

Conformational Lifetimes of the Helix—Coil Transition of Polypeptides

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ABSTRACT: In order to explain the separate α -CH peaks in NMR spectra of polypeptides in the helix—coil transition region, the lifetimes of molecules in two sites (one being a totally random-coil conformer and the other, comprising all other conformers containing at least one helix unit), were calculated on the basis of the one-dimensional Ising model in accordance with a two-site model. A lifetime greater than 10^{-2} s was not obtainable for a totally random-coil conformer for chain lengths ranging from 10 to 85. It was concluded that the occurrence of the separate peaks could not be attributed to the slow exchange rate between the two sites. Polydispersity in the chain length seems to be major consequence of such separate peaks.

KEY WORDS Conformational Lifetime / Helix—Coil Transition /
Polypeptide / One-Dimensional Ising Model / Two-Site Model /

For the kinetic consideration of the helix—coil transition of polypeptides, several theories¹⁻³ have been proposed, in which the initial rate of the transition was treated by perturbation after equilibrium. The mean relaxation time predicted by these theories can be used to interpret experimental kinetic data obtained by various approach-to-equilibrium measurements, such as the temperature jump,⁴ the ultrasonic absorption,⁵ and the dielectric relaxation.⁶ These measurements yielded relaxation times of the order of 10^{-5} — 10^{-8} s. But high-resolution nuclear magnetic resonance spectroscopy has often been used to study the helix—coil transition of polypeptides.⁷ The NMR observation of separate helix and coil peaks of α -CH in the transition region, separated by chemical shift difference of the order of 10^3 Hz, may suggest the presence of lifetimes of about 10^{-2} s or greater for each form.⁸ Recently, two theoretical models have been proposed to investigate such discrepancy in relaxation times. Ullman,⁹ Bradbury, *et al.*,¹⁰ and Nagayama and Wada⁷ have explained the NMR peaks in terms of molecular weight polydispersity. Ferretti and Jernigan,^{8,11} and Miller¹² have concluded that the low-field peak may be assigned to the totally random-coil conformer and the high-field peak to all other conformers containing at least one helix unit, and

that the separate peaks are a result of slow nucleation of the helix unit from a random-coil polypeptide. The latter authors concluded that the fast times obtained from both experiments and theories are related to the time for adding or melting one helix unit to or from an existing helical sequence, and that the slow times obtained by NMR spectra are related to the formation of an initial helical sequence from a random-coil segment of the polypeptide. Moreover, Ferretti and Jernigan⁸ investigated the NMR spectra on the basis of the two-site model, and evaluated the lifetimes associated with the separate peaks by the total line-shape analysis method.

In this paper, in accordance with the two-site model, the lifetimes of totally random-coil conformer (C-site) and of the all other conformers containing at least one helix unit (H-site) were calculated by using the nearest neighbor one-dimensional Ising model for polypeptide chains with the chain lengths ranging from 10 to 85, and the results obtained were compared with the experimental ones reported by Ferretti, *et al.*⁸

KINETICS OF ONE HELIX-REGION MODEL

This paper is concerned with the lifetime of the helix—coil transition for short chain polypeptides,

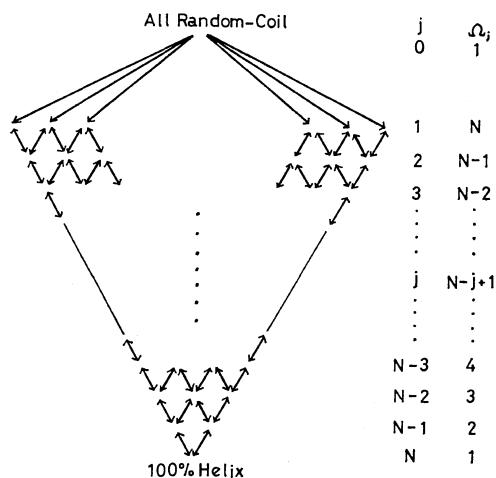


Figure 1. Schematic illustration of the transition of the one helix-region model. j indicates the number of helix units in the chain and Ω_j the degeneracy of the j -th state.

and thus, it is sufficient to consider¹³ only one helix-region restricted to the interior of the chain. This one helix-region model is equivalent for the random walk model^{12,14} with two interacting walkers for the helix—coil transition.

In the one helix-region model, the basic reaction sequence of the kinetics of the helix—coil transition of polypeptide is shown schematically in Figure 1. In Figure 1, j indicates the number of helix units in the chain, and Ω_j , the degeneracy of the state for the polypeptide chain having j helix units (*i.e.*, the degeneracy of the j -th state). Let p_j indicate the probability that the polypeptide is in the j -th state. According to the statistical mechanics of the helix—coil transition developed by Zimm and Bragg,¹⁵ and Poland and Scheraga,¹³ p_j at the equilibrium condition is given by

$$P_{0,\text{eq.}} = 1/Z$$

$$P_{j,\text{eq.}} = \Omega_j \sigma s^j / Z \quad (1 \leq j \leq N) \quad (1)$$

where s and σ are, respectively, the standard Zimm—Bragg helix stability having a statistical weight and the nucleation parameter, and Z is the partition function for the one helix-region model and is given by

$$Z = 1 + \sigma \sum_{j=1}^N \Omega_j s^j$$

and

$$\Omega_j = N - j + 1 \quad (2)$$

For a short chain, the s value at a transition midpoint (*i.e.*, the point at which the helical content θ is equal to 0.5) is greater than unity, *e.g.*, $s=1.211$, 1.107, and 1.057 for the polypeptides whose chain lengths are 34, 55, and 85, respectively. Therefore from eq 1 it is shown that the 0-th state (*i.e.*, the totally random-coil conformer) is most stable and that 1-st state (*i.e.*, conformer carrying only one helix unit) is the least probable state or the highest energy-level state. In view of these considerations, the description for the kinetics of the helix—coil transition by the two-site model may be regarded as an analogy of the dissociation of a diatomic molecule in which dissociation occurs when the molecules acquire a certain critical energy, as is considered so by Schwarz and Poland¹⁶ for dissociation of multi-strand complexes.

CONFORMATIONAL LIFETIMES

In consideration of the above, the lifetime of the molecules in H-site may be regarded as the first passage time¹⁷ for a molecule, initially in the H-site, to reach C-site, under the initial condition that the initial probability distribution, p_j , is of the Boltzmann type.

Jernigan and Ferretti³ derived master equations for describing the time and chain length dependence of p_j .

$$\frac{1}{k} \frac{dp_1}{dt} = - \left(q_b + \frac{2N-2}{N} q_r \right) p_1 + 2q_b p_2$$

$$\vdots$$

$$\frac{1}{k} \frac{dp_j}{dt} = \frac{2(N-j)+2}{N-j+2} q_r p_{j-1}$$

$$- \left(2q_b + \frac{2(N-j)}{N-j+1} q_r \right) p_j + 2q_b p_{j+1} \quad (3)$$

$$\vdots$$

$$\frac{1}{k} \frac{dp_N}{dt} = q_r p_{N-1} - 2q_b p_N$$

where k is the rate at which a unit performs a transition from either state to the opposite one, and q_r and q_b are $s/(1+s)$ and $1/(1+s)$, respectively. The processes associated with the kinetics of the one helix-region model are indicated schematically in Figure 2. The rate constants k_r and k_b correspond to kq_r and kq_b , respectively in their notation. However this difference is not signi-

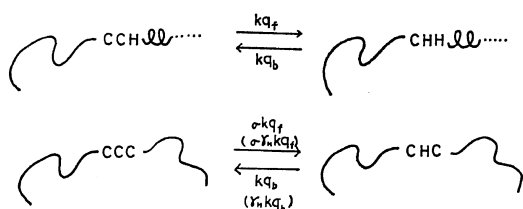


Figure 2. Types of transitions and rate constants of the transitions. H and C indicate helix and coil units, respectively. The rate constant in parentheses indicates that for which an additional parameter γ_H is taken into consideration.

ficant, because only the ratio of these two rate constants is guaranteed by the principle of detailed balance. Moreover our notation is suitable for comparing our results with those of Neves and Scott.²¹ In eq 3, the transition from the 0-th state to the 1-st state is omitted since the 0-th state is regarded as sink (or absorption wall) when the lifetime of molecules in the H-site is investigated.

The density function for absorption is given by

$$f_0 = \frac{dp_0}{dt} = - \sum_{j=1}^N \frac{dp_j}{dt} \quad (4)$$

The lifetime of the molecule in the H-site, $\langle T \rangle_H$, is given by

$$\begin{aligned} \langle T \rangle_H &= \int_0^\infty t \frac{dp_0}{dt} dt = - \sum_{j=1}^N \int_0^\infty t \frac{dp_j}{dt} dt \\ &= \sum_{j=1}^N \int_0^\infty p_j(t) dt \end{aligned} \quad (5)$$

Further, by the use of a matrix formulation, $\langle T \rangle_H$ may be given as follows (see Appendix)

$$\langle T \rangle_H = \frac{1}{k} eA^{-1}p(0) \quad (6)$$

where A becomes the transition matrix when eq 3 is rewritten into the matrix formulation as

$$\frac{1}{k} \frac{dP(t)}{dt} = -AP(t) \quad (7)$$

$P(0)$ is the initial value of the row vector $P(t) = (p_1(t), p_2(t), \dots, p_N(t))$,* and $p_j(0)$ is given by

$$p_j(0) = \frac{\sigma(N-j+1)s^j}{Z'} \quad (8)$$

where Z' is the partition function when the totally random-coil conformation is excluded from the population, and given by

$$Z' = \sigma \sum_{j=1}^N (N-j+1)s^j \quad (9)$$

and

$$e = (1, 1, \dots, 1) \quad (10)$$

The lifetime of the molecule in C-site is given by

$$\langle T \rangle_C = \frac{P_C}{P_H} \langle T \rangle_H \quad (11)$$

where P_C and P_H are the probabilities that the molecule is in C-site and the H-site at equilibrium, respectively.

In a similar manner, an equation is derived for the second moment, $\langle T^2 \rangle_H$, of $\langle T \rangle_H$

$$\langle T^2 \rangle_H = \frac{1}{k^2} eA^{-2}p(0) \quad (12)$$

RESULTS AND DISCUSSION

Schwarz and Poland¹⁶ have recently shown that one helix-region model is valid for the kinetics of the helix—coil transition as well as for the equilibrium statistical mechanics of the transition in a region near the transition midpoint when chain length N is less than $2\sigma^{-1/2}$. In mixed organic solvents, σ is of the order of 10^{-4} , so one helix-region model may be assumed valid for chains whose lengths are less than 200. Thus, a value 10^{-4} was adopted for σ for in making the calculations of this paper.

Calculated lifetimes, $k\langle T \rangle_H$, $k\langle T \rangle_C$, and $k\langle T^2 \rangle_H^{1/2}$ for a chain of 55 units, are indicated as functions of s in Figure 3. $\langle T \rangle_H$ has a strong dependence on s and shows nearly an exponential dependence on s . On the other hand, $\langle T \rangle_C$ has only a slight dependence on s and $k\langle T \rangle_C$ seems to approach 10^2 for a large s . The mean square root of $\langle T \rangle_H$ is nearly equal to $\langle T \rangle_H$ and this fact shows that the variance of $\langle T \rangle_H$ is very small even for a short chain.

In order to estimate the absolute values of the lifetimes, the value of k must be specified. Recently, Zana¹⁸ published summarized results on the relaxation times and concluded that the most probable value of the rate constant for the elemental step of helix growth is about 10^8 s^{-1} . He considers this process to be limited by the rate of rotation of short segments in the polypeptide chain around $C_\alpha-C'$ and $C_\alpha-N$ bonds, supported by the fact that the reciprocal relaxation time for

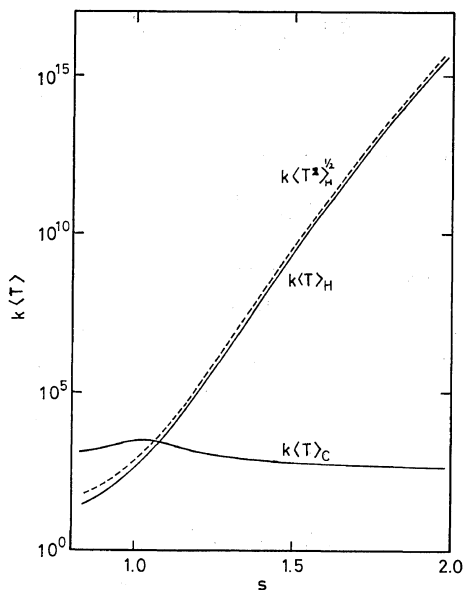


Figure 3. Conformational lifetimes, $k\langle T\rangle_{\text{H}}$, $k\langle T\rangle_{\text{C}}$, and $k\langle T^2\rangle_{\text{H}}^{1/2}$ (dashed curve), as functions of s for a chain of $N=55$ and $\sigma=10^{-4}$.

the rotation of a short segment of vinylic chain is of the same order of magnitude. According to the Zana's consideration, we adopt 10^8 s^{-1} for k value. Thus, we get $\langle T\rangle_{\text{H}}=5.6 \times 10^{-5} \text{ s}$ and $\langle T\rangle_{\text{C}}=2.2 \times 10^{-5} \text{ s}$ at the transition midpoint.

Recently, Ferretti and Jernigan⁸ investigated the NMR spectra on the basis of the two-site model and evaluated the average lifetime τ from the total line-shape analysis method, in which τ was defined by the equation:

$$\tau^{-1} = \langle T\rangle_{\text{H}}^{-1} + \langle T\rangle_{\text{C}}^{-1} \quad (13)$$

Their experimental and our calculated lifetimes are summarized in Table I. These lifetimes were calculated for two values of k , *i.e.*, 10^8 and $1.8 \times 10^5 \text{ s}^{-1}$. As shown in Table I, τ is almost independent of s in both the experiment and the calculation. When 1.8×10^5 is adopted as the k value, agreement between the calculated and experimental lifetimes is close. However, this k value (*i.e.*, 1.8×10^5) cannot be used to explain previously published data on the relaxation time, and moreover the conflict with the molecular mechanics of helix growth step suggested by several investigators^{18,19} from the chemical relaxation measurements cannot be explained. Therefore,

this value must be discarded.

The rate constant of the helix nucleation step in eq 3 is given by $\sigma k q_f$. However, Schwarz

Table I. Comparison of the calculated average lifetime τ_{cal} for a chain of $N=55$ and $\sigma=10^{-4}$ with experimental τ_{exp}

θ	s	τ_{exp} (s)	τ_{cal} (s) ^a	τ_{cal} (s) ^b
0.25	1.067	0.007	1.25×10^{-5}	0.007
0.40	1.091	0.008	1.50×10^{-5}	0.008 ₃
0.50	1.106	0.009	1.60×10^{-5}	0.008 ₉
0.60	1.124	0.013	1.66×10^{-5}	0.009 ₂
0.70	1.149	0.015	1.53×10^{-5}	0.008 ₅

^a For $k=10^8 \text{ s}^{-1}$. ^b For $K=1.8 \times 10^5 \text{ s}^{-1}$.

introduced a new factor γ_{H} , by which the helix nucleation rate constant may be denoted as $\sigma \gamma_{\text{H}} k q_f$. This γ_{H} factor corresponds to the kinetical difficulty in the helix nucleation step and/or additional energy barrier (additional activation energy) for the transition between the 0-th state and 1-st state. When the γ_{H} factor is taken into consideration, the first equation in eq 3 is converted to the following equation

$$\frac{1}{k} \frac{dp_1}{dt} = - \left(\gamma_{\text{H}} q_b + \frac{2N-2}{N} q_f \right) p_1 + 2q_b p_2 \quad (14)$$

The results on $k\langle T\rangle_{\text{H}}$ and $k\langle T\rangle_{\text{C}}$ calculated for various γ_{H} are indicated in Figure 4. In Figure 5, the lifetimes are plotted against γ_{H} at the transition midpoint. As shown in Figures 4 and 5, lifetimes are almost independent of γ_{H} in the region of $\gamma_{\text{H}} > 1$. On the contrary, they have a strong dependence on γ_{H} and are proportional to γ_{H} in the region of $\gamma_{\text{H}} \ll 1$. That is when γ_{H} is very small, the transition between the 0-th state and the 1-st state is the rate determining step. If 10^8 s^{-1} is adopted for the k value, it must be assumed that γ_{H} is $10^{-3} - 10^{-4}$ in order to obtain a good agreement between the calculated and experimental τ values. Though there is no knowledge about γ_{H} value at the present time, it seems impossible to assume γ_{H} of the order of $10^{-3} - 10^{-4}$. In view of the statistical mechanics of helix-coil transition, it is impossible to assume that the existence of an additional high energy barrier of the helix nucleation (in addition to the difficulty represented by σ) is enough to explain such a small γ_{H} value. Furthermore, if γ_{H} is considerably less than unity, it

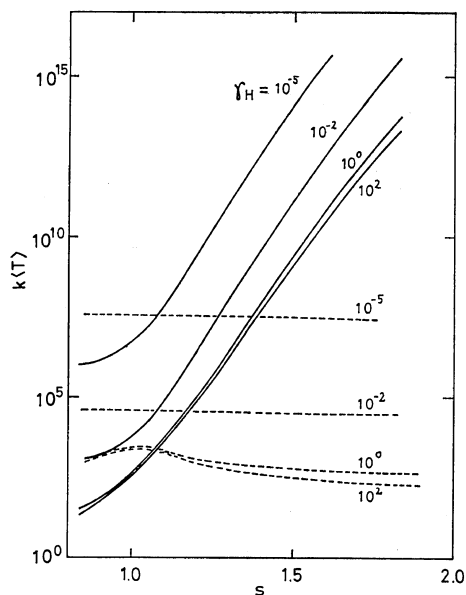


Figure 4. Conformational lifetimes, $k\langle T\rangle_H$ and $k\langle T\rangle_C$ (dashed curve), as functions of s at various γ_H values, for a chain of $N=55$ and $\sigma=10^{-4}$.

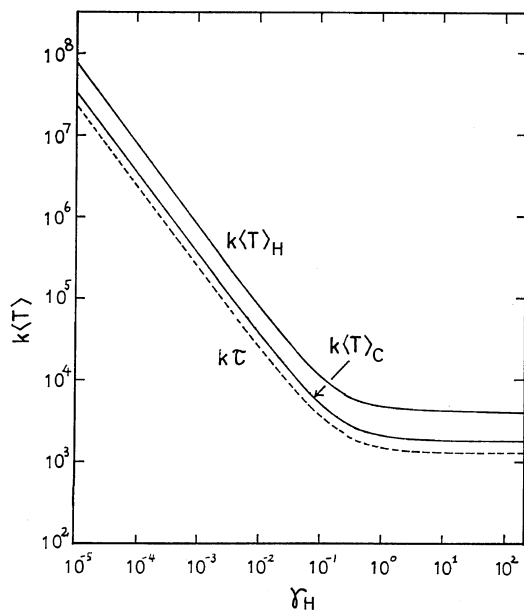


Figure 5. Dependences of conformational lifetimes $k\langle T\rangle_H$, $k\langle T\rangle_C$, and the average lifetime $k\tau$ (dashed curve), on γ_H at $s=1.1$, for a chain of $N=55$ and $\sigma=10^{-4}$.

should be assumed that the transition from the 1-st state to the 0-th state is much slower than the coil growth step. However, this is unreasonable since the helix unit in the 1-st state is bounded by two coil units on both sides, while the helix unit in the coil growth step is bounded by one coil unit and one helix unit on each side. Recently, Jernigan and Szu²⁰ have investigated the γ_H value theoretically, and suggested that the helix nucleation accompanies a frictional resistance from the rotational motion of the random-coil portion attached to the bond of the residue that performs transition. However, even if this frictional resistance is taken into consideration, the γ_H value is not of the order of 10^{-8} — 10^{-4} . Thus, the existence of a long lifetime of the order of 10^{-2} s is out of the question and the slow nucleation explanation for the separate peaks of α -CH NMR spectra is questionable.

In Figures 6 and 7, lifetimes $\langle T\rangle_H$ and $\langle T\rangle_C$ for chains with various lengths are indicated as functions of s . $\langle T\rangle_H$ has a strong dependence on the chain length, *i.e.*, it increases with increasing chain length, since the number of states of conformation of a polypeptide in a longer chain are more than

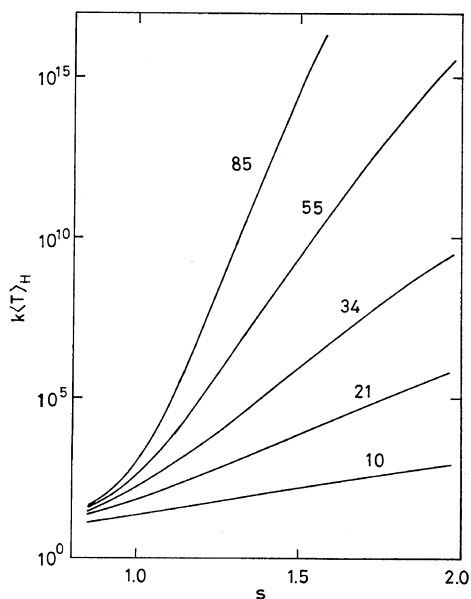


Figure 6. Conformational lifetime $k\langle T\rangle_H$ as a function of s for chains of various chain lengths. The numbers indicate the chain lengths.

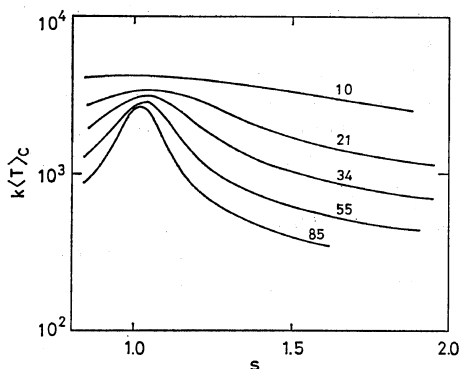


Figure 7. Conformational lifetime $k\langle T \rangle_C$ as a function of s for chains of various chain lengths. The numbers indicate the chain lengths.

in a shorter chain. On the other hand, $\langle T \rangle_C$ decreases with increasing chain length, since P_C is larger for a shorter chain than for a longer chain. Also, $\langle T \rangle_C$ has a maximum at an s value somewhat smaller than that of the transition midpoint.

Neves and Scott²¹ derived the same conclusion for the lifetime of a C-site by the Monte Carlo calculation. They obtained 5×10^{-4} s for the lifetime of the C-site, assuming 2.5×10^7 as the k value. It is invalid to assume nevertheless, that the lifetime of a totally random-coil conformer is different from the helix growth time scale by a factor of the order of σ as assumed by Ferretti and Jernigan.⁸

There may be different situations in the kinetics of the helix-coil transition not describable by the Ising model. Fujiwara and Saito²² investigated the rate of the coil nucleation from a completely helical polypeptide, taking consideration the frictional resistance caused by the motion of the helical rods of both sides of the unit that is transformed to a coil. They showed that the coil nucleation from a completely helical polypeptide rarely occurs, particularly for a unit located in the inner part of the helical chain. However, coil nucleation does not occur originally within the one helix-region model. And the helix and coil growth involve a rotation of a local coil part of polypeptide chain, as suggested by the value of the relaxation time obtained by the chemical relaxation measurements. Thus, a description of the kinetics of the helix-coil transition of polypeptides in terms of the one-dimensional Ising model seems

to be acceptable, at least for short chains for which the one helix-region model is valid.

APPENDIX

In order to calculate the integral of right-hand side of eq 5, we introduce Laplace transform of $p_j(t)$

$$L_j(u) = \int_0^{\infty} e^{-ut} p_j(t) dt \quad (\text{A-1})$$

From eq 5 and A-1, $\langle T \rangle_H$ can be described as follows

$$\langle T \rangle_H = \sum_{j=1}^N L_j(0) \quad (\text{A-2})$$

By the Laplace transformation of eq 9, and by substituting $u=0$ into the results, we get

$$L(0) = \frac{1}{k} A^{-1} P^{-1}(0) \quad (\text{A-3})$$

where $L(0)$ is the row vector: $L(0) = (L_1(0), L_2(0), L_N(0))^*$. From eq A-2 and A-3, we get eq 6 in the text.

The inverse of the matrix A is given as follows: Matrix A can be represented simply by

$$A = \begin{bmatrix} a_1 & -c_1 & 0 & \cdot & \cdot & \cdot \\ -b_2 & a_2 & -c_2 & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & -b_N & a_N \end{bmatrix} \quad (\text{A-4})$$

where the correspondence of elements a , b , and c to eq 3 is clear. The (i, j) -element of A^{-1} , $(A^{-1})_{i, j}$, is given by

$$\begin{aligned} \left[\prod_{k=j+1}^i b_k \right] \frac{D_{j-1} E_{N-i}}{D_N} & \quad (i > j) \\ (A^{-1})_{i, j} = \frac{D_{i-1} E_{N-i}}{D_N} & \quad (i = j) \\ \left[\prod_{k=i}^{j-1} c_k \right] \frac{D_{i-1} E_{N-j}}{D_N} & \quad (i < j) \end{aligned} \quad (\text{A-5})$$

where D_i is the determinant of the $i \times i$ matrix obtained from the first i rows and columns of A , and E_i is the determinant of the $i \times i$ matrix obtained from after elimination of the $N-i$ rows and columns. D_i and E_i are given by the following recurrent relations

$$\begin{aligned} D_0 &= 1 \\ D_1 &= a_1 \\ D_i &= a_i D_{i-1} - b_i c_{i-1} D_{i-2} \quad (2 \leq i \leq N) \end{aligned} \quad (\text{A-6})$$

$$E_0 = 1$$

$$E_1 = a_N \quad (\text{A-7})$$

$$E_i = a_{N-i+1}E_{i-1} - b_{N-i+2}c_{N-i+1}E_{i-2} \quad (2 \leq i \leq N)$$

REFERENCES

1. G. Schwarz, *J. Mol. Biol.*, **11**, 64 (1965).
2. D. Poland and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2071 (1966).
3. R. L. Jernigan and J. A. Ferretti, *J. Chem. Phys.*, **62**, 2519 (1975).
4. R. Lumry, R. Legare, and W. G. Miller, *Biopolymers*, **2**, 484 (1964).
5. A. Barksdale and J. Stuehr, *J. Am. Chem. Soc.*, **94**, 3334 (1972); R. Zana and J. Lang, *Biopolymers*, **12**, 79 (1973).
6. G. Schwarz and J. Seeling, *Biopolymers*, **6**, 1263 (1968).
7. K. Nagayama and A. Wada, *Biopolymers*, **12**, 2443 (1973); **14**, 2489 (1975); J. B. Milstein and J. A. Ferretti, *ibid.*, **12**, 2335 (1973).
8. J. A. Ferretti and R. L. Jernigan, *Macromolecules*, **6**, 687 (1973).
9. R. Ullman, *Biopolymers*, **9**, 471 (1970).
10. E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, **11**, 277 (1970).
11. J. A. Ferretti, B. W. Ninham, and V. A. Parsegian, *Macromolecules*, **3**, 34 (1970); R. L. Jernigan, J. A. Ferretti, and G. H. Weiss, *ibid.*, **6**, 684 (1973).
12. W. G. Miller, *Macromolecules*, **6**, 100 (1973).
13. D. Poland and H. A. Scheraga, "Theory of the Helix—Coil Transition in Biopolymers," Academic Press, New York, N.Y., 1970, Chapter 5.
14. M. Schwarz, Jr. and D. Poland, *J. Chem. Phys.*, **63**, 557 (1975).
15. B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
16. M. Schwarz, Jr. and D. Poland, *J. Chem. Phys.*, **65**, 2620 (1976).
17. For example, I. Oppenheim, K. E. Shuler, and G. H. Weiss, *Adv. Mol. Relaxation Processes*, **1**, 13 (1967—1968).
18. R. Zana, *Biopolymers*, **14**, 2425 (1975).
19. G. C. Hammes and A. C. Park, *J. Am. Chem. Soc.*, **91**, 956 (1969).
20. R. L. Jernigan and S. C. Szu, *J. Polym. Sci. Symposium*, **54**, 271 (1976).
21. D. E. Neves and R. A. Scott, *Macromolecules*, **10**, 339 (1977).
22. M. Fujiwara and N. Saito, *Polym. J.*, **9**, 625 (1977).