Thermodynamic Investigation of the Helix–Coil Transition

of a Polyribonucleotide System

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Some relative thermodynamic state functions of various types of structure which occur in the nucleic acid model system consisting of polyriboadenylic acid (poly A) and polyribouridylic acid (poly U) have been determined using the method of adiabatic calorimetry. Polymer concentrations have been calculated from the content of organic phosphorus of the components of the samples. The content of organic phosphorus has been determined by means of a chemical procedure following the method of Asmus and Baumert (1968). Since poly A is forming an intramolecular secondary structure in the temperature range covered by the calorimetric measurements, the measured transition enthalpies have been extrapolated to about 95°, where poly A exists almost entirely in the randomly coiled state. This extrapolation yields the values $\Delta H°$ (A + U) = 9.3 ± 0.5 kcal/mol of (A + U) for the helix–random coil transition of the helical duplex poly(A + U), and $\Delta H°$(A + 2U) = 13.5 ± 0.5 kcal/mol of (A + 2U) for the dissociation of the three-stranded complex poly(A + 2U). The calorimetric procedure (measuring continuously the heat capacity of the polymer solution as a function of temperature) involves the experimental determination of the fraction of structural change. Therefore the thermal helix–coil transition of the double-stranded poly(A + U) has been analyzed in terms of current theoretical approaches. Following the calculation procedure of Applequist (1967), the stacking parameter introduced by Crothers and Zimm (1964) is found to have a value of about 400, which is considered to be representative for this double-stranded polyribonucleotide system. Additionally, numerical values of some (average) properties of poly(A + U) at the conversion temperature are given. Appropriate interpolation along measured transition enthalpies yields an approximate value of $\Delta H°$(A) = 4.5 ± 2 kcal/mol of (A) for the transition of neutral poly A.

I. Introduction

Many reactions of biological significance are coupled with conformational changes of biopolymer molecules. The cooperative helix-to-random coil transition of the secondary structure observed in particular in nucleic acids and proteins may be considered as one of the most important processes in this field. Mutual structural conversions of this kind are postulated, for instance, to explain essential steps of the molecular mechanism of biological information transfer.[2]

Various types of secondary structure analogous to those found in natural biopolymers can be seen to occur in simple synthetic macromolecules, for instance polynucleotides which, in the simplest case, consist of a linear array of only one nucleotide residue. Due to uniform primary structures these polymers are quite suitable for quantitative investigations on the relative thermal stability of different conformations and for a theoretical analysis of the transition behavior of ordered polymers.

The synthetic polynucleotides polyriboadenylic acid (poly A) and polyribouridylic acid (poly U) represent a model system in which, under appropriate environmental conditions, some essential types of secondary structure found in natural nucleic acids are formed.[3] Poly A and poly U associate in aqueous salt solution to a double-stranded helical complex poly(A + U) and also to a three-stranded macromolecule poly(A + 2U). The

bihelical structure of poly(A + U) possesses some principal features of that of DNA and is quite similar to the double-stranded intercepts proposed in the transfer RNA's. A three-stranded complex has been discussed as a possible model for intermediary interaction between DNA and messenger RNA.[4] Poly A alone forms at neutral pH a single-stranded helix with stacked bases representing a model for single-stranded nucleic acids.[5–7]

It is possible to change thermally the secondary structure of polynucleotide molecules. One of the most convenient methods of following structural variations in this field is the measurement of the ultraviolet absorption as a function of temperature. In the case of cooperative transitions of structure the plot of the optical density at an appropriate wavelength vs. temperature often shows a characteristic approximately sigmoid profile within a limited range of temperature.

Heat effects accompanying thermal conversions of the

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(2) *Cf., e.g.,* J. D. Watson, "Molecular Biology of the Gene," W. A. Benjamin, Inc., New York, N. Y., 1965.

(3) R. F. Steiner and R. F. Beers, Jr., "Polynucleotides," Elsevier Publishing Co., Amsterdam, 1961.

(4) G. Felsenfeld and A. Rich, *Biochim. Biophys. Acta,* **26,** 457 (1957).

(5) (a) J. Brahms, *Nature,* **202,** 797 (1964); (b) J. Brahms and W. F. H. M. Mommaerts, *J. Mol. Biol.,* **10,** 73 (1964).

(6) C. C. McDonald and W. D. Phillips, *Science,* **144,** 1234 (1964).

(7) D. N. Holcomb and I. Tinoco, Jr., *Biopolymers,* **3,** 121 (1965).

cooperative type can be measured directly using the method of adiabatic calorimetry.[8,9] With the aid of measured transition enthalpies some relative thermodynamic-state functions of appropriate systems can be evaluated.

Since this calorimetric procedure involves the experimental determination of the fraction of structural change, it is possible to compare the measured thermal transition curves of the appropriate biopolymers with those calculated according to a theoretical model. In this way the thermal helix–coil transition of the poly-(A + U) double helix has been tentatively analyzed in terms of current theories resulting in numerical values for some characteristic parameters of this cooperative conversion.

Additionally, it can be shown that, at least in the case of the thermal conversion of poly(A + U), the course of the ultraviolet absorption with temperature differs considerably from the thermal transition curve obtained calorimetrically.

II. Calorimetric Method

The experimental method leading to the determination of the polynucleotide system is based on the continuous measurement of the heat capacity of the biopolymer solution as a function of temperature. The heat capacity measurements were carried out with recording adiabatic calorimeters. For a detailed description of the apparatus and experimental procedure, the reader is referred to the literature.[10–12]

A nearly linear dependence of the measured heat capacity on temperature was obtained for the pure solvent in the temperature range between room temperature and 100°. When the measurements were carried out with a solution of an appropriate biopolymer, however, for instance with a solution of poly(A + U) at neutral pH and low ionic strength, a characteristic peak appeared in the heat capacity vs. temperature curve in the same limited temperature range in which the ultraviolet absorption at 260 mμ of the solution showed an approximately sigmoid profile. This is shown in Figure 1; see also Figure 1 of ref 13. Obviously, the characteristic peak in the heat capacity vs. temperature curve is caused by the thermal conversion of the dissolved polymer. Hence, the area under the peak (limited by the measured curve and the dashed base line) is proportional to the additional enthalpy change accompanying the thermal conversion of the structure. As outlined in previous communications, the additional contribution to the total heat capacity is correlated to the first temperature derivative of the degree of conversion (cf. ref 10–12). For example, if the helical duplex poly(A + U) is converted to the randomly coiled state of the system, the hydrogen bonds between the complementary bases are disrupted, and the conformational order of the stacked bases is disturbed. If $\bar{m}$ is the
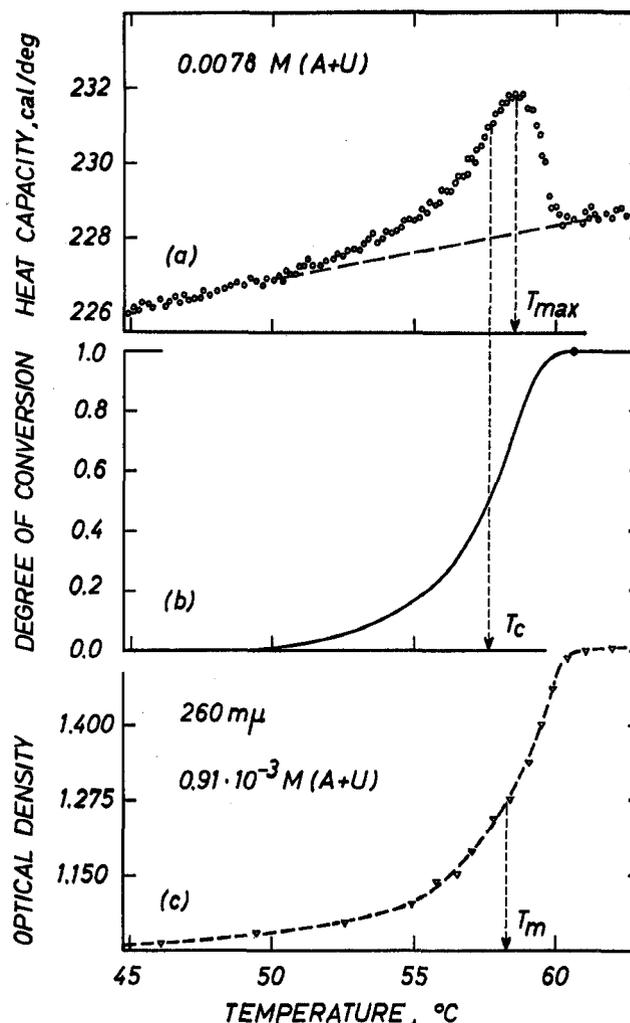
Figure 1.   Course of the helix–coil transition of poly(A + U): heat capacity (a), degree of conversion (b), and optical density at 260 mμ (c) all as a function of temperature (0.15 M aqueous salt solution containing 0.01 citrate, pH 6.8; T_m = midpoint of the change in absorbance).

average number of the base pairs which are in the non-bonded state, the degree of conversion, Θ, is defined as

$$\Theta = \bar{m}/N \qquad (1)$$

where N is the total number of base pairs which can be formed in the double helix. The degree of polymerization, N, is nearly the same for the two components poly A and poly U. As a first-order approxi-

(8) Cf., e.g., Th. Ackermann and E. Neumann, Biopolymers, 5, 649 (1967) (references).

(9) R. Danforth, H. Krakauer, and J. M. Sturtevant, Rev. Sci. Instrum., 38, 484 (1967).

(10) A. J. B. Cruickshank, P. A. Giguère, and Th. Ackermann in "Experimental Thermodynamics," Vol. I, J. P. McCullough and D. W. Scott, Ed., Butterworth and Co. Ltd., London, 1968.

(11) Th. Ackermann in "Biochemical Microcalorimetry," H. D. Brown, Ed., Academic Press, New York, N. Y., 1969.

(12) (a) Th. Ackermann, Z. Elektrochem., 62, 411 (1958); (b) E. Neumann, Ph.D. Thesis, University of Münster, 1967.

(13) E. Neumann and Th. Ackermann, J. Phys. Chem., 71, 2377 (1967).

mation, we assume that the molar transition enthalpy, ΔH, corresponding to the elementary process of structural conversion is temperature independent in the relatively small temperature range in which the cooperative conversion occurs. Thus, if the additional contribution to the heat capacity per mole of the stoichiometric unit (i.e., of the base pair) is C_c, we may write

$$C_c = \Delta H \frac{d\Theta}{dT} \qquad (2)$$

Hence, for a given temperature, the temperature derivative of the degree of conversion can be calculated from the difference between the measured heat capacity vs. temperature curve and the dashed base line (see Figure 1a). Graphical integration yields the Θ vs. T curve (see Figure 1b, for example).

III. The Polynucleotide System

Materials and Preparation of Samples. A brief description of the properties of the materials poly A (sodium salt) and poly U (ammonium salt) is given in a previous communication.[13] The polynucleotide components of the samples were dried under vacuum, and the content of organic phosphorus was determined by means of a chemical procedure following the method of Asmus and Baumert.[14] A weighed amount of the dried samples of poly A and poly U was dissolved in distilled water containing 0.01 M citrate buffer (i.e., at pH 6.8), and an appropriate amount of sodium salt was added. The liquid samples were kept at a constant temperature (0°) for 200 hr in order to achieve equilibrium conditions (cf. ref 15). The polymer concentrations were calculated from the content of organic phosphorus of the components of the samples. All concentrations are given in moles per kilogram of the pure solvent. Polymer concentrations are specified in moles of the appropriate chain units, i.e., (A + U) residue (base pair), (A + 2U) residue, or nucleotide residue, respectively, as indicated in the special case. The total salt concentration refers to moles of monovalent cations including those of the polymers, of trisodium citrate, and of sodium chloride.

The existence of the different conformations occurring in an equimolar mixture of poly A and poly U in aqueous solution at neutral pH depends on temperature and total salt concentration.[16] The helical duplex poly(A + U) formed at moderate salt concentrations and at low temperature converts on heating to the single strands according to the "over-all equation"

$$\text{poly}(A + U) = \text{poly A} + \text{poly U} \qquad (3)$$
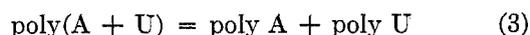
The calorimetric transition curve of such a cooperative conversion is shown in Figure 1a. The peak of the calorimetric curve shows an asymmetric profile with respect to T_c, the temperature of conversion, which is

defined by the temperature at which the degree of conversion equals 0.5

$$\Theta(T_c) = 0.5 \qquad (4)$$

The calorimetric transition curve corresponding to Figure 1a has been evaluated using eq 2. The result is given in Figure 1b. For comparison, the "optical" transition curve measured at 260 mμ is shown in Figure 1c. In this case the "conversion" appears to start at temperatures about 5° lower than indicated by the calorimetric method. An analysis of the recent results of Krakauer and Sturtevant[17] leads to a similar conclusion.

At the salt concentrations employed and in the temperature range considered here, poly U alone is known to be randomly coiled at neutral pH.[2,18] Poly A, however, displays an intramolecular secondary structure dependent on temperature. The thermal conversions of the multistranded complexes involving the liberation of poly A are therefore accompanied by the formation of secondary structure of this component. Because of the heat released in the partial helix formation of poly A, the measured transition enthalpies are reduced values.

The degree of order in neutral poly A does not depend on the salt concentration within relatively wide limits (cf. ref 16 and 19). The region of temperature in which the helix–coil transition of poly(A + U) occurs can be shifted by variation of the salt concentration. Thus, measurements of the heat capacity as a function of temperature carried out with poly(A + U) solutions at various salt concentrations (resulting in different values of T_c) should furnish a set of ΔH values, such that an extrapolation should be possible to about 95°, where poly A exists almost entirely in the randomly coiled state.

Actually, however, the application of this simple procedure is limited. At higher ionic strengths, the heat capacity vs. temperature curve (Figure 2) shows two separate peaks at temperatures where changes in the optical density at 280 mμ are correlated with the formation of poly(A + 2U) and poly A from poly(A + U) and, at higher temperatures, with the dissociation of the three-stranded complex into the single homopolymers. The first small peak observed at lower temperatures is caused by the thermal conversion[16,20]

$$\text{poly}(A + U) = \tfrac{1}{2}\text{poly}(A + 2U) + \tfrac{1}{2}\text{poly A} \qquad (5)$$
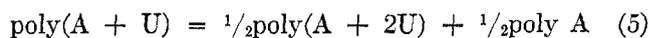
The second peak corresponds to the helix–coil transition of poly(A + 2U) according to

(14) E. Asmus and H. P. Baumert, Z. Anal. Chem., **233**, 252 (1968).

(15) R. D. Blake, J. Massoulié, and J. R. Fresco, J. Mol. Biol., **30**, 291 (1967).

(16) C. L. Stevens and G. Felsenfeld, Biopolymers, **2**, 293 (1964).

(17) H. Krakauer and J. M. Sturtevant, ibid., **6**, 491 (1968).

(18) H. Simpkins and E. G. Richards, ibid., **5**, 551 (1967).

(19) M. Leng and G. Felsenfeld, J. Mol. Biol., **15**, 455 (1966).

(20) H. T. Miles and J. Frazier, Biochim. Biophys. Res. Commun., **14**, 129 (1964).

$$\text{poly}(A + 2U) = \text{poly } A + 2\text{poly } U \qquad (6)$$

The conversion temperatures associated with the various structural conversions, eq 4–6, depend on the total salt concentration. A "phase diagram" (Figure 3) is obtained which resembles that found first by Stevens and Felsenfeld[16] and that obtained by Blake, et al.,[15] in a study of the influence of temperature on the ultraviolet absorption of an analogous set of samples.

Regarding eq 5, strand separation of poly(A + U) has to precede the formation of three-stranded poly-(A + 2U). Thus, the enthalpy change associated with the area under the first peak is only the difference between the enthalpy change involved in the endothermic strand dissociation of poly(A + U) and the ΔH value corresponding to the exothermic attachment of the released poly U to the duplex poly(A + U). Hence the enthalpy change ΔH(add) for the "addition" reaction

$$\text{poly}(A + U) + \text{poly } U = \text{poly}(A + 2U) \qquad (7)$$
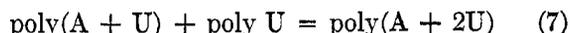
can be calculated from the measured transition enthalpy of poly(A + U) (eq 3) at a conversion temperature equal to the T_c value of the first peak.

Figure 3. Conversion temperatures for various salt concentrations, as obtained by calorimetric measurements ("phase diagram"; see text).

Figure 2. Heat capacity vs. temperature curve for an equimolar mixture of poly A and poly U (concentration of cations: 0.5 M; pH 6.8; see text).
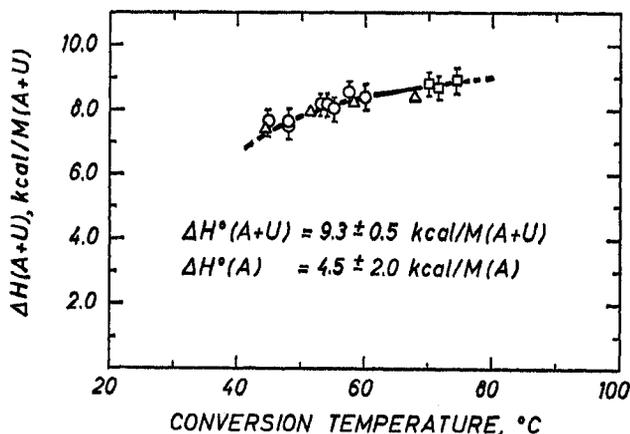
Figure 4. Transition enthalpies of poly(A + U), eq 3, at different conversion temperatures (corresponding to various salt concentrations): $\bigcirc$, measured; $\square$, calculated, see text; $\triangle$, values reported by Krakauer and Sturtevant.[17]

However, the application of the elementary relationships of thermochemistry requires some comments in this case. The electrostatic repulsion between the two negatively charged strands in poly(A + U) is lowered by an increase of the salt concentration. With respect to "hydrophobic" interactions, however, the separated single strands should be preferred at higher ionic strengths (cf. ref 21). We may expect that, to a first approximation, these two effects compensate each other. Thus, we assume that the variation of the measured transition enthalpies of poly(A + U), as shown in Figure 4, is mainly due to the formation of an intramolecular secondary structure of poly A accompanying the conversion process (cf. ref 16).

It turns out that the value of ΔH(add) is fairly insensitive to changes of salt concentration (cf. ref 22).

With the estimated value of ΔH(add), the transition enthalpy of poly(A + U) can then be evaluated from the area under the second peak of the heat capacity vs. T curve at a high conversion temperature where the three-stranded poly(A + 2U) is separated into the single homopolymers. (For the continuous heating method, a low heating rate (less than 0.05°/min) is required to keep the system in a steady state which is essentially the same as that required for equilibrium conditions. In particular, this requirement is im-

(21) See, e.g., G. Némethy, Angew. Chem., 79, 260 (1967).
(22) P. D. Ross and R. L. Scruggs, Biopolymers, 3, 491 (1965).

Table I: Calorimetric Data Obtained at Three Different Salt Concentrations Corresponding to the Upper Part of Figure 3 (cf. Figure 2, for Example)

	Salt concn, mol/kg of H_2O					
	0.46		0.50		0.57	
	First peak	Second peak	First peak	Second peak	First peak	Second peak
T_c, °C[a]	56.1	70.0	54.1	71.6	53.5	74.5
ΔH_o, kcal/mol of (A + 2U)[b]	4.1		3.8		3.8	
ΔH, kcal/mol of (A + 2U)[c]		12.9		13.0		13.1
ΔH, kcal/mol of (A + U) at T_c[d]	8.2		8.1		8.0	
ΔH, kcal/mol of (A + U) at T_c[e]		8.8		8.7		8.9
ΔH(add), kcal/mol of (A + 2U)[f]	−4.1		−4.3		−4.2	

[a]−[c] Measured values; b and c correspond to eq 5 and 6, respectively. [d] Values of Figure 4 (cf. eq 3). [e] Sum of (c) and (f). [f] Difference between d and b. For details of the calculation, see text.

portant for a calorimetric study of the thermal conversion process at low ionic strengths. At the salt concentrations employed here, the rate of the displacement reactions corresponding to eq 5 and 7 should be high enough, so that a heating rate in the range 0.06–0.1°/min can be applied.[23]

IV. Results and Discussion

Preliminary results of a study on the enthalpy changes associated with some conversions of structure occurring in the model system have been reported in a previous communication.[13] We can now present a more complete set of data.

For the transition enthalpy of the double-stranded complex poly(A + U), the results of the measurements and of the calculations (see section III) are shown in Figure 4. Within the present accuracy of the method, our results are in good agreement with those obtained in a detailed study by Krakauer and Sturtevant using a twin-cell differential microcalorimeter.[17]

The measured transition enthalpy ΔH(A + U) related to 1 mol of base pairs increases slightly with increasing conversion temperature. The extrapolation to about 95° yields $\Delta H°$(A + U) = 9.3 ± 0.5 kcal/mol of base pair. This value represents the molar transition enthalpy between the completely ordered poly(A + U) complex and the totally random homopolymers poly A and poly U in aqueous salt solution at neutral pH. The results of the calorimetric measurements obtained for higher salt concentrations are given in Table I. (The preliminary results reported in a brief communication to the editor (cf. ref 13) have been calculated for approximate values of the polymer concentrations which were evaluated from the weight of the polynucleotide samples without further chemical analysis. Hence, a considerable amount of water and impurities has not been taken into account in our preliminary study. The correct values presented in Table I are in fairly good agreement with those obtained by Krakauer and Sturtevant.[17])

As mentioned above, the average value of ΔH(add) (−4.2 ± 2 kcal/mol of (A + 2U) residue formed)

corresponding to eq 7 can be used to evaluate further extrapolated enthalpy data. Concerning the two- to three-strand conversion, eq 5, we have $\Delta H_c° = 5.1 ± 0.5$ kcal/mol of (A + 2U) formed. As to the helix-coil transition of poly(A + 2U) according to eq 6, we find $\Delta H°$(A + 2U) = 13.5 ± 0.5 kcal/mol of (A + 2U).

Poly A. From various studies of the temperature dependence of the optical quantities of the system it has been concluded that neutral poly A is forming a secondary structure which is nearly complete at temperatures in the range between −10 and 0°. At temperatures of about 90–95° poly A is considered to be randomly coiled. Within this range of temperature the profiles of the "optical" transition curves suggest that the thermal conversion of neutral poly A takes place almost gradually with temperature (cf. ref 15, 18, and 24).

Consequently, we can expect that at a temperature of about 42° neutral poly A should have only about 50% of its bases stacked (cf. ref 19 and 24). From the plot in Figure 4 it is obvious that the transition enthalpy for poly(A + U) measured at 42° would be smaller by approximately 2300 cal compared with the extrapolated value $\Delta H°$(A + U). Thus, for the thermal conversion of structure in neutral poly A we find an approximate value of the transition enthalpy $\Delta H°$(A) (4.5 ± 2 kcal/mol of nucleotide residue), as a result of a very rough interpolation. The relatively wide margin of error for $\Delta H°$(A) corresponds to the uncertainties in the estimation of the lower and the upper limiting value for the degree of stacking in neutral poly A. Since the secondary structure of neutral poly A arises from base stacking interactions, $−\Delta H°$(A) is equal to the molar stacking enthalpy. The numerical value of $\Delta H°$(A) is somewhat lower than that obtained by Epand and Scheraga[25] in a series of calorimetric measurements.

(23) (a) R. D. Blake, L. C. Klotz, and J. R. Fresco, *J. Amer. Chem. Soc.*, **90**, 3556 (1968); (b) R. D. Blake and J. R. Fresco, *J. Mol. Biol.*, **19**, 145 (1966).

(24) M. Leng and A. M. Michelson, *Biochim. Biophys. Acta*, **155**, 91 (1968).

Table II: Standard Values of the Relative Molar State Functions of the Model System (The Numerical Values Are Related to the Corresponding Structure Unit; See Text)

Relative state functions	poly(A + U)	Helix–coil transition of poly(A + 2U)	poly A
ΔH°, kcal/mol	9.3 ± 0.5	13.5 ± 0.5	4.5 ± 2
ΔS°, cal/mol deg	25.2 ± 1.5	36.8 ± 1.5	14.3 ± 6
$\Delta G^\circ(298)$, kcal/mol	1.8 ± 0.2	2.5 ± 0.3	0.25 ± 0.8

If the thermal conversion of structure of neutral poly A is considered as a slightly cooperative equilibrium of the isomeric states of the adenine bases being stacked or unstacked, the results of theoretical treatments of Gibbs and DiMarzio,[26] Zimm and Bragg,[27] and Applequist[28] can be used to calculate a "cooperative parameter." In this context, the cooperative parameter σ is related to the transition enthalpy $\Delta H^\circ(A)$ and the so-called van't Hoff heat of transition by

$$\Delta H(\text{van't Hoff}) = \Delta H^\circ(A)/\sigma^{1/2} \qquad (8)$$

If the temperature dependence of the optical quantity (*i.e.*, optical density or rotatory power) measured in the special case is roughly proportional to the temperature dependence of the degree of conversion (at least at the transition temperature), an approximate value of ΔH(van't Hoff) can be evaluated from the slope of the optical transition curve. (See, however, the differences between the calorimetric and the "optical" transition curves, as shown in Figure 1, for example.) The values of ΔH(van't Hoff), as reported in the literature[16,19,24,29] cover the range from about 6 up to 13 kcal/mol of nucleotide residue.

If an average value of 8000 cal is inserted in eq 8 for ΔH(van't Hoff), one obtains $\sigma \simeq 0.3$. Since the calculation of σ is based on a set of roughly estimated data, the physical meaning of this numerical value may be questionable. Nevertheless, our approximate values of $\Delta H^\circ(A)$ and σ may be compared with those reported by Poland, *et al.*,[30] and by Applequist and Damle,[31] but the estimated value of σ can only be interpreted as the slightest suggestion that σ is less than unity.

Relative State Functions. Cooperative conversions of structure similar to those occurring in the model system may be classified as "diffuse" phase transitions. With respect to thermodynamics these conformational conversions are usually treated analogous to phase transitions of first order. At the conversion temperature of such a cooperative conversion, the free enthalpy of conversion equals zero, $\Delta G(T_c) = 0$. The entropy change at T_c is therefore given by $\Delta S(T_c) = \Delta H/T_c$. If we assume that ΔS calculated in this manner is to a first approximation independent of temperature, we are able to determine $\Delta G(298°K)$. The results of these calculations for the three model structures discussed here are summarized in Table II.

Referring the entropy changes to 1 mol of nucleo-

tide residue we obtain a value of about 12–19 eu with respect to all three types of structure. This agrees well with the calculation of Longuet-Higgins and Zimm,[32] who have predicted a value between 14 and 28 eu for the configurational entropy change associated with the helix–coil transition.

It should be mentioned that the quantities of Table II reflecting the thermodynamics of the complex helix–coil transitions are relative state functions. They correspond quantitatively to variations of the total system, biopolymers in aqueous salt solution. In this context, we remember that the values of the molar transition enthalpies are obtained as the additional enthalpy changes from the additional heat capacity data of the polymer solution. The measured enthalpy change is therefore immediately related to the additional increment of the apparent molar enthalpy of the dissolved polymer.

The thermodynamic state functions used in theoretical treatments are partial molal quantities. Since in a recent study[33] a dependence on concentration of the measured molar transition enthalpies over a range of about 10^{-2} to 10^{-3} M could not be detected, we consider the measured ΔH values to be independent of polymer concentration. Since the relatively small polymer concentrations are not changed during the thermal conversions studied with our calorimetric method, the concentration-dependent terms which occur in the relationship between the apparent and partial molal transition enthalpies are comparatively small.[34] Thus, we use the extrapolated values of the measured transition enthalpies as the standard values of the

(25) R. M. Epand and H. A. Scheraga, *J. Amer. Chem. Soc.*, **89**, 3888 (1967).

(26) J. H. Gibbs and E. A. DiMarzio, *J. Chem. Phys.*, **30**, 271 (1959).

(27) B. H. Zimm and J. K. Bragg, *ibid.*, **31**, 526 (1959).

(28) J. Applequist, *ibid.*, **38**, 934 (1963).

(29) J. Brahms, A. M. Michelson, and K. E. Van Holde, *J. Mol. Biol.*, **15**, 467 (1966).

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

(31) J. Applequist and V. Damle, *J. Amer. Chem. Soc.*, **88**, 3895 (1966).

(32) H. C. Longuet-Higgins and B. H. Zimm, *J. Mol. Biol.*, **2**, 1 (1960).

(33) H.-J. Hinz, O. J. Schmitz, and Th. Ackermann, *Biopolymers*, in press.

(34) *Cf.*, *e.g.*, G. N. Lewis and M. Randall, "Thermodynamics," McGraw-Hill Book Co., Inc., New York, N. Y., 1961.

partial molar enthalpy changes of the polymers. The total amount of the enthalpy change is correlated to the following processes, all having a share in the double helix–coil transition: breaking of hydrogen bonds; separation of the strands, each assuming a randomly coiled configuration, in which the individual purine and pyrimidine residues are individually immersed in the aqueous solvent and hydrogen bonded to the solvent; abolishing of stacking interactions and changes in the intensity of electrostatic forces. Most of the enthalpy change is associated with the stacking interactions (cf. ref 35). (Up to the present time none of the experimental techniques applied in this field is capable of revealing detailed information on the various contributions to the stabilization energies resulting from different processes. Nmr measurements as performed by McDonald and Phillips[36] may help to resolve this problem.)

V. Helix–Coil Transition of Poly(A + U)

The theoretical analysis of the cooperative conversions investigated experimentally has been restricted in this section to the helix–coil transition of poly(A + U), eq 3. Essentially, the results of current theoretical treatments[37–45] of these structural conversions are used in an attempt to interpret the experimental transition curves in terms of some characteristic parameters. The problems arising from the approximative character of these theoretical treatments are discussed in particular by Flory and Miller.[46]

Due to cooperativity, conversion equilibria of this type cannot be described by only a single equilibrium constant. Since the observed "sharpness" of the transition is assumed to be due to cooperative stacking interactions between adjacent base pairs, Crothers and Zimm[40] have introduced a mean stacking parameter, τ, which is related to the molar stacking free energy, ϵ, by

$$\epsilon = -RT \ln \tau \qquad (9)$$

The stacking parameter τ (a measure of the cooperativity) is considered to be constant within the temperature range of the transition. The reciprocal of τ represents the mean nucleation parameter associated with the initiation of a helical sequence from the randomly coiled state of the chains.[41]

By experiment, we obtain immediately the degree of conversion, Θ, as a function of temperature. (See eq 1 and 2 and Figure 1b.) Theoretically, a statistical approach provides finally an expression in which Θ is given as a function of essentially only two parameters, s and τ, where τ itself is explicitly involved in another quantity denoted by σ_j. The terms s and σ_j, originally introduced as statistical weight factors,[38] can be interpreted as "intrinsic" equilibrium constants.

The stability constant, s, represents the equilibrium constant for the process of enlarging an existing helical

sequence of consecutive stacked base pairs by one further pair of bases from the randomly coiled state. The reverse step of conversion of one base pair at the end of a helical sequence from the bonded stack state to the nonbonded unstacked randomly coiled one is correlated to the molar enthalpy change $\Delta H^\circ(A + U)$ for the "elementary process" of the helix-to-random coil transition.

The interruption constant, σ_j, is formally the equilibrium constant for the conversion of a single helical sequence into two helical sequences separated by a random sequence (ring or loop) of a total of j nucleotide residues of both strands without changing the total number of bonded base pairs.[42]

In the case of poly(A + U) a loop may consist of two single-stranded regions in which the number of nucleotide residues in the one-strand intercept differs from that of the other. This possibility is taken into account by the so-called imperfect matching model,[37,40,43] which is used for the calculation of the configurational partition function $Q(N)$ in terms of statistical weight factors for a molecule poly(A + U) with N repeating (A + U) residues.

At constant τ, we obtain the relation[27]

$$\Theta = 1 - \frac{1}{N}\left(\frac{\partial \ln Q(N)}{\partial \ln s}\right)_\tau \qquad (10)$$

With respect to poly(A + U), Litan and Lifson have mentioned a resemblance to a one-dimensional two-component systems,[43] but as the two strands poly A and poly U are assumed to have equal numbers of nucleotide residues, a simple approximation is possible. Concerning the structural change in poly(A + U), the adenylic acid residue is regarded to be functionally equivalent to that of uridylic acid, each one contributing an additional factor $a^{1/2}$ to the partition function.[40,42] Thus, as to the mathematical procedure, the imperfect matching model is treated in the same manner as the perfect matching model (which itself allows only "symmetrical" loops). The differences between the two models are expressed by the special form of a func-

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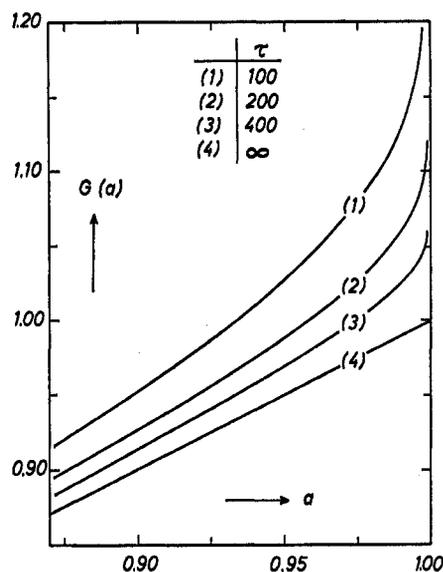
Figure 5. The functions $G(a)$ for some values of τ; see text.

tion $G(a)$ which occurs in the explicit form of the partition function.[39]

The functions $G(a)$ contain the interruption constant σ_j. Quite analogous to the expression of Crothers and Zimm,[40] σ_j is approximated by

$$\sigma_j = \frac{j+1}{\tau(j/2 + 1)^2} = \frac{4(j+1)}{\tau(j+2)^2} \qquad (11)$$

The factor $4/\tau$ is identical with the quantity b_2 introduced by Applequist,[42] and the equations derived by Applequist are used to calculate Θ. Thus, we have (modified in notation)

$$G(a) = a - \frac{4}{\tau}\left[\frac{a}{4} + \ln(1 - a^{1/2}) + \sum_{j=0}^{\infty} \frac{(a^{1/2})^{j+1}}{(j+1)^2}\right] \qquad (12)$$

and, using (12), the explicit form of Θ as a function of s and τ (in parametric representation by the variable $a \leq 1$)

$$\Theta = 1 - \frac{1}{s}\left[a_0 + \frac{2}{\tau}\left(\frac{a_0^{1/2}}{1 - a_0^{1/2}} - \frac{a_0}{2} + \ln(1 - a_0^{1/2})\right)\right]^{-1} \qquad (13)$$

where a_0 is defined by

$$sG(a_0) - 1 = 0 \qquad (14)$$

(cf. ref 39).

In order to obtain a_0, the functions $G(a)$ have been calculated according to eq 12 for some given values of τ in the limiting range of interest. The results are shown in Figure 5.

Recalling the definition of Θ as the fraction of broken bonds and that of s as the "intrinsic" stability constant, we derive at constant τ from the van't Hoff relation

$$\frac{\partial \Theta}{\partial T} = \left(-\frac{\partial \Theta}{\partial \ln s}\right)_\tau \frac{\Delta H}{RT^2} \qquad (15)$$

The term $(-\partial\Theta/\partial \ln s)_\tau$ is directly related to experimental data. The combination of (2) with (15) leads to

$$\left(-\frac{\partial \Theta}{\partial \ln s}\right)_\tau = C_c(T)R\left(\frac{T}{\Delta H}\right)^2 \qquad (16)$$

affording the possibility of comparing theory with experimental results.

A possible procedure is to calculate Θ with the aid of eq 13 using a graphical representation of the solutions of eq 12, Figure 5, and to plot Θ as a function of $\ln s$ for some values of τ. Now, at a given τ, the slope values of the calculated functions $\Theta(\ln s)$ are compared point by point with the right-hand side of eq 16. For instance, we may start the comparison at the "transition point" obtaining the amount of $\ln s$ at T_c. Taking $\ln s(T_c)$ as a "fixed" point, we can calculate the values of $\ln s(T)$ from the experimental curve, Figure 1b, with the aid of the van't Hoff relation. Thus, with respect to Θ both the calculated curve and the experimental data can be associated.

In Figure 6 the plot of Θ as a function of $\ln s$ is shown. The best fit to the points obtained from the experimental transition curve of Figure 1b is given using $\tau = 200 \pm 20$. Hence, according to eq 9, $\epsilon(298°K) = -3.2$ kcal/mol of $(A + U)$.

In addition, the theoretical treatment leads to a set of equations, from which some other molecular properties can be calculated. The average number $\langle h \rangle$ of base pairs in a helical sequence is given by $\langle h \rangle = (1 - a_0 s)^{-1}$.[40] Using $\tau = 200$, we find that at T_c, $\langle h \rangle = 18 \pm 2 (A + U)$ residues.

In the course of the helix–coil transition nonbonded nucleotide residues in the interior of a double-stranded molecule form rings (loops) while at the ends there are "open" single-stranded chain regions. Although theory
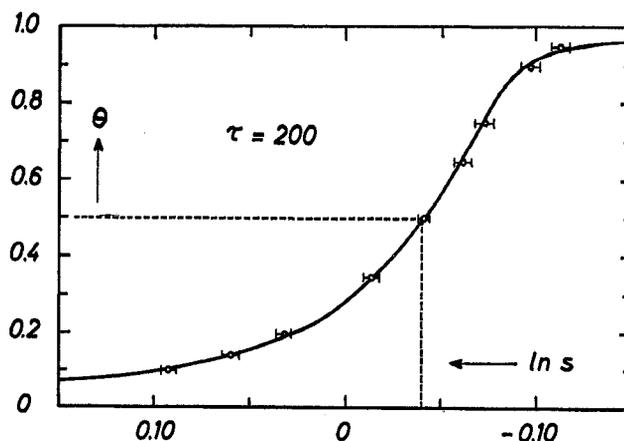
Figure 6. The degree of conversion, Θ, calculated as a function of $\ln s$ at $\tau = 200$; O, calculated from experimental data of Figure 1a and b.

has developed relations valid only for very long molecules where end effects are negligible, it is possible to calculate the mean number $\langle i \rangle$ of pairs of complementary bases at each of both ends, $\langle i \rangle = a_0 s (1 - a_0 s)^{-1}$. At T_c, we have $\langle i \rangle = 17 \pm 2$ pairs of bases in each of the two open ends of a poly(A + U) molecule.

We have found that at lower salt concentration, for instance 0.057 M, the analysis of the corresponding transition curve yields a τ value of about 300. From a tentative extrapolation to zero ionic strength we estimate that τ amounts to about 400. With respect to the special form of the approximation for σ_j, eq 11, this value of the mean stacking parameter, $\tau = 400$, is considered to be representative for this polyribonucleotide system. Hence the mean nucleation parameter is $\tau^{-1} = 2 \times 10^{-3}$ l./mol of (A + U), and $\epsilon(298°K) = -3.6$ kcal/mol of (A + U).

According to the approximation of Crothers and Zimm (cf. eq 15 and 18 of ref 40), the numerical value of τ is obtained from the slope of the Θ vs. T curve at T_c, but as eq 11 of this paper is modified with respect

to the original expression given by Crothers and Zimm, the values of τ evaluated using eq 18 of ref 40 are larger by a factor of about 2 than those given above.

The reliability of numerical values, especially that of τ, depends on the assumption that the polymers used have the required high degree of polymerization.

With regard to the theoretical approach, among the restricting assumptions especially the expression for the statistical weight of a loop depending on its size, eq 11, is rather crude,[40,46] but, in principle, from Figure 6 it can be seen that the course of the thermal helix–coil transition of poly(A + U) can be described using only one additional parameter besides the "intrinsic" stability constant. Thus, the helix–coil transition of poly(A + U) represents a simple model reaction for the relatively complicated cooperative conversion of a double-stranded nucleic acid.

The Radiolysis of Solutions of Ethylene in Ethane[1]

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The radiation chemistry of pure liquid ethane at low doses and of solutions of C_2D_4 in liquid ethane has been studied in order to obtain more information on the relative importance of scavenging of H atoms and of positive ions by olefins in hydrocarbon solutions. At low concentrations of product C_2H_4 or of added C_2D_4, H-atom scavenging is the predominant reaction and the effect of dose on the major products from pure ethane obeys a simple kinetic expression. At higher concentrations of added C_2D_4 an ionic reaction, which appears to be simple charge transfer, becomes increasingly significant. The concentration dependence of this latter reaction is adequately described by an expression which other workers have found to apply to several cases of electron and positive-ion scavenging. A few experiments with added oxygen lead to the conclusion that it acts as a simple radical scavenger only at very low concentrations.

Introduction

The role of solutes in irradiated solutions of liquid hydrocarbons has been studied very intensively in the past few years. It has been shown that several solutes which had been thought to scavenge only free radicals, in fact, also react with electrons, positive ions, or excited species. More specifically, though in several studies with ethylene as solute the results were explained on the basis that ethylene scavenges only hydrogen atoms,[4,5] recent work indicates that an H_2-transfer reaction from hydrocarbon parent ions to ethylene is important.[6,7] From kinetic analysis of hydrogen yields from cyclo-

hexane solutions of propylene, Robinson and Freeman[8] concluded that the results could be quantitatively

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