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Thermodynamics of the One-Stranded Helix–Coil Equilibrium in Polyadenylic Acid

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Abstract: The broad hypochromic "melting" curves of high molecular weight polyadenylic acid and its oligomers (degree of polymerization 2 through 11) at neutral pH were interpreted in terms of the one-dimensional Ising lattice as a model for the equilibrium between random coil and the one-stranded helix known to be present at low temperatures. For the purpose of taking into account the dependence of hypochromicity on helix length, exact equations for the sequence length distribution in the finite and infinite Ising models were derived. The experimental data yielded the thermodynamic parameters for the helix-coil transition by computer solution of the equations. The enthalpy and entropy of helix formation were found to be, respectively, -9.4 ± 2 kcal/residue-mole and -29.3 ± 6 cal/deg residue-mole. The helix interruption constant was found to be 0.6 ± 0.3, indicating that cooperative effects are small, as is predicted on molecular grounds.

Polyadenylic acid (poly-A) in aqueous solution at neutral pH is believed to be a one-stranded polymer with a secondary structure that is nearly complete at temperatures near 0° and that melts gradually over a broad temperature range. The early evidence for this view has been summarized by Steiner and Beers.[1] The most reasonable current hypothesis is that this secondary structure consists of a one-stranded helix stabilized by the stacking of the bases with their planes more or less perpendicular to the helix axis. Several observations have been cited in support of this structure: the nuclear magnetic resonance of the adenine protons showed chemical shifts that were similar to those found in other systems of stacked aromatic rings;[2] the circular dichroism[3] and the optical rotatory dispersion[4] in the ultraviolet showed the behavior predicted for a system of chromophores stacked with their transition moments perpendicular to the helix axis; low-angle X-ray scattering[5] revealed a rod-like structure for poly-A at neutral pH, and the linear density corresponded to one nucleotide per 3.5 A, which is very nearly the crystallographic thickness of the aromatic group.

A novel feature of the secondary structure is its broad melting range,[6] since most nucleic acid helices melt relatively sharply, including the two-stranded helical form of poly-A occurring at acid pH.[6] For the one-dimensional Ising model, the sharpness of a temperature-induced transition has been shown[7,8] to be governed by two factors: the enthalpy change for the transition and the helix interruption constant, which measures the cooperativeness of the transition. A broad transition could result from either a small value of the former or a large value of the latter. The importance of cooperative effects can be determined if the melting behavior is known as a function of chain length, as has been demonstrated in the cases of poly-γ-benzyl-

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[1] R. F. Steiner and R. F. Beers, Jr., "Polynucleotides," Elsevier Publishing Co., Amsterdam, 1961, Chapter 7.

[2] C. C. McDonald and W. D. Phillips, *Science*, **144**, 1234 (1964).

[3] J. Brahms, *Nature*, **202**, 797 (1964); J. Brahms and W. F. H. M. Mommaerts, *J. Mol. Biol.*, 10, 73 (1964).

[4] D. Holcomb and I. Tinoco, Jr., *Biopolymers*, 3, 121 (1965).

[5] V. Luzzati, A. Mathis, F. Masson, and J. Witz, *J. Mol. Biol.*, 10, 28 (1964).

[6] J. R. Fresco and E. Klemperer, *Ann. N. Y. Acad. Sci.*, 81, 730 (1959).

[7] L. Peller, *J. Phys. Chem.*, **63**, 1194 (1959).

[8] J. Applequist, *J. Chem. Phys.*, **38**, 934 (1963).

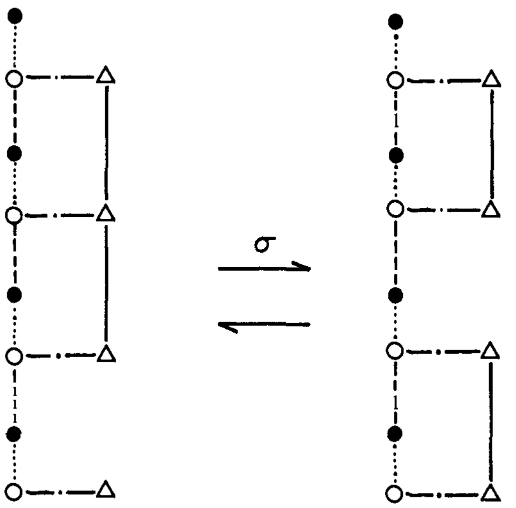

Figure 1. The helix interruption constant σ, illustrated for $N = 4$. – – ● · · represents the C3′–O–P–O–C5′–C4′ linkage between ribose (O) units. O– · –Δ is the glycosidic linkage to the base (Δ). Δ——Δ represents stacked bases.

L-glutamate[9] and the two-stranded poly-A helix.[10] The necessary data for the one-standed poly-A case have been obtained by Fresco, Blake, and Doty[11] in their study of ultraviolet hypochromicities of fractionated oligoadenylic acids (oligo-A) (dimers through 11-mers). We have found by examining their data in the light of what is known about the nature of the hypochromic effect and the nature of cooperative helix–coil equilibria that this transition behaves in an almost noncooperative manner, that the helix interruption constant is approximately as predicted for the theoretical model, and that thermodynamic parameters for the transition can be evaluated by solution of the theoretical equations. The purpose of this paper is to derive the necessary equations and to present an analysis of the data of Fresco, *et al.*

During the course of this work two reports of a closely related nature have appeared. Van Holde, Brahms, and Michelson[12] have examined the thermodynamics of base stacking in diadenylic acid in a straightforward interpretation of circular dichroism measurements. Poland, Vournakis, and Scheraga[13] have observed the melting of various oligo-A's by means of ultraviolet optical rotation, and have interpreted their data according to a model which is identical with ours, *i.e.*, the one-dimensional Ising model. A novel feature of our treatment, however, is the exact derivation of the distribution of helical sequence lengths, and the use of this distribution to take into account the dependence of the observed quantity (hypochromicity) on sequence length.

Theory

The molecular model to assumed in this paper is that of a single polynucleotide strand which is capable of taking on a helical conformation stabilized by the interaction among bases arranged in a linear array. It will be assumed that each base interacts only with its two nearest neighbors. For concreteness, it can be assumed that the interaction produces a stacked array, with the planes of the bases more or less perpendicular to the helix axis. The interaction between two bases that are so stacked will be called an "S bond." The accessible states of the molecule include all possible sequences of formed and broken S bonds along the chain. (No such thing as a partially formed S bond will be considered.) The problem is therefore that of the one-dimensional Ising lattice.[7,8,14] The phrasing of the problem in terms of conformational transitions in polymers by Zimm and Bragg[15] is particularly appropriate to the present case. Following their notation, we define s as the equilibrium constant for the addition of one S bond to the end of a sequence of existing S bonds (the "stability constant"), and σ as the equilibrium constant for the formation of an interruption in a sequence of S bonds in such a way as to maintain a constant number of S bonds (the "helix interruption constant").

The significance of σ is shown schematically in Figure 1. When an isolated S bond is formed between two neighboring bases, the covalent bonds in the sugar phosphate backbone (C3′-O-P-O-C5′-C4′) and two glycosidic bonds (C1′-N) lose their freedom of rotation. When one more S bond is added adjacent to the first, as shown on the left side of Figure 1, only one C1′-N bond loses its rotational freedom in addition to the backbone bonds. If, however, this additional S bond is formed elsewhere along the chain, as shown on the right side of Figure 1, two C1′-N bonds lose their freedom of rotation. The formation of an interruption in a sequence of S bonds is therefore accompanied by an entropy change ΔS_i, which includes the entropy loss for one C1′-N bond, as well as any solvent entropy changes involved in the process. Since the total number of S bonds is the same in the two structures shown in Figure 1, the enthalpy change on formation of an interruption may be assumed to be zero as a first approximation. Therefore

$$\sigma = e^{\Delta S_i/R} \tag{1}$$

where ΔS_i is in units per mole of interruptions.

A value of $\sigma < 1$, corresponding to $\Delta S_i < 0$, would give rise to a cooperative effect, which may be defined as a tendency for residues in the same state to group together. This would be the situation if the solvent contribution to ΔS_i is negligible and if there is appreciable freedom of rotation about the C1′-N bond in the random coil form. Donohue and Trueblood[16] suggest that such internal rotation is possible, and that two broad potential energy minima exist for the rotation. Making the assumption (probably oversimplified), then, that restriction of the rotation reduces the number of configurations about the C1′-N bond by a factor of 2, one estimates $\sigma = 0.5$. In other words, the system would be expected to show little cooperative effect. The experimental data will be shown to be consistent with this estimate of σ.

A lack of cooperativeness implies that helical regions of a molecule will tend to be short. The average number of S bonds per helical region at the midpoint of the

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(10) J. Applequist and V. Damle, *J. Am. Chem. Soc.*, **87**, 1450 (1965).

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(12) K. E. Van Holde, J. Brahms, and A. M. Michelson, *J. Mol. Biol.*, **12**, 726 (1965).

(13) D. Poland, J. N. Vournakis, and H. A. Scheraga, *Biopolymers*, **4**, 223 (1966).

(14) E. Ising, *Z. Physik*, **31**, 253 (1925).

(15) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

(16) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

transition has been shown[8] to be $1 + \sigma^{-1/2}$, or probably between two and three for the present case. The effect of helix length on hypochromicity therefore has a particularly large influence on the experimental observations, and the approach we have adopted previously[10] in treating more cooperative equilibria should be especially well suited to the present problem. This approach consists of expressing the hypochromicity $\bar{G}$ as a weighted average over all helix lengths.

$$\bar{G} = \sum_{k=1}^{N-1} G_k x_k \qquad (2)$$

Here $\bar{G} = (\epsilon' - \epsilon)/\epsilon'$, where ϵ is the residue-molar extinction coefficient of the system and ϵ' is that of the isolated nucleotides; $G_k = (\epsilon' - \epsilon_k)/\epsilon'$, where ϵ_k is the residue-molar extinction coefficient for a helix with k S bonds ($k + 1$ nucleotides); x_k is the fraction of bases in the system in helical segments containing k S bonds ($k + 1$ bases); and N is the degree of polymerization of the sample. The G_k's are obtained empirically, and the x_k's are derived below by extension of the exact treatment of the Ising model.

It should be noted that eq 2 would apply to other types of observation as well, where G represents the measured quantity. If G_k is independent of k, then the observed $\bar{G}$ is proportional to the fraction of bases in helical segments, and the analysis becomes greatly simplified. It can further be shown that if G_k is proportional to $k/(k + 1)$, then $\bar{G}$ is proportional to the fraction of S bonds formed, and the analysis is likewise relatively simple. The latter condition is equivalent to the approximation made by Poland, *et al.*,[13] and their analysis was thus equivalent to the available treatment of the one-dimensional Ising model. The hypochromicity data are only roughly approximated by the same condition (see eq 22), and more detailed treatment is required for these data.

The distribution of helical sequence lengths, as expressed by x_k, has been derived for modifications of the Ising model appropriate to the α-helix of polypeptides by Nagai[17] and by Lifson and Roig,[18] using matrix methods in both cases. Their results should apply approximately to the present case. However, we have instead derived exact expressions for the simple version of the Ising model, as is appropriate to the present case, and will demonstrate here a relatively simple method of carrying out this derivation. The method is based on results obtained by Lifson[19] employing sequence generating functions and is closely related to the method described previously[8] for the derivation of the exact partition function for finite chains.

Lifson's method of generating functions is applied as follows. Let u_i be the configurational partition function of a sequence of i residues, none of which is S-bonded to its neighbor to the left along the chain, assuming the residues to be arranged in a line from left to right. Let v_j be the configurational partition function of a sequence of j residues, each of which is S-bonded to its neighbor to the left. One can, without loss of generality, arbitrarily set $u_i = 1$ for all i, whereupon $v_j = \sigma s^j$ for $j \geq 1$ and $v_0 = 1$. The sequence generating functions are defined in terms of an arbitrary variable y by

$$U(y) = \sum_{i=1}^{\infty} u_i y^{-i} = \frac{1}{y - 1} \qquad (3)$$

$$V(y) = \sum_{j=1}^{\infty} v_j y^{-j} = \frac{\sigma s}{y - s} \qquad (4)$$

$$V_0(y) = \sum_{j=0}^{\infty} v_j y^{-i} = 1 + \frac{\sigma s}{y - s} \qquad (5)$$

$V_0(y)$ is the function applying to the helical sequence at the right end of the molecule and contains a term for $j = 0$ since this sequence may contain no residues, *i.e.*, in complexions (microscopic states) in which the right-terminal sequence is randomly coiled. The left-terminal residue is nonbonded to the left in all complexions; therefore, the function $U(y)$ applies to the left-terminal sequence, as well as to all other random-coil sequences in the molecule. A power series $\Gamma(y)$ is written as

$$\Gamma(y) = \sum_{N=0}^{\infty} Z_N y^{-N} \qquad (6)$$

where Z_N is the configurational parition function of a chain of N residues. Lifson's result[19] applicable to the present case is

$$\Gamma(y) = \frac{U(y)V_0(y)}{1 - U(y)V(y)} \qquad (7)$$

which becomes, on inserting eq 3–5

$$\Gamma(y) = \frac{y - s + \sigma s}{(y - 1)(y - s) - \sigma s} \qquad (8)$$

$\Gamma(y)$ aids in determining the x_k in the following way. We have

$$x_k = \frac{(k + 1)\langle \nu_k \rangle}{N} \qquad (9)$$

where $\langle \nu_k \rangle$ is the average number of helical sequences with k S bonds per molecule. Since each term in the partion function Z_N contains v_k raised to the power of the number of sequences of k S bonds found in the corresponding complexion, we have

$$\langle \nu_k \rangle = \frac{v_k}{Z_N}\frac{\partial Z_N}{\partial v_k} = \frac{\sigma s^k}{Z_N}\frac{\partial Z_N}{\partial v_k} \qquad (10)$$

It follows from eq 6 that $\partial Z_N/\partial v_k$ is the coefficient of y^{-N} in the series expansion of $\partial \Gamma(y)/\partial v_k$. From eq 7 we have

$$\frac{\partial \Gamma(y)}{\partial v_k} = \frac{U(1 + U)}{y^k(1 - UV)^2} \qquad (11)$$

$$= \frac{(y - s)^2}{y^{k-1}[(y - 1)(y - s) - \sigma s]^2} \qquad (12)$$

The expansion in a power series is accomplished by separating the expression on the right-hand side of eq 12 into partial fractions after first factoring the polynomial in the denominator in the form

$$[(y - 1)(y - s) - \sigma s]^2 = (y - \lambda_0)^2(y - \lambda_1)^2 \qquad (13)$$

where

$$\lambda_0 = \frac{1}{2}\{1 + s + [(1 - s)^2 + 4\sigma s]^{1/2}\} \qquad (14)$$

$$\lambda_1 = \frac{1}{2}\{1 + s - [(1 - s)^2 + 4\sigma s]^{1/2}\} \qquad (15)$$

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(17) K. Nagai, *J. Chem. Phys.*, 34, 887 (1961).
(18) S. Lifson and A. Roig, *ibid.*, 34, 1963 (1961).
(19) S. Lifson, *ibid.*, **40**, 3705 (1964).

The separation of eq 12 into partial fractions gives

$$\frac{\partial\Gamma(y)}{\partial v_k} = \frac{1}{y^{k-1}}\left[\frac{A_1}{(y-\lambda_0)^2} + \frac{A_2}{y-\lambda_0} + \frac{B_1}{(y-\lambda_1)^2} + \frac{B_2}{y-\lambda_1}\right] \qquad (16)$$

where

$$A_1 = (\lambda_0 - s)^2/(\lambda_0 - \lambda_1)^2$$
$$A_2 = 2(\lambda_0 - s)(s - \lambda_1)/(\lambda_0 - \lambda_1)^3$$
$$B_1 = (\lambda_1 - s)^2/(\lambda_0 - \lambda_1)^2$$
$$B_2 = 2(\lambda_1 - s)(s - \lambda_0)/(\lambda_1 - \lambda_0)^3 = -A_2$$

We then employ the following expansions

$$\frac{1}{y-a} = \sum_{i=1}^{\infty}\frac{a^{i-1}}{y^i} \qquad (17)$$

$$\frac{1}{(y-a)^2} = \sum_{i=2}^{\infty}\frac{(i-1)a^{i-2}}{y^i} \qquad (18)$$

The coefficient of y^{-N} in the expansion of $\partial\Gamma(y)/\partial v_k$ is thus found to be

$$\frac{\partial Z_N}{\partial v_k} = (N-k)\left[(\lambda_0 - s)^2\lambda_0^{N-k-1} + (\lambda_1 - s)^2\lambda_1^{N-k-1}\right]/(\lambda_0 - \lambda_1)^2 + 2(\lambda_0 - s)(s - \lambda_1)(\lambda_0^{N-k} - \lambda_1^{N-k})/(\lambda_0 - \lambda_1)^3 \qquad (19)$$
$$k = 1, 2, \ldots, N$$

Similarly, Z_N is found as the coefficient of y^{-N} in the expansion of eq 8. This is the method used elsewhere,[3,14] with the result[20]

$$Z_N = \frac{\lambda_0^N(1-\lambda_1) - \lambda_1^N(1-\lambda_0)}{\lambda_0 - \lambda_1} \qquad (20)$$

The x_k's are therefore obtained by combination of eq 9, 10, 19, and 20. It is shown in the Appendix that these formulas reduce to those derived for a random arrangement of bonds along the chains in the case $\sigma = 1$.

The results take a simpler form in the limit of very large N. It can be verified that $\lambda_0 > 1$ and $0 \leq \lambda_1 < 1$ for all $s > 0$, $\sigma > 0$. Thus as $N \rightarrow \infty$, $\lambda_1^N \rightarrow 0$. Using eq 9 we find x_k approaches the limit

$$x_k = (k+1)\sigma s^k(\lambda_0 - s)/\lambda_0^{k+1}(\lambda_0 - \lambda_1) \qquad (21)$$

Results

The ultraviolet absorption measurements of Fresco, *et al.*,[11] on oligo-A and poly-A were made at pH 7.3 in 0.15 *M* sodium chloride + 0.015 *M* sodium citrate. Concentrations were in the range of 30–65 × 10^{-6} residue-molar. The hypochromicity was calculated from the absorbance at 257 mμ, on the assumption that a residual hypochromicity of 0.020 exists at 90°, the highest temperature at which the absorbance was measured. (This figure is consistent with approximate extinction coefficient measurements at 90° and is based primarily on the fact that theoretical curves calculated to fit the data at lower temperatures always showed hypochromicities of 0.013 to 0.025 at 90°.) The data will be given here as relative hypochromicities, $\bar{G}/G_\infty$, where G_∞ was found to be 0.253 from the absorbance of high molecular weight poly-A at 2°, making the correction for residual hypochromicity.

Values of G_k/G_∞ were determined from the hypochromicities near 0° of samples whose degree of polymerization $N\ (= k + 1)$ varied from 2 to 11. The data are found to follow the empirical relation

$$\frac{G_k}{G_\infty} = 1 - \frac{2.32}{k+1} + \frac{3.82}{(k+1)^2} \qquad (22)$$

for $k \geq 2$. A comparison of the experimental data with eq 22 is given in Table I. In applying eq 2, eq 22 is used as a smoothing function and as a means of extrapolating to longer helix lengths. For terms in $k = 1$, the experimental value $G_1/G_\infty = 0.524$ is used. It is of interest that most of the values of G_k/G_∞ are in approximate agreement with those calculated by Rich and Tinoco[21] for two-stranded helical oligomers.

Table I. Relative Hypochromicites of Various Oligomers

	G_k/G_∞	
k	Exptl	Eq 22
1	0.524	. . .
2	0.647	0.651
3	0.664	0.659
4	0.710	0.689
5	0.677	0.720
7	0.639	0.770
8	0.800	0.789
9	0.801	0.806
10	0.769	0.821

The above theory gives $\bar{G}/G_\infty$ for a given N as a function of s and σ. For each experimental value of $\bar{G}/G_\infty$, the theoretical equations were solved numerically (using an IBM 7094 computer at the Columbia University Computer Center) to give s for each of several trial values of σ. For each sample the data were taken only from the temperature region where $\bar{G}$ varies rapidly with temperature, since s is determined most accurately in this region. From the temperature dependence of s, values of the standard enthalpy $\Delta H°$ and standard entropy $\Delta S°$ for formation of the helix from the random coil were determined corresponding to each trial value of σ. If the experimental data rigorously followed the theory, one would expect to find a unique σ, $\Delta S°$, and $\Delta H°$ valid for all samples. Unfortunately, the calculated data did not reveal an obviously unique solution, most likely owing to experimental errors, but it was possible to determine values of the parameters that give a reasonable fit to most of the data. These are $\sigma = 0.6$, $\Delta H° = -9.4$ kcal/residue-mole, and $\Delta S° = -29.3$ cal/deg residue-mole. In Figure 2 the theoretical curves for these parameters are shown in comparison with experimental data for a group of samples felt to be representative. Of the samples omitted, those with $N = 6$ and 8 showed poorer agreement than illustrated here. However, the fact that reasonable agreement is obtained for most of the samples supports the validity of our analysis. The uncertainties in the parameters used can be estimated from the fact that $\Delta H°$ and $\Delta S°$ can be varied by perhaps as much as

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[20] Equation 20 gives the exact partition function for the Ising model of finite length. To our knowledge it was given first by Zimm and Bragg,[15] although its existence has been known since Ising[14] described the major features of the derivation in 1925.

[21] A. Rich and J. Tinoco, Jr., *J. Am. Chem. Soc.*, **82**, 6409 (1960).

20%, and σ by as much as 50%, before the over-all fit between experiment and theory becomes clearly poorer.

Van Holde, *et al.*,[12] obtained $\Delta H^\circ = -8.4$ kcal and $\Delta S^\circ = -28.5$ eu from their study of diadenylic acid in 0.1 M NaCl + 0.1 M tris(hydroxymethyl)aminomethane, pH 7.4. Poland, *et al.*,[13] obtained $\Delta H^\circ = -6.5$ kcal, $\Delta S^\circ = -21.4$ eu, and $\sigma = 0.7$ from their study of oligo-A's in 0.15 M KCl + 0.1 M phosphate buffer, pH 6.83. The agreement among these estimates is as good as might be expected, considering that in all cases it is necessary to make some sort of extrapolation to determine the values of the measured quantities for the complete helix and the complete random coil. There is good agreement as to the degree of cooperativeness in this system, supporting the hypothesis that the freedom of rotation about the Cl′–N bond should give rise to a cooperative effect.

The fraction of possible S bonds formed, f, is also shown in Figure 2 for the "infinite" polymer, as given by the equation[8]

$$f = {}^1/_2 + (s - 1)/2[(1 - s)^2 + 4\sigma s]^{1/2} \qquad (23)$$

taking

$$s = \exp\left(\frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT}\right) \qquad (24)$$

As mentioned above, this curve would coincide with that for $\bar{G}/G_\infty$ only if G_k were proportional to $k/(k + 1)$.

Discussion

Thermodynamics. The thermodynamic parameters obtained here invite comparison with those found by a similar analysis of data for the two-stranded helix–coil equilibrium.[10] The necessary data are summarized in Table II. C represents a nucleotide residue in the random-coil form, Hx_1 a residue in a one-stranded helix, and Hx_2 a pair of residues in a two-stranded helix. The fraction of adenine groups protonated is given in parentheses following the symbol. Citric acid is represented as H_3Ct. The polynucleotides are understood to be in their hypothetical standard states of unit activity. Where hydrogen ion appears, its activity is $10^{-4.00}$, since the reactions involving H^+ are relevant to reaction I at pH 4.00.[10] For simplicity, we assume that only the second ionization of citric acid is involved at this pH. (The pK_a's are 3.13, 4.76, and 6.40.[22] All ionizations play some role in the actual system, but the approximation made here is not serious because the enthalpies are very similar for all three.) From titration data[23] it can be estimated that the concentration of H_2Ct^- is 0.0132 M and that of HCt^{2-} is 0.0018 M at pH 4.00. These concentrations may be understood wherever each substance appears, although the precise values do not affect the calculations. The ΔS and ΔH values are not standard values in I, III, IV, and V, except in the sense that the polynucleotide species are in their standard states. Uncertainties shown for the thermodynamic quantities are estimated limits of error.

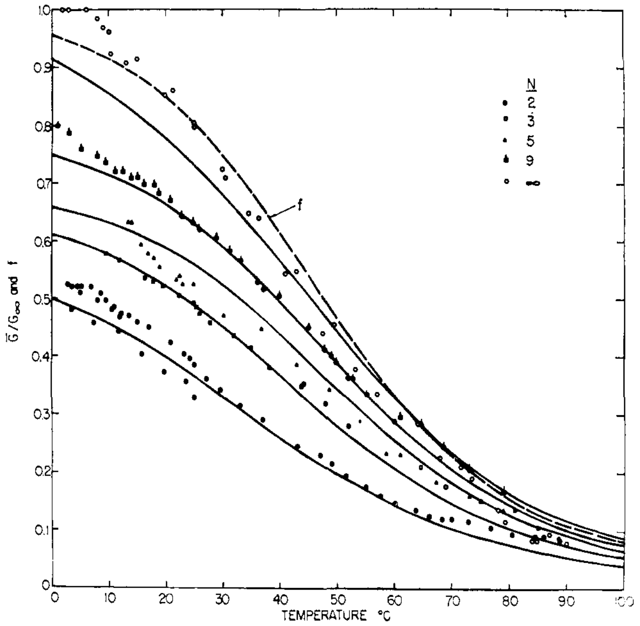

Figure 2. Relative hypochromicities of oligo- and polyadenylic acids; data of Fresco, *et al.*,[11] at 257 mμ in 0.15 M NaCl + 0.015 M sodium citrate, pH 7.3. Solid curves are calculated from eq 2 using $\Delta H^\circ = -9.4$ kcal/residue-mole, $\Delta S^\circ = -29.3$ eu, $\sigma = 0.6$, for the chain lengths shown. The fraction of stacking interactions formed, f, was calculated from eq 23, using the same parameters.

We wish to focus attention of reaction VI, for which the data are obtained as shown from those for reactions I through V. Reaction VI may be thought of as the transfer of a residue from a one-stranded helical state at the end of a two-stranded helical segment to the two-stranded helical state by pairing with its mate in the opposite strand, the degree of protonation being zero in both states. It is interesting that a positive ΔS of about 17 eu is associated with this change. It appears unlikely from the structures that a significant entropy change in the polymer would occur, since there is no internal rotation in either state. We therefore conclude that the 17-eu

Table II. Thermochemical Data for One- and Two-Stranded Helix–Coil Transitions in Polyadenylic Acid

		ΔS, eu	ΔH, kcal
(I)	$C(\alpha') + (\alpha - \alpha')H_2Ct^- \rightarrow {}^1/_2\,Hx_2(\alpha) + (\alpha - \alpha')HCt^{2-}$	-10.5 ± 1^a	-4.0 ± 0.4^a
(II)	$C(0) \rightarrow Hx_1(0)$	-29.3 ± 6^b	-9.4 ± 2^b
(III)	$C(1) \rightarrow C(0) + H^+$	$+\ 9.8 \pm 0.8^c$	$+2.64 \pm 0.2^d$
(IV)	${}^1/_2Hx_2(1) \rightarrow {}^1/_2Hx_2(0) + H^+$	$+\ 3.7 \pm 0.5^c$	$+2.4 \pm 0.2^e$
(V)	$H_2Ct^- \rightarrow HCt^{2-} + H^+$	$+\ 3.0 \pm 0.07^f$	$+0.90 \pm 0.02^g$
(VI)	$Hx_1(0) \rightarrow {}^1/_2Hx_2(0)$	$+16.7 \pm 8^h$	$+6.0 \pm 2.7^h$
(VII)	$C(0) \rightarrow {}^1/_2Hx_2(0)$	-12.6 ± 2^i	-3.4 ± 0.7^i

[a] Reference 10. [b] This work. [c] Calculated from $\Delta S = \Delta H/T + 2.3R(\text{pH} - \text{p}K_a)$, with pH 4.00. For III, pK_a is taken as 3.8, as found for adenosine phosphates: D. O. Jordan, "The Chemistry of Nucleic Acids," Butterworths, Washington, D. C., 1960, p 137. For IV, pK_a is taken as 4.95, estimated from pK_a = pH + log $\alpha/(1 - \alpha)$, with $\alpha = 0.9$ at pH 4.00.[6] [d] Value reported for 5′-deoxyriboadenylic acid: M. Rawitscher and J. M. Sturtevant, *J. Am. Chem. Soc.*, **82**, 3739 (1960). [e] Determined in 0.1 M KCl.[26] [f] Calculated from $\Delta S = \Delta H/T$, since equilibrium exists and $\Delta G = 0$. [g] From heat of neutralization of monosodium citrate at infinite dilution (−12.8 kcal/mole) [T.W. Richards and B. J. Mair, *J. Am. Chem. Soc.*, **51**, 740 (1929)] and heat of ionization of water (+13.7 kcal/mole). Corrections to appropriate concentrations with heats of dilution were negligible. [h] (VI) = (I) − (II) − α'(III) + α(IV) − ($\alpha - \alpha'$) (V); $\alpha = 0.9$, $\alpha' = 0.4$ (footnote *c*). [i] (VII) = (II) + (VI).

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(22) R. G. Bates and G. D. Pinching, *J. Am. Chem. Soc.*, 71, 1274 (1949).

(23) National Research Council, "International Critical Tables," Vol. 1, McGraw-Hill Book Co., Inc., New York, N. Y., 1926, p 82.

change arises from an increased disorder of the solvent molecules upon formation of the two-stranded helix. The process is perhaps similar in some respects to the transfer of an aromatic hydrocarbon from aqueous solution to the liquid phase, for which the entropy change is in the range of 14 to 20 eu for a number of substances.[24] (The close agreement with our value is no doubt fortuitous.) The analogy suggests that hydrophobic bonding is a contributor to the stability of the two-stranded helix, as has been suggested by several authors.[25] It should be noted that the observed entropy change could arise either from an unusual degree of solvent disorder in the vicinity of the two-stranded helix or from an unusual degree of order in the vicinity of the one-stranded helix.

The origin of the 6-kcal enthalpy for reaction VI is less obvious, but it presumably has contributions from base-pairing interactions, electrostatic repulsions between strands due to the charged phosphate groups, and reorganization of hydrogen bonds in the solvent.

Reaction VII represents the transition of a residue in the randomly coiled state at the end of a two-stranded helical segment to the two-stranded helical state, the degree of protonation being zero in both states. The data are included here because we expressed reluctance to pursue this calculation in our earlier work.[10] The ΔH of -3.4 kcal is to be compared with the value of -1.55 kcal obtained for the same process by Rawitscher, *et al.*,[26] from calorimetric data on poly-A in 0.1 M KCl. The discrepancy is probably real, since it is beyond the estimated limits of experimental error. The value of -3.4 kcal was obtained at a higher ionic strength (0.15 M NaCl + 0.015 M sodium citrate); therefore, the greater electrostatic shielding between strands would be expected to produce a discrepancy in the observed direction.

On the Cooperative Effect. An important feature of the system studied here is the small magnitude of the cooperative effect. Thus the thermal transition is relatively broad, even though the enthalpy for the process is fairly large. It is evident that a large stacking enthalpy is not, by itself, capable of producing a cooperative effect, as Poland, *et al.*,[13] have also noted.

This conclusion is interesting in the light of the finding by Crothers and Zimm[27] that, in the case of a two-stranded helix, the free energy of stacking is a major contributor to the cooperative effect. This is a question that deserves more discussion than we are able to give here, but it should be clear that the conclusions of Crothers and Zimm do not apply to the one-stranded case.

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Appendix

The Noncooperative Case. If the helix–coil equilibrium were completely noncooperative, all S bonds would form and break independently of their neighbors. The fraction of S bonds formed, f, is therefore the probability that any S bond be formed, regardless of the state of its neighbors. From simple probability theory it follows that

$$\langle \nu_k \rangle = (N - k - 2)f^k(1 - f)^2 + 2f^k(1 - f)$$
$$k = 1, 2, \ldots, N - 2 \qquad (25)$$

where the first term on the right is the average number of sequences of k S bonds per molecule other than those such sequences located at the ends, and the second term is the number of such sequences located at the ends. Equation 25 may be rearranged in the form

$$\langle \nu_k \rangle = (N - k)f^k(1 - f)^2 + 2f^{k+1}(1 - f)$$
$$k = 1, 2, \ldots, N - 2 \qquad (26)$$

For the completely bonded case we have

$$\langle \nu_{N-1} \rangle = f^{N-1} \qquad (27)$$

The noncooperative case corresponds to the Ising model with $\sigma = 1$. Under this condition, $\lambda_0 = 1 + s$, $\lambda_1 = 0, Z_N = (1 + s)^{N-1}$, and

$$f = \frac{1}{N-1}\frac{\partial \ln Z_N}{\partial \ln s} = \frac{s}{1+s} \qquad (28)$$

Equation 28 was shown by Zimm and Bragg[15] to hold for the infinitely long chain and is seen to be also valid for finite chains, as one might expect. It may be readily verified that for $\sigma = 1$, eq 10, 19, and 20 reduce to eq 26 and 27, taking into account eq 28, and taking care to note that the expression λ_1^{N-k-1}, which appears in eq 19, is unity for all σ when $k = N - 1$, but is zero for $\sigma = 1, k \neq N - 1$.

Postscript. Two additional studies[28,29] of the helix–coil equilibrium discussed here appeared at the time of completion of this manuscript. In addition, our attention has been directed to an earlier analysis of the system.[30] In all of these studies, the system is assumed to behave noncooperatively. The thermodynamic parameters obtained by Brahms, Michelson, and Van Holde[29] appear to be consistent with this assumption. The data of Leng and Felsenfeld[28] would, however, be consistent with a small degree of cooperativeness, as we and Poland, *et al.*,[13] have found. Taking this into account, the magnitude of $\Delta H°$ found by Leng and Felsenfeld agrees well with our value. Appropriate comments on the experimental errors encountered in this type of study have been made by Leng and Felsenfeld, and to some extent their remarks apply to all of the methods reported to data. Most of the apparent discrepancies in results will probably be traceable to such errors, although the weight of the evidence seems to favor the existence of a detectable cooperative effect. While we cannot claim to have improved the precision in the thermodynamic parameters with the present study, we hope that our contribution of another paper on the subject is justified by our description of a theoretically rigorous method of interpreting the data.

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