages of interest in terms of all three eigenvalues of the probability matrix and their corresponding eigenvectors. Thus, one obtains a solution to the polypeptide helix-coil transition problem for low as well as intermediate molecular weights, and a clearer insight into the effect of the various eigenvalues on the quasi-phase-transition in finite, one-dimensional systems.

CONFIGURATIONAL PARTITION FUNCTION

We consider a chain molecule of $n+2$ peptide units (amino acid residues), embedded in a solvent. The units are numbered, $0, 1, \cdots, i, \cdots, n, n+1$, with the zeroth and the $(n+1)$ units carrying the free carboxyl and the free amine, respectively. The peptide bonds connecting the adjacent peptide units are known to have a partial double-bond character, and are assumed to be fixed. The two other bonds of each peptide have a partial freedom of rotation, and the instantaneous values of the respective internal rotation angles (ϕ_i, ψ_i) determine the microscopic configuration of the chain (see Fig. 1). The nitrogen of the zeroth unit is assumed to form an intramolecular hydrogen bond with the oxygen of the fourth unit if, and only if, the units 1, 2, and 3

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§ On leave from the Institute of Physical Chemistry, C.S.I.C., Madrid, Spain.

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[4] (a) B. H. Zimm and J. K. Bragg, J. Chem. Phys. **28**, 1246 (1958); (b) **31**, 526 (1959).

[5] (a) J. H. Gibbs and E. A. DiMarzio, J. Chem. Phys. **28**, 1247 (1958); (b) **30**, 271 (1959).

[6] S. A. Rice and A. Wada, J. Chem. Phys. **29**, 233 (1958).

[7] T. L. Hill, J. Chem. Phys. **30**, 383 (1959).

[8] K. Nagai, J. Phys. Soc. Japan **15**, 407 (1960).

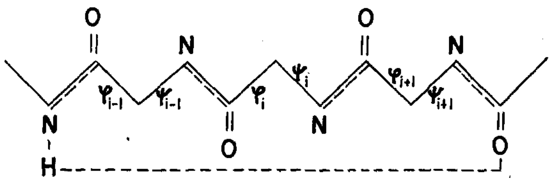

FIG. 1. Polypeptide chain, a schematic representation in the extended zigzag configuration.

are in the α-helix conformation.[9] Similarly, any pair of units $(i-2, 1+2)$ is bonded by a hydrogen bond provided the three peptide units $i-1$, i, $i+1$, are in the α-helix conformation. The energy of the intramolecular hydrogen bonds together with the interaction between the R groups of the amino acid residues, tend to stabilize the α-helix structure, while the free energy of the partial freedom of internal rotation and the solvent-polymer interactions tends in general to favor the random, i.e., coiled, conformation. By calculating the configurational partition function of the system, one obtains a quantitative measure of the interplay between these two tendencies.

The solvent polypeptide interactions may be of various kinds, including solvent peptide hydrogen-bonding. We shall not discuss them here in detail. For our purpose it is sufficient to consider the total configurational energy $V^{(n)}(\phi_1, \psi_1, \cdots, \phi_n, \psi_n)$ as a function of the $2n$ internal rotation angles, obtained after properly averaging[10] over all possible configurations of the solvent molecules, as well as over the internal rotation angles of the peptide units 0 and $(n+1)$ which do not participate directly in stabilizing the helical structure.

We shall assume that the configurational energy $V^{(n)}$ is given by

$$V^{(n)}=\sum_{i=1}^{n} V_i^{(1)}(\phi_i, \psi_i)$$
$$+\sum_{i=2}^{n-1} V_i^{(3)}(\phi_{i-1}, \psi_{i-1}, \phi_i, \psi_i, \phi_{i+1}, \psi_{1+1}). \quad (1)$$

The terms $V_i^{(1)}(\phi_i, \psi_i)$ represent the potential of hindered rotation, namely, the part of the energy of each peptide unit which is independent of other units. They also include the energy of solvent-peptide hydrogen bonds or other particular interactions whenever such interactions are present. The terms $V_i^{(3)}(\phi_{i-1}, \cdots, \psi_{i+1})$ represent the energy of formation of the helix.[11] They include the energy change accompanying the formation of a hydrogen bond between the NH group of the $(i-2)$ peptide unit and the oxygen of the $(i+2)$ unit, and also other interactions such as interactions between side chains which help to stabilize the α helix and are presumably essential in determining the right- or left-handedness of the helix.[12] The term $V_i^{(3)}$ is assumed to be different from zero if, and only if, all three pairs of internal rotation angles have the values $\phi_{i-1}^{(h)}\cdots\psi_{i+1}^{(h)}$ assigned to them in the α-helix conformation, within a narrow range of variation

$$\Delta\phi_{i-1}^{(h)}, \quad \Delta\psi_{i-1}^{(h)}, \cdots, \Delta\phi_{i+1}^{(h)}, \quad \Delta\psi_{i+1}^{(h)}.$$

The configurational partition function of the system is given by

$$Z=\int_{\phi_1=0}^{2\pi} \cdots \int_{\psi_n=0}^{2\pi} \exp[-\beta V^{(n)}]d\phi_1\cdots, d\psi_n, \quad (2)$$

where $\beta=1/kT$.

Evidently, the multiple integral of Eq. (2) cannot be factorized; however, it can be reduced to a sum of products and the summation performed with the help of matrix methods. For this purpose we divide the integration region of each pair of internal rotation angles (ϕ_i, ψ_i) into two parts: the coil region c_i and the helix region h_i. These regions are shown schematically in Fig. 2.

The integration over the coil region c_i can be performed over each pair of angles independent of the state of the adjacent peptide units, since $V_i^{(3)}=0$ holds over the entire region c_i, and we may write

$$\int_{c_i} \exp(-\beta V_i^{(1)})d\theta_i d\psi_i=u, \qquad i=1 \text{ or } n \qquad (3)$$

$$\int_{c_i} \exp(-\beta V_i^{(1)}-\beta V_i^{(3)})d\phi_i d\psi_i=u, \qquad i=2, \cdots, n-1. \qquad (4)$$

The integration over the helical region h_i does depend on the state of the adjacent peptide units. When both the $i-1$ and $i+1$ peptide units are in the helix region,

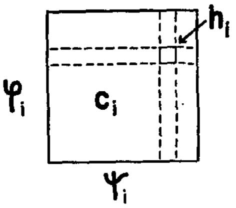

FIG. 2. Range of integration of a pair of internal rotation angles (ϕ_i, ψ_i).

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[9] L. Pauling, R. B. Corey, and H. R. Branson, Proc. Natl. Acad. Sci. U. S. **37**, 205, 241 (1951).

[10] A detailed discussion of the nature of $V^{(n)}$ for polymer molecules in general is given in a recent paper by S. Lifson and I. Oppenheim, J. Chem. Phys. **33**, 109 (1960), where $V^{(n)}$ is shown to be the potential of the average torque acting on the internal rotation angles. It has, roughly speaking, the nature of a free energy with respect to the solvent molecules and of an internal energy with respect to the intra-polypeptide interactions.

[11] The attributions of $V_i^{(3)}$ $(\phi_{i-1}\cdots\psi_{i-1})$ to the ith unit is arbitrary. We could just as well attribute it to any of the units between $i-2$ and $i+2$. It has, however, the advantage of symmetry. We shall sometimes say that the ith unit is hydrogen bonded meaning merely that $v_i^{(3)}\neq 0$.

[12] E. R. Blout, C. de Lozé, S. M. Bloom, and G. D. Fasman, J. Am. Chem. Soc. **82**, 3787 (1960).

the energy $V_i^{(3)}$ is different from zero, and we have

$$\int_{h_i} \exp(-\beta V_i^{(1)} - \beta V_i^{(3)}) d\phi_i d\psi_i = w,$$

$i=2, \cdots, n-1$; both $i-1$ and $i+1$ in the helix region. (5)

Otherwise the energy $V_i^{(3)}$ vanishes, and we have

$$\int_{h_i} \exp(-\beta V_i^{(1)} - \beta V_i^{(3)}) d\phi_i d\psi_i = v,$$

$i=2, \cdots, n-1$; either $i-1$ or $i+1$ or both are in the coil region. (6)

Similarly, integration over the helical regions of the peptide units at both ends gives always

$$\int_{hi} \exp(-\beta V_i^{(1)}) d\phi_i d\psi_i = v, \qquad i=1 \text{ or } n, \qquad (7)$$

irrespective of the state of the adjacent unit.

Consider now a compound state of the polypeptide chain defined by specifying the state of each peptide unit as being either in the helix region or in the coil region. The contribution of such a state to the partition function can be expressed as a product of n factors, each of which is either u, v, or w. As an example, let us examine a sequence with $n=15$ in the state described by

$$hhhhhcccchhchccc, \qquad (8)$$

[the units 0 and 16 were omitted; see the discussion preceding Eq. (1)], in which the first five peptide units are in the helix region, the three succeeding units are in the coil region, etc. The contribution of this state to the partition function is seen to be

$$vwwwvuuuvvuvuuu. \qquad (9)$$

The rules in constructing such terms follow directly from Eqs. (3)-(7):

(1) A peptide unit in the coil state always contributes a factor u.

(2) A peptide unit at the beginning or at the end of an uninterrupted sequence of helical states contributes a factor v.

(3) A peptide unit at the interior of an uninterrupted sequence of (more than two) helical states contributes a factor w.

The product given in (9) is proportional to the probability of occurrence of the sequence (8), the the proportionality factor being Z^{-1}. The factors u, v, and w represent *conditional* probabilities of occurrence of their corresponding events, the conditions being given by the above three rules. Since the terms u, v, and w are not normalized, only ratios between them are significant. If one of these factors is arbitrarily set equal to unity, this is equivalent to fixing arbitrarily the zero point of the energy in Eq. (1), and does by no means limit the generality of the discussion. *We shall later make use of this freedom, by setting $u=1$, and shall consider the different quantities of interest as functions of v and w only.*

Note also that, in general,

$$v \ll u \qquad (10)$$

because the region h is much smaller than the region c. Also

$$v \ll w \qquad (11)$$

because $V^{(3)}$ must be negative if a helix-coil transition is to be expected.

At this point it seems instructive to compare the set of statistical weights, or conditional probabilities, introduced by Zimm and Bragg with those employed here. Our w corresponds to their s. Also our v corresponds to their $\sigma^{\frac{1}{2}}$. This last correspondence is somewhat disguised by the fact that they attribute the factor σ to the *beginning* of a helical sequence, while we attribute a factor v, namely, $\sigma^{\frac{1}{2}}$, to both the beginning and the end of a helical sequence which, nevertheless, amounts to the same final effect. Our statistical weight of the coil region, u, however, does not correspond exactly to their statistical weight of the unbonded segment. It follows from our definitions of u, v, and w that the (unnormalized) probability of a segment to be unbonded[11] is $u+v$ if either of its neighbors is in the coil region, but it reduces to u if its two neighbors are both in the helical region. Zimm and Bragg did not distinguish between these two possibilities and attributed the same statistical weight (unity) to the unbonded segment, irrespective of the state of its neighbors. The effect of this approximation is small as long as $v \ll u$. The approximation, however, is unnecessary since the more rigorous treatment is also simpler. It should be noted that the above remarks hold, with minor differences, with respect to Gibbs and DiMarzio,[5] and Nagai.[8]

To obtain the partition function we sum over all possible terms which can be constructed following the rules 1 through 3. To do this we define a variable index ρ which stands for either h or c, and introduce the following notation

$$\int_{\phi_i=0}^{2\pi} \int_{\psi_i=0}^{2\pi} d\phi_i d\psi_i = \sum_{\rho_i=h,c} \int_{\rho_i} d\phi_i d\psi_i. \qquad (12)$$

The partition function can be written now as

$$Z = \sum_{\rho_1=h,c} \cdots \sum_{\rho_n=h,c} \int_{\rho_1} \cdots \int_{\rho_n} \exp(-\beta V_1^{(1)}) \prod_{i=2}^{n-1}$$
$$\times \exp(-\beta V_i^{(1)} - \beta V_i^{(3)}) \exp(-\beta V_n^{(1)}) d\phi_1 d\psi_1 \cdots d\phi_n d\psi_n \qquad (13)$$

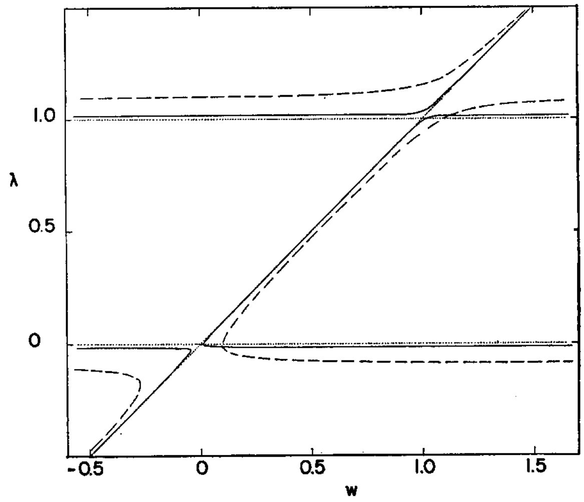

FIG. 3. Secular equation for $v=0.1$ (dashed lines) and (see footnote 15 in text) $v=0.0141$ (solid lines). The dotted lines represent the asymptotic behavior in the limit $v\rightarrow 0$.

or

$$Z=\sum_{\rho_1=h,c}\cdots\sum_{\rho_n=h,c} u_{\rho_1}w_{\rho_1\rho_2\rho_3}\cdots w_{\rho_{n-2}\rho_{n-1}\rho_n}u_{\rho_n}, \tag{14}$$

where $u_{\rho_i}(i=1 \text{ or } n)$ is either u or v according to whether ρ_i is c_i [Eq. (3)] or h_i [Eq. (7)]. Similarly $w_{\rho_{i-1}\rho_i\rho_{i-1}}$ is either u or v or w according to whether the sequence $\rho_{i-1}\rho_i\rho_{i+1}$ corresponds to the conditions of Eqs. (4), (6), or (5), respectively.

The values of $w_{\rho_{i-1}\rho_i\rho_{i+1}}$ may be represented by a 3×3 matrix $\mathbf{W}_i=\{W_{rs}\}$ in which the rows correspond to the value of $\rho_{i-1}\rho_i$ and the columns to those of $\rho_i\rho_{i+1}$:

$$\mathbf{W}_i=\{W_{rs}\}=\begin{array}{cc|ccc} & \rho_{i+1} & h & c & h\cup c\\ \rho_{i-1} & \rho_i & h & h & c\\ \hline h & h & w & v & 0\\ h & c & 0 & 0 & u\\ c & h\cup c & v & v & u\end{array} \tag{15}$$

The notation $h\cup c$ means "h or c." The third row is denoted by $(c, h\cup c)$ which means that ρ_{i-1} is determined, $\rho_{i-1}=c$, while ρ_i can be either h or c and is determined for each element in the row by the column to which it belongs. Similarly, the third column has $\rho_i=c$, while ρ_{i+1} is either h or c. As long as $\mathbf{W}_i$ is considered alone, the value of ρ_{i+1} in the third column is not determined and is also irrelevant. However, when we form, for example, the matrix product $\mathbf{W}_i\mathbf{W}_{i+1}$, elements of the third row of $\mathbf{W}_{i+1}$ always multiply elements of the third column of $\mathbf{W}_i$ and thereby the value of ρ_{i+1} becomes determined for each element of the product.

By the rules of matrix multiplication the sum in Eq. (14) is represented exactly by the product

$$Z=\mathbf{u}\mathbf{W}^{n-2}\mathbf{u}^+, \tag{16}$$

where $\mathbf{u}$ and $\mathbf{u}^+$ are, respectively, the row and column vectors

$$\mathbf{u}=(v, v, u), \qquad \mathbf{u}^+=(v, u, v+u)^*, \tag{17}$$

and represent the peptide units at the two ends of the chain. We use the asterisk to denote a transpose of a vector or matrix, but note that $\mathbf{u}^+$ is not the transpose of $\mathbf{u}$. The elements of the vector $\mathbf{u}$ correspond to the states h, h, c which are the values of the variable index ρ_{i-1} in $\mathbf{W}_i$. Similarly, the elements of the vector $\mathbf{u}^+$ correspond to the states h, c, $h\cup c$ of ρ_{i+1} in $\mathbf{W}_i$.

The introduction of the logical symbol $\cup$, as in the third row and column, serves to reduce the four possible values of $\rho_{i-1}\rho_i$ (as well as of $\rho_i\rho_{i+1}$) to three.[13] Its applicability is quite general; it helps to reduce considerably the high orders of matrices which represent the partition function of more complicated problems, such as the detailed balance between intra- and intermolecular hydrogen bonding, or between right-handed and left-handed helices when both are feasible. A detailed discussion of such systems will be published separately.

In very small chains, where the end effects are of major importance, the terms v and u in $\mathbf{u}$ and $\mathbf{u}^+$ might be considered as different from those in the interior of the chain, mainly due to the particular structure of the end groups. If, however, we do not wish to make this distinction, it appears preferable for later applications to define the row and column vectors

$$\mathbf{e}=(0,0,1), \qquad \mathbf{e}^+=(0,1,1)^* \tag{18}$$

so that

$$\mathbf{u}=\mathbf{e}\mathbf{W}, \qquad \mathbf{u}^+=\mathbf{W}\mathbf{e}^+, \tag{19}$$

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[13] Instead, we might represent $w_{\rho_{i-1}\rho_i\rho_{i+1}}$ by a 4×4 matrix. Let the rows and columns correspond to the 4 pairs *hh*, *ch*, *hc*, *cc*. The partition function is then given by

$$Z=\mathbf{u}'\mathbf{W}'^{n-2}\mathbf{u}'^+=(vuvu)\begin{pmatrix} w & 0 & v & 0\\ v & 0 & v & 0\\ 0 & u & 0 & u\\ 0 & u & 0 & u\end{pmatrix}^{n-2}\begin{pmatrix} v\\ v\\ u\\ u\end{pmatrix}.$$

To show that this expression is equivalent to Eq. (16) we introduce a similarity transformation

$$\mathbf{X}=\begin{pmatrix} 1 & 0 & 0 & 0\\ 0 & -1 & 1 & -1\\ 0 & 1 & 0 & 0\\ 0 & 1 & 0 & 1\end{pmatrix}, \qquad \mathbf{X}^{-1}=\begin{pmatrix} 1 & 0 & 0 & 0\\ 0 & 0 & 1 & 0\\ 0 & 1 & 0 & 1\\ 0 & 0 & -1 & 1\end{pmatrix},$$

and obtain $Z=(\mathbf{u}'\mathbf{X})(\mathbf{X}^{-1}\mathbf{W}'\mathbf{X})^{n-2}(\mathbf{X}^{-1}\mathbf{u}'^+)$ which is equal to $\mathbf{u}\mathbf{W}^{n-2}\mathbf{u}^+$ of Eq. (16), except that it has all 4th rows and columns zero. Since the matrix $\mathbf{W}$ is regular, there can be no further reduction of order.

and the partition function may be written as

$$Z=\mathbf{e}\mathbf{W}^n\mathbf{e}^+, \tag{20}$$

where each peptide unit is represented by a matrix **W**.

An evaluation of Z is effected by transforming **W** into a diagonal matrix **Λ** according to the transformation

$$\mathbf{T}^{-1}\mathbf{W}\mathbf{T}=\mathbf{\Lambda}. \tag{21}$$

The diagonal elements λ_1, λ_2, λ_3, of the diagonal matrix **Λ** are the eigenvalues of the matrix **W**, namely, solutions of the secular equation

$$f(\lambda;w,v,u)=(\lambda-w)(\lambda^2-u\lambda-uv)-uv^2=0. \tag{22}$$

After introducing $\mathbf{W}=\mathbf{T}\mathbf{\Lambda}\mathbf{T}^{-1}$ into Eq. (20), the partition function Z can be written as

$$Z=\mathbf{e}\mathbf{T}\mathbf{\Lambda}^n\mathbf{T}^{-1}\mathbf{e}^+. \tag{23}$$

The columns of **T** are right-hand eigenvectors of **W** which satisfy the equations $\mathbf{W}\mathbf{x}_r^*=\lambda_r\mathbf{x}_r^*(r=1,2,3)$, and are seen to be proportional to $[uv/(\lambda_r-w),u,\lambda_r]^*$; the proportionality factor will be set here as λ_r^{-1},

$$\mathbf{x}_r^*=[uv/\lambda_r(\lambda_r-w),u/\lambda_r,1]^*,\qquad r=1,2,3. \tag{24}$$

Similarly the rows of $\mathbf{T}^{-1}$ are left-hand eigenvectors of **W** which satisfy the equations $\mathbf{y}_r\mathbf{W}=\lambda_r\mathbf{y}_r$ and are given by

$$\mathbf{y}_r=[uv/\lambda_r(\lambda_r-w),(1-u/\lambda_r),u/\lambda_r]C_r. \tag{25}$$

The normalizing factor $C_r=C_r(\lambda_r)$ is determined by $\mathbf{T}^{-1}\mathbf{T}=1$ which requires $\mathbf{y}_r\mathbf{x}_r^*=1$, and is given by

$$C_r^{-1}=u^2v^2/\lambda_r^2(\lambda_r-w)^2+u(2\lambda_r-u)/\lambda_r^2, \tag{26}$$

or

$$C_r=\lambda_r^2(\lambda_r-w)/u(\lambda_r-\lambda_s)(\lambda_r-\lambda_t);\qquad r\neq s\neq t=1,2,3, \tag{26'}$$

or

$$C_r=\lambda_r^2v^2/[\mu_r^2+(2\lambda_r-u)uv^2], \tag{26''}$$

where

$$\mu_r=\lambda_r^2-u\lambda_r-uv. \tag{27}$$

The various forms of C_r are introduced for later application and are obtained by using the secular equation (22) and the symmetric functions of its coefficients

$$\sum_{r=1}^{3}\lambda_r=w+u,\qquad \sum_{r<s}^{3}\lambda_r\lambda_s=u(w-v),$$

$$\lambda_1\lambda_2\lambda_3=-uv(w-v). \tag{28}$$

By using Eqs. (24)–(26) to obtain **eT** and $\mathbf{T}^{-1}\mathbf{e}^+$,

$$\mathbf{e}\mathbf{T}=(1,1,1),\qquad \mathbf{T}^{-1}\mathbf{e}^+=(C_1,C_2,C_3)^*, \tag{29}$$

the partition function Z is finally derived, after introducing Eq. (29) into Eq. (23):

$$Z=\sum_{r=1}^{3}Z_r \tag{30}$$

with

$$Z_r=\lambda_r^nC_r. \tag{31}$$

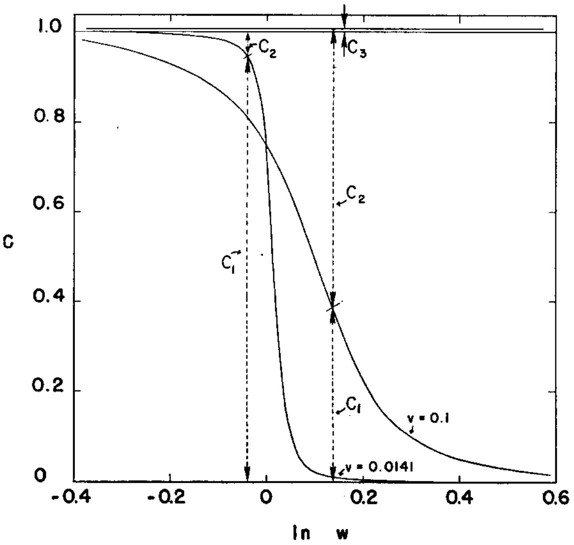

FIG. 4. Dependence of C_r on $\ln w$ for $v=0.1$ and $v=0.0141$.

A fair insight into the problem of the helix-coil transition and its relation to the eigenvalues of the probability matrix **W** can be obtained by analyzing the dependence of the three eigenvalues λ_1, λ_2, and λ_3 on the parameters w and v. We shall put $u=1$ since, as indicated before, only ratios between the parameters w, v, and u have physical significance.[14] The secular equation may be rearranged for that purpose in the form

$$w=\lambda-[v^2/(\lambda^2-\lambda-v)],\qquad (u=1). \tag{32}$$

In Fig. 3, $\lambda(w)$ is plotted from this equation, for two values of v, $v=0.1$ and $v=0.0141$.[15] The dotted lines indicate the asymptotic behavior of $\lambda(w)$ in the limit $v\to 0$. The point of intersection of the dotted lines is the point of a true sharp phase transition which may occur only in the limit $v\to 0$, as was pointed out by Zimm and Bragg.[4] The region of the actual diffuse transition is in the vicinity of that point,[16] where the values of the eigenvalues λ_1 and λ_2 deviate from their asymptotic values to an extent dependent on the value of v. In this region the difference between λ_1 and λ_2 is

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[14] The relationship between these parameters and observable quantities such as temperature or chemical potential has been discussed by other authors and will not concern us here; the significance of ln w is briefly mentioned in Eq. (33).

[15] This value was chosen for comparison with Zimm and Bragg's results (see footnote 4).

[16] The midpoint of the transition for large n is approximately $w=1+v$, $\lambda_1=1+2v$, $\lambda_2=1$. We shall refer sometimes to this point as the transition point or simply the transition.

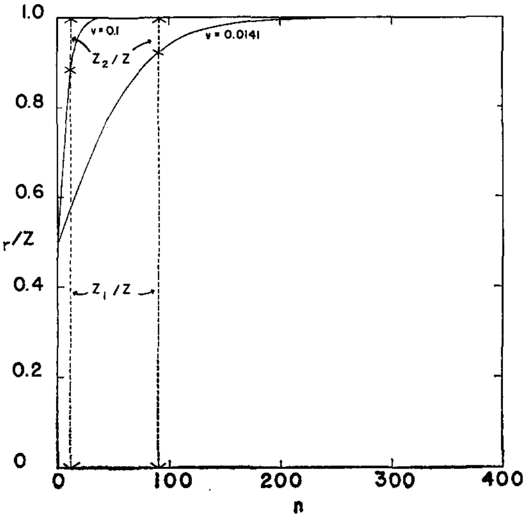

FIG. 5. Dependence of Z_1/Z and Z_2/Z on n for $v=0.1$ and $v=0.0141$ at $w=1+v$.

small, of the order of v, and the contribution of λ_2 to the partition function, the degree of helicity etc., may not be neglected unless n is very large. On the other hand, λ_3 is seen to be always small and essentially independent of w. Its contribution to the transition, therefore, is negligible except at extreme conditions of very small n and relatively large v. Since λ_3 is always negative, its main contribution to the partition function changes sign according to whether n is odd or even. A corresponding significant difference between the properties of polypeptides with odd and even numbers of peptide units evidently may occur only when n is small.

The mathematical procedure leading from the formulation of the configurational partition function, Eq. (2), to its final evaluation, Eq. (30), may be viewed as a series of transformations of the state variables which determine the state of the peptide units of the polypeptide chain. The first transformation replaced the $2n$ continuous variables ϕ_i and ψ_i by n discrete state variables, one for each unit, each with two values c and h. Represented by these variables, the partition function, Eq. (14), was given as a sum over the corresponding 2^n configurations of the whole system. When we next applied the matrix representation of the partition function, Eq. (20), we introduced in fact as our new state variables the states of the $n-1$ pairs of adjacent units, each with three values, (h, h), (h, c), and $(c, h\cup c)$. The representation of the 2^n configurations of the whole system was unaltered by this transformation. Finally, the similarity transformation **T**, which diagonalized the matrix of conditional probabilities **W**, may also be considered as a transformation to new $n-1$ state variables whose values determine the state of the n units of the system. Since the matrix of conditional probabilities is diagonal in these variables, it follows that the whole system can be found, in this representation, in one of the three states $r=1$, 2, 3 with a probability Z_r/Z for each. We shall consider Z_r as the partition function for the rth state. It is to be expected, therefore, that any average molecular property should be calculated by averaging its values over these three states, with Z_r/Z as the corresponding statistical weights. In the next section we shall derive explicitly such formulas for various average molecular properties.

The expression $Z_r=\lambda_r{}^nC_r$ suggests that λ_r is the contribution of each unit to Z_r while C_r is a factor which represents the contribution of end effects to the partition function Z_r. In Fig. 4 we see the dependence of these end effects on $\ln w$; this variable was chosen because of its correlation with the temperature, which we assume to be

$$\ln w=-\Delta H/kT+\Delta S/k. \qquad (33)$$

Here ΔH is the energy change accompanying the formation of an intramolecular hydrogen bond, and ΔS is the corresponding charge of entropy. Both ΔH and ΔS include, in general, contributions from solvent interactions. It is easy to show that $C_1+C_2+C_3=1$ and that $C_3<0$. The latter is essentially independent of w and is very small; for $v=0.1$, C_3 is less than 1% of the largest of C_1 or C_2 and for smaller values of v, such as $v=0.0141$, it is completely negligible. On the other hand, the relative importance of C_1 and C_2 depends strongly on $\ln w$; at low values of w (coil region) C_1 is near unity, whereas C_2 is small, the end effect thus being appreciable only in Z_2. On the other hand, at large values of $\ln w$ (helical region) only Z_1 is appreciably reduced by the end effect while C_2 is near unity; at the transition point, $C_1\simeq C_2$.

The three partition functions Z_r are strongly dependent on n through the factor $\lambda_r{}^n$. Even for very low values of n, the value of $\lambda_3{}^n$ is small; this together with the behavior of C_3 makes Z_3 completely negligible. The relative magnitude of Z_1 and Z_2 is determined essentially by $(\lambda_2/\lambda_1)^n$; as seen from the secular equation (22) and Fig. 3, this factor has a maximum at the transition point. The sharpness of this maximum increases and its value decreases when n and/or v increases and makes Z_2 important only in the transition region, i.e., around $w=1+v$, and even then only if n and/or v are not too large. In Fig. 5 the values of Z_1/Z and Z_2/Z at $w\cong 1+v$ (where $C_1=C_2$) are plotted vs n, which show the change of their relative magnitude with increasing n for $v=0.1$ and for $v=0.0141$. The value of n at which Z_2 is negligible within a specified degree of approximation can be calculated as follows, according to Eqs. (30), (31), and (26′):

$$Z\cong Z_1[1+(\lambda_2/\lambda_1)^n(C_2/C_1)]$$
$$\cong Z_1[1+(\lambda_2/\lambda_1)^{n+1}(w-\lambda_2)/(\lambda_1-w)], \qquad (34)$$

if we assume that $Z_3 \cong 0$ and that $(\lambda_1-\lambda_3)/(\lambda_2-\lambda_3) \cong \lambda_1/\lambda_2$. The maximum of $(\lambda_2/\lambda_1)^{n+1}$ occurs at the transition point but that of the bracket is shifted slightly toward larger w since C_2/C_1 is an increasing function of w as can be seen from Fig. 4. This shift is very small, though; at a point such as $\lambda_1 \cong 1+3v$, $(w \cong 1+\frac{5}{2}v$, $\lambda_2 \cong 1+\frac{1}{2}v)$, at which $(w-\lambda_2)/(\lambda_1-w) \cong \frac{4}{5} \cong 1$ the derivative $\partial(Z_2/Z_1)/\partial w$ is already negative for any value of v and n. Then,

$$(Z_2/Z_1)_{\max} \cong (\lambda_2/\lambda_1)_{\max}{}^{n+1} \cong (1+2v)^{-n} = \exp[-n\ln(1+2v)] \cong e^{-2nv}. \tag{35}$$

Therefore, to have $(Z_2/Z_1)_{\max} < 0.01$, n has to be such that $nv > 2.3$. For $nv \geq 3$, the term Z_2/Z_1 is negligible within an error of less than 1/300, and one might adopt this as a criterion for the negligibility of Z_2, i.e.,

$$Z_2 \cong 0, \qquad Z_1 \cong Z, \qquad \text{if } nv \geq 3. \tag{36}$$

To sum up the above discussion, we observe that among the three terms which comprise Z, one, Z_3, is practically always negligible. Among the others, Z_2 contributes to the partition function only in the transition region, i.e., in the vicinity of $w=1+v$, and provided $nv<3$; the effect of the ends of the chain, through C_2/C_1, is negligible at the coil region, but tends to increase slightly the relative weight of Z_2 at the helix side of the transition, within the narrow range $1+v \leq w \leq 1+2.5v$. Beyond this range Z_2 is negligible, unless nv is significantly smaller than 3.

MOLECULAR AVERAGES

Degree of Intramolecular Hydrogen Bonding

The average number of intramolecular hydrogen bonds, $\bar{n}_{\text{bond}}$, and the relative number or degree of hydrogen bondings, $\theta=\bar{n}_{\text{bond}}/n$, are both functions of n, w, and v (we set $u=1$ without loss of generality). By standard methods of statistical mechanics we have

$$\theta = n^{-1}\partial \ln Z/\partial \ln w. \tag{37}$$

On using Eq. (30) we may write

$$\theta = \sum_{r=1}^{3} \theta_r Z_r/Z, \tag{38}$$

where θ_r is given by

$$\theta_r = n^{-1}\partial \ln Z_r/\partial \ln w. \tag{39}$$

Thus, it appears that θ_r is the same function of Z_r as θ is of Z and may, therefore, be considered as the degree of intramolecular hydrogen bonding for the rth state. Equation (38) then gives θ as the average value of θ_r with statistical weights Z_r/Z, as pointed out in the preceding section. After introducing Eq. (31) into Eq. (39) we obtain

$$\theta_r = \partial \ln\lambda_r/\partial \ln w + n^{-1}\partial \ln C_r/\partial \ln w \equiv \theta_r{}^0[1+n^{-1}\theta_r{}'], \tag{40}$$

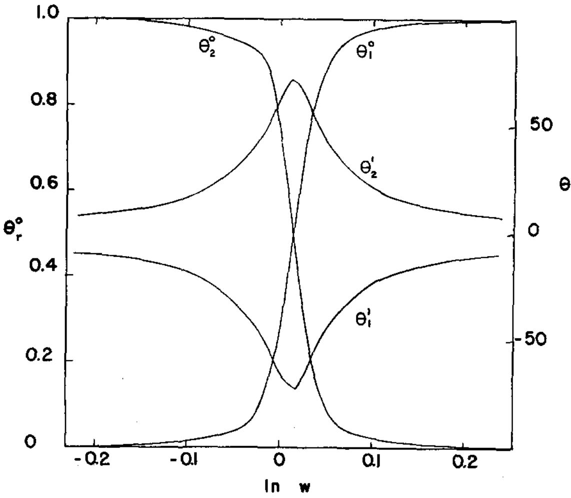

FIG. 6. Dependence of $\theta_1{}^0$, $\theta_2{}^0$, $\theta_1{}'$, and $\theta_2{}'$ on $\ln w$ for $v=0.0141$.

where the last term, $n^{-1}\theta_r{}'$, is a correction due to end effects, linear in n^{-1}, whereas $\theta_r{}^0$ represents the limiting value of θ_r for large n. The explicit expressions for $\theta_r{}^0$ and $\theta_r{}'$ are easily found from Eqs. (40), (26″), (27), and (22):

$$\theta_r{}^0 = \frac{\mu_r}{\lambda_r}\,\frac{\lambda_r\mu_r - v^2}{v^2(2\lambda_r-1)+\mu_r{}^2}$$

$$\theta_r{}' = 2\left[1-\lambda_r\frac{\mu_r(2\lambda_r-1)+v^2}{v^2(2\lambda_r-1)+\mu_r{}^2}\right]. \tag{41}$$

Thus, we have $\theta_r{}^0$ and $\theta_r{}'$ in terms of v and $\lambda_r(w, v)$, i.e., as functions of the variables of physical interest, v and w. The molecular weight dependence of θ is represented explicitly by the factors Z_r/Z in Eq. (38) and the factor n^{-1} in Eq. (40), while $\theta_r{}'$ are independent of n. As Z_3 is usually negligibly small, only $\theta_0{}^0$, $\theta_1{}'$, $\theta_2{}^0$, and $\theta_2{}'$ will be considered. In Fig. 6 these functions are plotted vs $\ln w$, with $v=0.0141$. It is seen that the contributions of $\theta_2{}^0$ and $\theta_2{}'$ to θ counteract those of $\theta_1{}^0$ and $\theta_1{}'$, respectively, $\theta_2{}^0$ tends to zero when $\theta_1{}^0$ tends to one, and vice versa, while $\theta_2{}'$ and $\theta_1{}'$ have opposite signs.[17] It should be noted also that $\theta_2{}'$ and $\theta_1{}'$ are rather large in absolute value in the transition region, and increase with decreasing v.

We can now see in a more quantitative fashion at which value of n and v can $\theta_2{}^0$ and the end effects be considered negligible within a certain error. Equation (38) can be rewritten as

$$\theta = \theta_1 \frac{1+\theta_2 Z_2/\theta_1 Z_1}{1+Z_2/Z_1}. \tag{42}$$

By using Eqs. (40), (41), (31), (26″), (22), and (28) and by approximating again $(\lambda_1-\lambda_3)/(\lambda_2-\lambda_3)$ by

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[17] In fact, it can be shown rigorously that $\Sigma_r\theta_r{}^0=1$, and that $\theta_3{}^0$ and $\theta_3{}'$ are relatively negligible, thus $\theta_2{}^0 \cong 1-\theta_1{}^0$, $\theta_2{}' \sim -\theta_1{}'$.

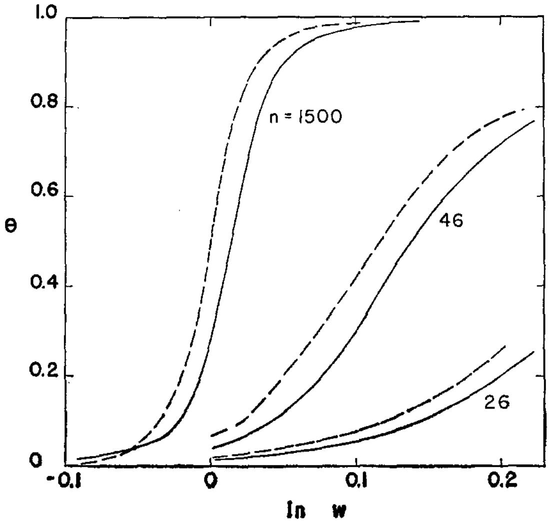

FIG. 7. Dependence of the degree of intramolecular hydrogen bonding, θ, on $\ln w$ for $v=0.0141$ and $n=26$, 46, and 1500. Comparison of the present theory (solid lines) with that (see footnote 4 in text) of Zimm and Bragg (dashed lines).

λ_1/λ_2 we have

$$\frac{\theta_2 Z_2}{\theta_1 Z_1}\cong(\lambda_2/\lambda_1)^n\frac{n(\lambda_1-\lambda_2)+2\lambda_2}{n(\lambda_1-\lambda_2)-2\lambda_1}. \tag{43}$$

The last fraction shows a maximum near the midpoint of the transition $w\cong1+v$ and tends to unity with increasing values of n and/or v since then $n(\lambda_1-\lambda_2)$ becomes larger. The maximum value of the whole expression, θ_2Z_2/θ_1Z_1, then lies quite near the point $w\cong1+v$, as can be shown by making $\partial(\theta_2Z_2/\theta_1Z_1)/\partial w=0$. The condition for maximum is, after minor simplifications,

$$\lambda_1=(\lambda_1+\lambda_2)(\partial\lambda_1/\partial w).$$

This equation cannot be solved analytically for w but it can be shown that the solution lies in the narrow range: $(1-\frac{1}{2}v)<w<1+v$ in which the last fraction in Eq. (43) is very close to 2, if $nv=3$. Then, according to Eq. (35) and its discussion,

$$(\theta_2Z_2/\theta_1Z_1)_{\max}\cong2e^{-2nv}$$

and

$$\theta=\theta_1=\theta_1^0(1+n^{-1}\theta_1') \quad \text{if } nv\geq3, \tag{44}$$

within an error of less than 1%.

The influence of the end effects, included in θ_1', however, is still important and vanishes only at relatively large values of n, at which $n^{-1}\theta_1'\cong0$. The actual value of n at which that happens is easily found from Eqs. (40), (41), and the secular equation; according to them,

$$\theta_1=\theta_1^0\left(1-\frac{2\lambda_1^2}{\lambda_1-\lambda_3}\frac{1}{n(\lambda_1-\lambda_2)}\right).$$

Obviously the maximum value of the fraction will occur at the transition, where $\lambda_1-\lambda_2\cong2v$, as seen in Fig. 6. Then, when $1/nv$ is negligible compared with 1, Eq. (38) becomes

$$\theta=\theta_1^0 \quad \text{if } nv\gg1, \tag{45}$$

that is, θ reaches its limiting value. This result could be obtained directly from Eq. (37) if we assume that, at high values of n, $\ln Z\cong n \ln\lambda_1$.

As a summary of the results, θ has been plotted vs $\ln w$ in Fig. 7 for $v=0.0141$ and several values of n together with the results of Zimm and Bragg,[4] (for $\sigma=2\times10^{-4}$ in their notation). Except for a shift in the scale of $\ln w$, the agreement between the two theories in fairly good. While in our theory the transition point is at $w=1+v$, in the theory of Zimm and Bragg it occurs at $w=1$. The difference can be traced to the different ways of assigning conditional probabilities, which has been discussed in the preceding section.

The experimental data of Doty and Young,[18] and Zimm, Doty and Iso,[19] on the optical rotation of poly-γ-benzyl-L-glutamate were analyzed according to our theory, by using Eq. (33) to determine the relation of $\ln w$ to T. We obtain $\Delta H=950$ cal/mol, which is comparable to $\Delta H=850$ cal/mol obtained by Zimm, Doty, and Iso.

Average Number of Helical Sequences and Number-Average Length of a Sequence

When the degree of hydrogen bonding θ is different from its extreme values 0 and 1, the question arises how strong is the tendency of helical states to group into long sequences. The smaller the average number of sequences for a given θ, the longer is the average length of a sequence. Let $\bar{n}_{\text{seq}}$ denote the average number of helical sequences having at least 2 helical states in a sequence, then the number-average length $\bar{l}_n$ of a helical sequence is given by

$$\bar{l}_n=(\bar{n}_{\text{bond}}+2\bar{n}_{\text{seq}})/(n)_{\text{seq}}=\theta/\eta+2, \tag{46}$$

where $\eta=\bar{n}_{\text{seq}}/n$. The term $2\bar{n}_{\text{seq}}$ represents the two nonbonded units in the helical state which start and end each sequence.

To obtain η (or $\bar{n}_{\text{seq}}$), we make the following observation. In the matrix **W**, Eq. (15), the three elements (31), (12), and (32), though having the same value v, represent three distinguishable states of nonbonded helical units. Let us denote them, respectively, by v_{31}, and v_{12}, and v_{32}. Then v_{31} belongs to a peptide unit which starts a helical sequence. This is evident from the values of $\rho_{i-1}\rho_i\rho_{i+1}$ for this element, namely chh. Similarly v_{12} belongs to a peptide unit at the end of a helical sequence because it is determined by $\rho_{i-1}\rho_i\rho_{i+1}=hhc$, while v_{32} corresponds to $\rho_{i-1}\rho_i\rho_{i+1}=chc$ and, there-

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[18] P. Doty and J. T. Yang, J. Am. Chem. Soc. **78**, 498 (1956).

[19] B. H. Zimm, P. Doty, and K. Iso, Proc. Natl. Acad. Sci. U. S. **45**, 1601 (1959).

fore, belongs to a lonely helical state. Since each helical sequence of two or more units has one beginning and one end, the average number of v_{31}, denoted by $\bar{n}_{31}$, equals the average number $\bar{n}_{12}$ of v_{12} and the average number of sequences, $\bar{n}_{\mathrm{seq}}$,

$$\bar{n}_{12}=\bar{n}_{31}=\bar{n}_{\mathrm{seq}}. \qquad (47)$$

To obtain these averages we distinguish between the three v's in the secular equation (22), and thus have

$$f(\lambda; w, v_{12}, v_{31}, v_{32}) = (\lambda-w)(\lambda^2-\lambda-v_{32})-v_{12}v_{31}=0. \qquad (48)$$

Now λ_r and Z are functions of the different v's and we have

$$\eta=\bar{n}_{12}/n=n^{-1}\partial \ln Z/\partial \ln v_{12}. \qquad (49)$$

By following the same reasoning which led from Eqs. (37) to (40), we have

$$\eta=\sum_{r=1}^{3}\eta_r Z_r/Z, \qquad (50)$$

where

$$\eta_r=\eta_r^0(1+n^{-1}\eta_r'). \qquad (51)$$

Again η_r represents the relative number of helical sequences for the rth state. Explicit expressions can be obtained by using Eqs. (51), (26″), (27), (22), and (41):

$$\eta_r^0=\frac{v^2\mu_r}{\lambda_r[v^2(2\lambda_r-1)+\mu_r^2]}=\theta_r^0[(\lambda_r-w)/w]$$

$$\eta_r'=\theta_r'+(\lambda_r\mu_r/v^2). \qquad (52)$$

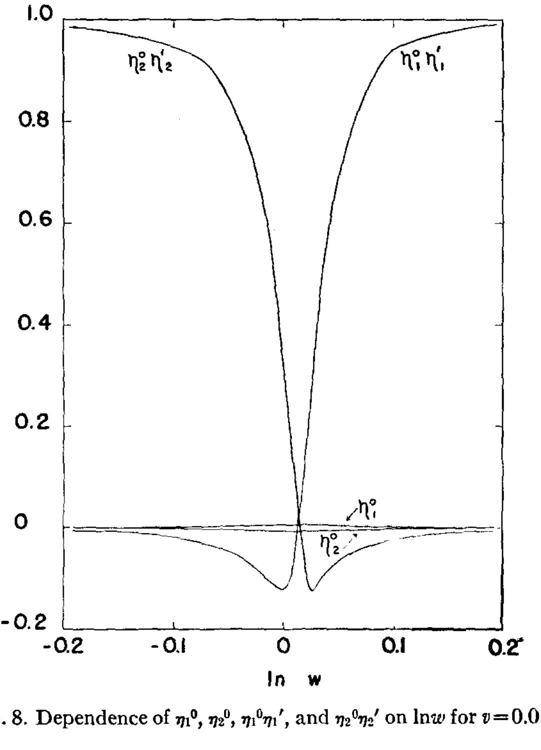

FIG. 8. Dependence of η_1^0, η_2^0, $\eta_1^0\eta_1'$, and $\eta_2^0\eta_2'$ on $\ln w$ for $v=0.0141$.

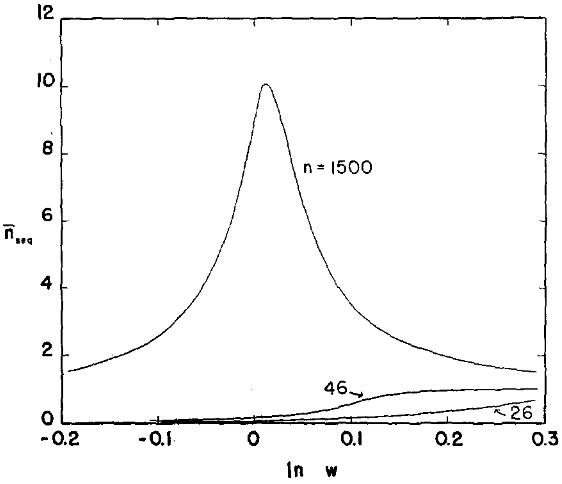

FIG. 9. Dependence of the average number of helical sequences, $\bar{n}_{\mathrm{seq}}$, on $\ln w$ for $v=0.0141$ and $n=26$, 46, and 1500.

We neglect η_3 since its statistical weight Z_3/Z is very small; therefore, only η_1 and η_2 have to be considered. According to Eq. (52) η_r^0 is always small, because when θ_r^0 tends to unity, $(\lambda_r-w)/w$ tends to zero. Both η_1^0 and $-\eta_2^0$ have a shallow maximum in the transition region, as seen in Fig. 8. On the other hand η_r' undergoes very large changes. For example, η_1' increases indefinitely with increasing w, because of the term $\lambda_1\mu_1/v^2$, while η_1^0 tends at the same time to zero. It appears more instructive, therefore, to consider the molecular weight dependence of η_r through the product $\eta_r^0\eta_r'$ which is plotted vs $\ln w$ in Fig. 8. We see that $\eta_1^0\eta_1'$ and $\eta_2^0\eta_2'$ are approximately symmetric with respect to a line through the transition point. At the extreme helical region, when θ tends to unity, $\eta_1^0\eta_1'$ tends also to unity and η tends to $1/n$; namely, the molecule consists of a single helical sequence. In following arguments similar to those used in the discussion of θ, we find that η is given to a good approximation by η_1 provided nv is of the order of three or larger,

$$\eta=\eta_1=\eta_1^0(1+n^{-1}\eta_1') \quad \text{for } nv\geq 3. \qquad (53)$$

As a summary of these results, the average number of helical sequences, $\bar{n}_{\mathrm{seq}}$, is plotted in Fig. 9 vs $\ln w$ for $v=0.0141$ and $n=26$, 46, and 1500. All three curves tend to zero in the coil region, and to unity (single, unbroken helix) in the helical region, but only when the molecular weight is high is the average number of helical sequences significantly larger than one, and its maximum value occurs in the transition region. The number average length of a helical sequence [Eq. (46)] increases with increasing helicity monotonously and smoothly irrespective of whether η has a maximum in the transition region or not. This is seen in Fig. (10), where $\bar{l}_n/n$ is plotted vs $\ln w$ with the values of n and v the same as in Fig. 9.

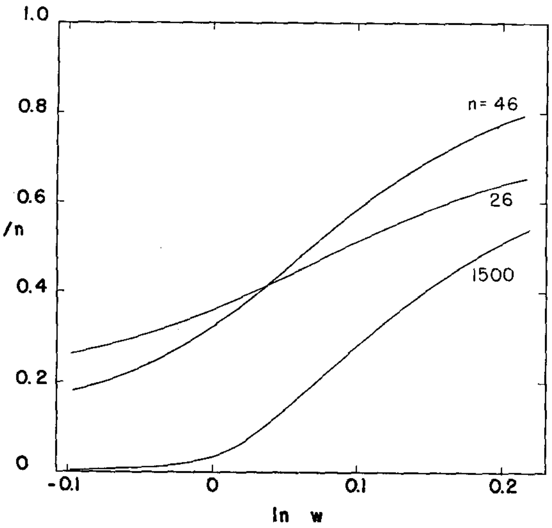

FIG. 10. Dependence of the relative number average length of a helical sequence, $\bar{l}_n/n$ on $\ln w$ for $v=0.0141$ and $n=26$, 46, and 1500.

Distribution of Lengths of Helical Sequence

We now ask what is the average number $\bar{n}_x$ of helical sequences of length x (i.e., sequences of x peptide units) for a molecular chain of n units, and given values w and v. To answer this and other similar questions we need a more general method of averaging over molecular properties than that used in the preceding examples. We shall now derive such a method, which is a modification of a method used by one of us[20] in calculating average dimensions of polymers, and which takes advantages of the simplicity of the matrix representation of the partition function.

It was observed before, that the three v's in the matrix **W** represent three distinguishable states of nonbonded helical units. The same is true for each element of the matrix, i.e., the corresponding state is determined by the sequence $\rho_{i-1}\rho_i\rho_{i+1}$ to which it belongs. For example, the element u_{23} [in position (23)] starts a random sequence, the element u_{33} continues such a sequence while w evidently belongs to a bonded unit in the interior of a helical sequence. (A better distinction between different u's is obtained when the 4×4 matrix representation[13] is followed.)

We ask now, what is the probability $p_{rs}(i)$ that a particular peptide unit, the ith, will be found in one of the above-mentioned positions. It should be clear that the answer cannot be u, v, or w since these are conditional probabilities determined by the unit under consideration *and* its neighbors, while we are interested in the probability of finding the ith element in a given state (rs) irrespective of, and therefore averaged over, all the admissible positions of all other peptide units.

The probability of finding the whole chain in a specified state, $\rho_1\cdots\rho_n$, is given by the summand in Eq. (14). To obtain $p_{rs}(i)$ we fix the ith unit in the state (rs) and sum over all the states of the $i-1$ units on the left and the $n-i$ units on the right, which are consistent with the state (rs) of the ith unit. In matrix notation,

$$p_{rs}(i)=\mathbf{e}\mathbf{W}^{i-1}(W_{rs}\mathbf{E}_{rs})\mathbf{W}^{n-i}\mathbf{e}^{+}/Z, \tag{54}$$

where W_{rs} is the element (rs) of **W**, and $\mathbf{E}_{rs}$ is a 3×3 matrix with the digit one in position (rs) and zero elsewhere. Note that $\mathbf{E}_{rs}$ does not commute with **W**. The factor $W_{rs}\mathbf{E}_{rs}$ represents our requirement that the ith elements be in the (rs) position, while $\mathbf{e}\mathbf{W}^{i-1}$ and $\mathbf{W}^{n-i}\mathbf{e}^{+}$ represent the summation, or averaging, over all possible states of the other peptide units, consistent with the ith unit in (rs); $1/Z$ is the normalization factor. That these probabilities are normalized, i.e.,

$$\sum_{r,s}p_{rs}(i)=1; \qquad i=1,\cdots,n \tag{55}$$

follows directly from the identity

$$\mathbf{W}=\sum_{r,s}W_{rs}\mathbf{E}_{rs}. \tag{56}$$

Equation (54) can be transformed to enable direct summation over i. We first diagonalize **W** by Eq. (21), and obtain

$$p_{rs}(i)=W_{rs}\mathbf{e}\mathbf{T}\mathbf{\Lambda}^{i-1}\mathbf{T}^{-1}\mathbf{E}_{rs}\mathbf{T}\Lambda^{n-1}\mathbf{T}^{-1}\mathbf{e}^{+}/Z. \tag{57}$$

Now let $\mathbf{l}_\alpha$ and $\mathbf{l}_\alpha{}^*$ be a row and a column vector, respectively, whose components are the diagonal elements of Λ^α, where α is any integer; and let $\mathbf{T}_r{}^+$ and $\mathbf{T}_s$ be diagonal matrices whose diagonal elements are, respectively, the rth column of $\mathbf{T}^{-1}$ and the sth row of **T**. Let us further denote

$$\mathbf{a}_r=\mathbf{e}\mathbf{T}\mathbf{T}_r{}^{+} \qquad \mathbf{a}_s{}^{+}=\mathbf{T}_s\mathbf{T}^{-1}\mathbf{e}^{+}. \tag{58}$$

Equation (56) can then be written as

$$p_{rs}(i)=W_{rs}\mathbf{a}_r\mathbf{l}_{i-1}{}^*\cdot\mathbf{l}_{n-i}\mathbf{a}_s{}^{+}/Z, \tag{59}$$

where $\mathbf{l}_{i-1}{}^*\cdot\mathbf{l}_{n-1}$ is the diadic product of the two vectors.

As examples of the use of the probabilities p_{rs} we shall first obtain the functions θ and η derived already above, Eqs. (38) and (50). Thus, the degree of hydrogen bonding θ is given by

$$\theta=n_{\mathrm{bond}}/n=\sum_{i=1}^{n}p_{11}(i)/n=\mathbf{a}_1\mathbf{L}_{n-1}\mathbf{a}_1{}^*/Zn, \tag{60}$$

where $\mathbf{a}_1$ and $\mathbf{a}_1{}^*$ are obtained from Eq. (58) with the help of Eqs. (24), (25), and (29), setting $u=1$,

$$(\mathbf{a}_1)_r=vC_r/\lambda_r(\lambda_r-w); \tag{61}$$

$\mathbf{a}_1{}^*=\mathbf{a}_1{}^+$ is the transpose of $\mathbf{a}_1$; and $\mathbf{L}_{n-1}$ is the symmetric matrix

$$\mathbf{L}_{n-1}=\sum_{i=1}^{n}\mathbf{l}_{i-1}{}^*\cdot\mathbf{l}_{n-i}; \qquad (\mathbf{L}_{n-1})_{rr}=n\lambda_r{}^{n-1};$$

$$(\mathbf{L}_{n-1})_{rs}=(\lambda_r{}^n-\lambda_s{}^n)/(\lambda_r-\lambda_s). \tag{62}$$

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[20] S. Lifson, J. Chem. Phys. **30**, 964 (1959).

The equivalence of Eqs. (60) and (38) can be easily shown, and will not be given here.

Similarly η can be obtained by

$$\eta=\bar{n}_{12}/n=\sum_{i=1}^{n}p_{12}(i)/n=\mathbf{a}_1L_{n-1}\mathbf{a}_2{}^{+}/nZ, \tag{63}$$

where $a_2{}^{+}$ is obtained like $a_1{}^{+}$ above, and is given by

$$(\mathbf{a}_2{}^{+})_r=C_r/\lambda_r, \tag{64}$$

and again Eqs. (63) and (50) can be shown to be equivalent.

This formulation of probabilities can be applied to states involving more than one peptide unit. For example, we may ask what is, in terms of the matrices $\mathbf{W}$ and $\mathbf{E}_{rs}$, the probability $p(x, i)$ that a helical sequence of length x (i.e., having x peptide units) starts at the unit $i-x+1$ and ends at i, $(x\leq i)$. The above considerations lead directly to the answer

$$p(x,i)=\mathbf{e}\mathbf{W}^{i-x}(v\mathbf{E}_{31})(w\mathbf{E}_{11})^{x-2}(v\mathbf{E}_{12})\mathbf{W}^{n-i}\mathbf{e}^{+}/Z, \qquad 2\leq x\leq i, \tag{65}$$

which is reduced to

$$p(x,i)=(v^2w^{x-2})\,\mathbf{e}\mathbf{W}^{i-x}\mathbf{E}_{32}\mathbf{W}^{n-i}\mathbf{e}^{+}/Z, \qquad 2\leq x\leq i \tag{66}$$

by using the identities

$$\mathbf{E}_{rt}\mathbf{E}_{ts}=\mathbf{E}_{rs}. \tag{67}$$

We are now in a position to answer the question stated at the beginning of this section: The average number $\bar{n}_x$ of helices of length x is given by

$$\bar{n}_x=\sum_{i=x}^{n}p(x,i)=(v^2w^{x-2})\,\mathbf{a}_3\mathbf{L}_{n-x}\mathbf{a}_2{}^{+}/Z \qquad 2\leq x\leq n, \tag{68}$$

where $\mathbf{L}_{n-x}$ is obtained from Eq. (62) by replacing n by $n-x+1$; $\mathbf{a}_3$ is obtained from Eq. (58) with the help of Eqs. (25) and (29), and the result is the same as Eq. (64), i.e., $\mathbf{a}_3=(\mathbf{a}_2{}^{+})^{*}$. Thus,

$$\bar{n}_x=(v^2w^{x-2}/Z)\,\mathbf{a}_3\mathbf{L}_{n-x}\mathbf{a}_3{}^{*}. \tag{69}$$

For the special case $x=1$, when $\bar{n}_1$ is the average number of lonely helical states, Eq. (66) has to be slightly modified: The factor v replaces (v^2w^{x-2}), so that

$$\bar{n}_1=(v/Z)\,\mathbf{a}_3L_{n-1}\mathbf{a}_3{}^{*}. \tag{70}$$

It is seen that $\bar{n}_x$ of a chain of n units is related to $\bar{n}_1$ of a chain of $n-x+1$ units by

$$\bar{n}_x{}^{(n)}=(vw^{x-2}Z^{(n-x+1)}/Z^{(n)})\,\bar{n}_1{}^{(n-x+1)}, \tag{71}$$

where superscripts such as (n) and $(n-x+1)$ will denote henceforth, when necessary, the respective numbers of peptide units in the whole chain. An expression for $\bar{n}_1$ may be obtained also by using the common method of calculating molecular averages

$$\bar{n}_1=(v_{32}/Z)\,\partial Z/\partial v_{32}, \tag{72}$$

where Z is a function of v_{32} through the eigenvalues of the secular equation given in Eq. (48). By following again the same path which led to Eq. (38) for θ, we obtain for any x

$$\bar{n}_1{}^{(x)}/x=\sum_{r=1}^{3}\xi_rZ_r{}^{(x)}/Z^{(x)}, \tag{73}$$

where

$$\xi_r=\xi_r{}^{0}(1+x^{-1}\xi_r{}'), \tag{74}$$

$$\xi_r{}^{0}=\partial\ln\lambda_r/\partial\ln v_{32}, \tag{75}$$

$$\xi_r{}'=(\partial\ln C_r/\partial\ln v_{32})/(\partial\ln\lambda_r/\partial\ln v_{32}). \tag{76}$$

By introducing Eq. (73) into Eq. (71) we have after replacing x by $n-x+1$ in Eq. (71),

$$\bar{n}_{n-x+1}{}^{(n)}=(vw^{n-1}/Z^{(n)})\sum_{r=1}^{3}C_r\xi_r{}^{0}(x+\xi_r{}')(\lambda_r/w)^{x}. \tag{77}$$

If $nv\geq 3$ we may write, according to Eq. (36), $Z^{(n)}=Z_1{}^{(n)}$, and

$$\bar{n}_{n-x+1}{}^{(n)}=(v/\lambda_1)(w/\lambda_1)^{n-1}\sum_{r=1}^{3}(C_r/C_1)\,\xi_r{}^{0}(x+\xi_r{}')(\lambda_r/w)^{x}. \tag{78}$$

For a large part of the range of variation of x we may also reduce the sum in Eq. (78) to only its first term, and we obtain then for most of the distribution

$$\bar{n}_x{}^{(n)}=(v/\lambda_1)\,\xi_1{}^{0}(n+1-x+\xi_1{}')(w/\lambda_1)^{x-2}. \tag{79}$$

Equations (77)–(79) indicate that the distribution of lengths of helical sequences is rather broad on both sides of the transition region, although the actual value of all $\bar{n}_x$ increases steeply with the transition from the coil to the helix region.

After having derived $\bar{n}_x$ we obtain directly the distribution of peptide units among the various helical sequences

$$\bar{m}_x=x\bar{n}_x \tag{80}$$

and the "weight-average" length $\bar{l}_A$ of a helical segment, where the statistical weight of each sequence is proportional to the number of peptide units comprising the sequence. Since the distribution $\bar{n}_x$ is rather broad, $\bar{l}_A$ is, in general, much larger than $\bar{l}_n$. It is given by

$$l_w=\sum_{x=2}^{n}x\bar{m}_x\Big/\sum_{x=2}^{n}\bar{m}_x=\sum_{x=2}^{n}x^2\bar{n}_x\Big/\sum_{x=2}^{n}x\bar{n}_x$$

$$=\sum_{x=2}^{n}x^2\bar{n}_x/n(\theta+2\eta). \tag{81}$$

The distribution of helical sequences or the weight-average length of such sequences may be helpful in the study of the hydrodynamic behavior of polypeptides at and above the transition region. According to Eq. (79) $\bar{n}_x$ tends to zero when x is large enough for any fixed value $w/\lambda_1<1$. Therefore, when n is large enough the polypeptide molecule must exhibit a hydrodynamic behavior of a kinked chain of rigid rods, whereas a short or medium length molecule might behave as a rigid helix under the same conditions of temperature and solvent. It is appropriate to note in this connection that Nagai,[8] has discussed the hydrodynamic behavior of polypeptide chains in the transition region by calculating the mean-square end-to-end distance of a partially helical chain. His results may be simplified considerably by replacing his 7×7 matrices by our 3×3 ones.

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Theory of Solutions. III. Thermodynamics of Aggregation or Polymerization*

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Polymeric and colloidal systems are usually polydisperse, containing possibly hundreds or thousands or more, of subspecies differing only in degree of polymerization n. The required thermodynamic approach is that appropriate to a highly multicomponent system. Let $P(n)$ be the fraction of polymer molecules of size n. The distribution function $P(n)$ will depend on one or more parameters, for example, the mean $\bar{n}$ and standard deviation σ in the case of a Gaussian distribution. The thermodynamic problem becomes tractable if we replace, as composition variables, the numbers of molecules of all the polymer subspecies by the total number of polymer molecules N and the parameters of $P(n)$. The new set of variables may be only two, three, etc., in number. Our main interest in this paper is to investigate how the thermodynamic functions of the system change when the distribution function $P(n)$ changes, as, for example, in a kinetic study in which $P(n)$ evolves sufficiently slowly with time or in thermodynamic studies on different samples of the same polymer.

I. INTRODUCTION

IN systems such as a solution of a natural or synthetic polymer, a gas of liquid droplets or nuclei, inorganic colloidal particles or nuclei suspended in a liquid, etc., the "polymer," which is the general term we shall use for brevity in all these cases, does not exist, in general, as a single component but rather as a whole series of components differing in the degree of polymerization or aggregation, i.e., in molecular weight. In other words, the polymer is usually polydisperse (heterogeneous). The required thermodynamic approach here is that appropriate to a highly multicomponent system: the number of polymer subspecies may range, say, from ten to thousands or more.

Let n be the number of monomers (repeat units) or molecular units (e.g., water molecules in a water droplet) in a given polymer subspecies, and let $P(n)$ be the fraction of all polymer molecules containing n monomers. In this paper we shall be concerned primarily with the question of how the thermodynamic functions of the system change when the distribution function $P(n)$ changes. Such a discussion should be of use in equilibrium studies comparing different samples [with differing distributions $P(n)$] of the same polymer or in kinetic studies on a polymeric system in which the distribution evolves with time (e.g., polymerization of a polymer, aggregation of a colloidal suspension, or formation of condensation nuclei in a gas). In the kinetic case the rate must be sufficiently slow so that quasi-equilibrium is attained at each stage of evolution.

In any given application the mathematical form of $P(n)$ must be sufficiently general to encompass the complete range of alterations in $P(n)$ to be encountered. Thus P will be a function not only of n but also of a sufficient number of parameters $\alpha_1, \alpha_2, \cdots$. The number of parameters in practical cases would usually be in the range one to four. Ordinarily the degree of polymerization will be sufficiently large so that a continuous distribution $P(n)$[normalized by $\int P(n)dn=1$] can

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