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A CALORIMETRIC INVESTIGATION OF SINGLE STRANDED BASE STACKING IN THE RIBO-OLIGONUCLEOTIDE A_7 *

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Differential scanning calorimetry has been employed to determine the energy change associated with single stranded base stacking in the ribo-oligonucleotide A_7. A total enthalpy change of 20.3 kcal (mole of heptamer)$^{-1}$ was measured. This corresponds to 2.9 kcal (mole of adenine)$^{-1}$ or 3.4 kcal (mole of A-A stack)$^{-1}$ if one assumes that all six stacking interactions are energetically equivalent. These results represent the first direct determination of this important parameter for a ribo-oligonucleotide. It is noted that the calorimetrically determined value reported here is considerably lower than any of the previously published van 't Hoff enthalpies but is consistent with values that can be derived from other calorimetric data.

1. Introduction

The existence of ordered structure in poly-, oligo-, and even diribo-nucleotides has been known for some time. Since these molecules can serve as models for RNA polymer structure, the detailed nature of their ordered single stranded structure has been the object of much research [1–7]. Most of these earlier investigations have made use of a variety of optical techniques such as ultraviolet and infrared spectroscopy, circular dichroism, and optical rotary dispersion. Other experimental approaches such as NMR, intrinsic viscosity, sedimentation coefficients and low angle X-ray diffraction also have been employed.

The most widely investigated system to date involves polyadenylic acid and oligoadenylic acids of various chainlengths. Practically all reported studies conclude that their results are consistent with the existence of ordered single stranded structure. The temperature dependence of the various observables indicates that the single stranded structure "melts" over a rather broad temperature range. This low degree of cooperativity is usually contrasted with the high degree of cooperativity generally observed for the melting of double-stranded structures.

In order better to understand the molecular forces responsible for such single stranded structure, various statistical treatments have been developed in attempts to derive indirectly thermodynamic information from optical data [4,8–11]. The treatments usually consider the single stranded structure to be the result of "stacking" interactions between adjacent adenine bases. The thermodynamic parameter most readily calculated is the enthalpy change associated with this stacking interaction. It is disturbing to note that the literature contains quite a broad range of values for the enthalpy of stacking between two adenine bases [12]. Certainly an accurate enthalpy value for single-stranded stacking is required if this important form of structure is to be fully understood. As a result, differential scanning calorimetry has beem employed in order to determine directly the enthalpy change associated with the order-disorder transition of ribo-A_7.

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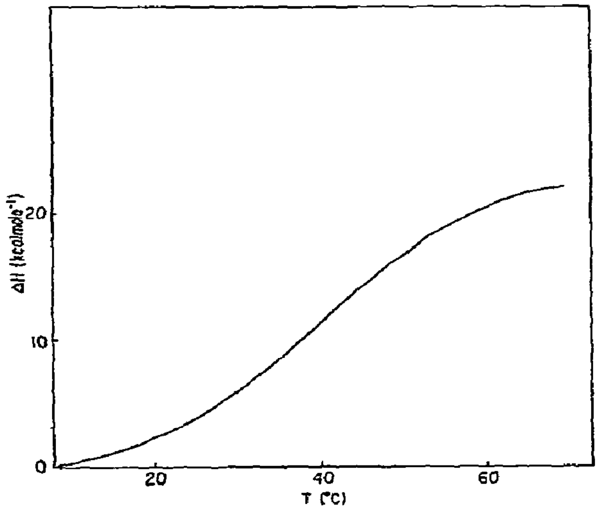

Fig. 1. Variation of calorimetric enthalpy with temperatures for rA_7 in 1M Na^+. The strand concentration is 1.26×10^{-3} M.

2. Materials and methods

2.1. Materials

Synthesis of ribo-A_7. A mixture of $(Ap)_n$ oligomers was obtained by limited alkaline hydrolysis of poly A. This mixture of oligomers was treated with alkaline phosphatase in order to remove the 3' terminal phosphates. The various chainlengths were then tractionated on a DEAE Sephadex A25 column by elution with a linear gradient of 0–0.3 M NaCl in 7M urea. The desired $(Ap)_6A$ peak was identified by paper chromatography and the appropriate tubes were pooled.

2.2. Methods

The calorimetric and optical measurements reported in this paper were carried out in a buffer system consisting of 1M NaCl, 0.01 M sodium phosphate, and 10^{-4} M sodium EDTA, adjusted to pH 7.

The transition calorimetry was carried out at Yale University on an instrument which previously has been described in detail [13,14]. There are two platinum cells, one of which serves as the reaction cell while the other serves as the solvent reference cell. In a given transition experiment, one obtains data at temperature intervals of one minute on the total energy fed back to the reaction cell. These data (along with the known concentration of the solute) permit the construction of an enthalpy $mole^{-1}$ versus temperature curve as shown in fig. 1.

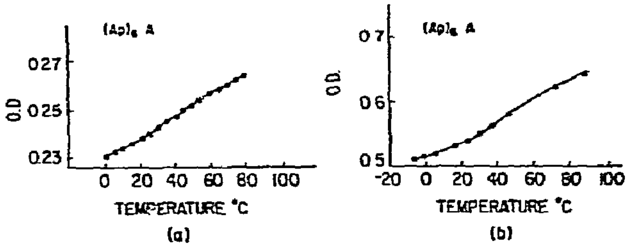

Fig. 2. Equilibrium absorbance versus temperature profiles for the transition of rA_7. (a) in 1M Na^+ monitored at 257 mμ; This work. (b) In 0.1 M Li^+ monitored at 258 mμ; from Leng and Felsenfeld [2].

The concentration of the oligomer was spectroscopically determined by measuring the absorbance at 25° C and using the extinction coefficients reported by Singer et al. [15].

The solution used for the calorimetric experiments reported here had a concentration of 1.26×10^{-3} M in heptamer. This corresponds to 2.8 mg/ml. Based upon the results of previous studies on poly rA [16–18] involving concentrations several times that used in this work, we can conclude that the calorimetric data are not significantly affected by aggregation. Furthermore, no concentration dependence was observed for the UV melting curves obtained in this investigation.

The optical melting curve shown in fig. 2a was measured at 257 nm using an automatic recording spectrophotometer equipped with a programmable, thermoelectrically heated and cooled cell. The temperature was increased at a rate of 0.5°C/min.

Treatment of data. Leng and Felsenfeld [2] previously have studied the thermally induced order-disorder transition of rA_7 by UV spectroscopy. They obtained the melting curve shown in fig. 2b which is very similar to the melting curve obtained in this work (fig. 2a). Both curves exhibit a hypochromic effect of approximately 15% between 0 and 75°C. In order to extract thermodynamic data from such curves they must be subjected to a van 't Hoff analysis. This requires defining points corresponding to 0 and 100 percent reaction; that is, extrapolating the data to low and high temperature "base lines". As emphasized by Leng and Felsenfeld, this assignment undoubtedly represents the limiting factor in the accurate determination of van 't Hoff enthalpies from optical melting curves.

Furthermore, once the baselines are established, one must assume a model for the transition so that the optical curve can be converted into a transition curve reflecting the fractional change in the degree of reaction per unit change in temperature. The shape of this transition curve can then be analyzed so that a value for the van 't Hoff enthalpy change can be calculated. Clearly, the enthalpies obtained by this procedure should be very sensitive to the choice of baselines and, to a lesser extent, to the model selected to describe the transition. Consequently, it is not surprising that the published baseline-dependent and model-dependent van 't Hoff enthalpies for single stranded base stacking cover a rather broad range [12].

In contrast, the calorimetric experiment directly provides a model-independent enthalpy change for the order-disorder transition of rA_7 (see fig. 1). We will refer to the *total* enthalpy change that occurs upon heating a solution of rA_7 from 0° to 70°C. This treatment is preferred to assigning arbitrarily low and high temperature baselines in an attempt to "correct" for any heat capacity contribution from either the initial or the final state. As a result, it might be more appropriate to refer to fig. 1 as a heat up-take curve rather than a transition curve.

If one were to assign to fig. 1 initial and/or final baselines possessing positive slopes, the value for the calorimetric enthalpy accompanying the order-disorder transition would be reduced. Therefore, the value reported here can be considered to be an upper limit.

3. Results and discussion

Table 1 summarizes the results of the calorimetric experiments on rA_7. The reported enthalpy change is simply the total heat absorbed between 0° and 70.0°C as obtained from the calorimetric transition curve shown in fig. 1. This corresponds to 2.9 kcal (mole of adenine)$^{-1}$ or 3.4 kcal (mole of A-A stack)$^{-1}$ if one assumes that all six stacking interactions are energetically equivalent.

Table 1
Calorimetrically determined enthalpy changes

Experiment no.	ΔH (kcal mol^{-1})
1	18.6
2	22.0
average	20.3

The shape of the calorimetric curve has been analyzed as previously described [19] using horizontal baselines to yield an average van 't Hoff enthalpy of 18 kcal. The good agreement between the van 't Hoff and the calorimetric enthalpies reported here is consistent with a transition approaching two-state behavior [20].

Fig. 2 shows the corresponding optical melting curve obtained by Leng and Felsenfeld [2] for rA_7. Significantly, both the calorimetric and the optical melting curves have similar broad shapes. Analysis of the shape of the optical melting curve provided Leng and Felsenfeld with a van 't Hoff enthalpy of 11.4 kcal ("mole")$^{-1}$. In general, published van 't Hoff enthalpies obtained from optical melting curves of oligoadenylic acids and polyadenylic acid range from 5 to 13 kcal (mole of A-A stack)$^{-1}$. Some of these previously published values are presented in table 2.

The significant observation is that in all cases the calculated van 't Hoff enthalpies are considerably larger than the calorimetrically determined enthalpy change reported here. Klump [21] recently reported a similar disparity between calorimetric and van 't Hoff enthalpy data for the order-disorder transition of polyguanylic acid.

Clearly it is of interest to compare our results with previously reported calorimetric data. Since the present study represents the first direct calorimetric investigation of single stranded base stacking interactions in a ribo-oligonucleotide, only indirect comparisons with published calorimetric data are possible.

Rawitscher et al. [22] studied the order-disorder transition of poly A so that they could correct the heat of interaction of poly A + poly U for single stranded stacking in poly A. Their approach involved calorimetric titrations of poly A from neutral to acid pH. After correcting for heats of protonation and using optical data to define the percentage of helical structure, they calculate a value of 2.7 kcal (mole of

Table 2
Some previously published van 't Hoff enthalpies for single stranded stacking between two adenine bases

Compound(s) studied	Solution conditions	Experimental method employed	ΔH (kcal/mole)	Refs.
ApA (A_2)	25.2% LiCl, 0.004M Tris, pH 7.1	ORD melting curve	−5.3	[12]
A_2, A_4 and A_6	0.1M KCl 0.05M phosphate, pH 6.83	ORD melting curve	−6.5	[4]
poly rA		Curve fitting on UV	−7.9	[9]
A_2, A_3, A_5, A_7, A_{12}	0.1M NaCl, 0.01M Tris, pH 7.4	CD melting curves	−7.8 to −8.1	[3]
A_2	25.2% LiCl, 0.004M Tris, pH 7.1	UV melting curve	−8.5	[12]
A_2	1M NaCl, 0.005M phosphate, pH 7.8	UV, CD and ORD melting curve	−8.5	[10]
A_2-A_{11} and poly rA	0.15M NaCl, 0.015M Citrate, pH 7.3	UV melting curves	−9.4	[8]
A_2, A_3, A_7 and poly rA	0.1M LiCl, 0.01M cacodylate, pH 7	UV melting curves	−10 to −13	[2]
poly rA	0.5M $LiClO_4$, 0.002M TES, pH 7.5	ORD and UV melting curves	−11.6	[7]

adenine)$^{-1}$ for the complete transition of poly A from the ordered to the disordered state at 25°C in 0.1 M KCl.

Klump (unpublished manuscript) calorimetrically determined the enthalpy changes accompanying the double strand to single strand transitions of the alternating copolymer poly (A-U) and the homopolymer poly A • poly U. He interpreted the energy difference between the two transitions as being the result of ordered single stranded structure in poly A which does not exist in the single strands of the alternating copolymer poly (A-U). With the aid of optical data for defining the percentage of the helical structure, Klump calculated the enthalpy change associated with the order-disorder transition of poly A to be 2.5 kcal (mole of adenine)$^{-1}$.

Neumann and Ackermann [23] have calorimetrically determined the energy change accompanying the helix to coil transition of the homopolymer poly A • poly U. However, poly A possesses some intramolecular secondary structure in the temperature range covered by the calorimetric experiment. Therefore it was necessary to correct for this additional energy contribution. To this end, Neumann and Ackermann were able to obtain indirectly an approximate value for the single stranded base stacking in poly A of 4.2 ± 2 kcal (mole of adenine)$^{-1}$ by examining the transition enthalpies of poly A • poly U at different conversion temperatures (corresponding to different salt concentrations).

Epand and Scheraga [17] performed a series of calorimetric experiments on poly A from which they indirectly calculated the enthalpy associated with single stranded base stacking. Specifically, they determined the heats of solution of poly A at two temperatures and corrected the data by making heat capacity measurements on the solid and on the solution. With the aid of optical data (used to define the extent of helical structure), these investigators obtained a value of −9 kcal(mole of adenine)$^{-1}$ for the enthalpy change associated with completely disrupting single stranded base stacking in poly A.

Table 3
Calorimetrically derived enthalpies for single stranded stacking between adenine bases

System(s) studied	Solution conditions	ΔH kcal(mole of adenine)$^{-1}$	Refs.
poly(A, U) • poly(A, U) and polyA • polyU	0.1M NaCl	−2.5	Klump
poly A	0.1M KCl, 0.01M cadcodylate, pH 7.0	−2.7	[22]
A_7	1.0M NaCl, 0.01M phosphate, pH 7.0	−2.9	this work
polyA • polyU	0.5M NaCl, 0.01M citrate, pH 6.8	−4.2 ± 2	[23]
polyA	0.1M NaCl, 0.01M Tris, pH 7.3	−9	[18]

The results on the calorimetric experiments just described are summarized in table 3. It is encouraging to note the good agreement between the direct calorimetric results of this investigation and the enthalpies derived from previous calorimetric data. At present it is not clear why the results of Epand and Scheraga are so different from the other studies.

In connection with the results reported here, it is interesting to note the theoretical calculations of Appleby and Kallenbach [11]. These investigators re-analyzed the optical melting curves obtained for short self-complementary RNA oligomers in order to take explicitly into account contributions from single stranded stacking. They describe a fitting procedure whereby the enthalpy changes associated with single stranded base stacking can be extracted from optical melting curves. Significantly they report a value of 3.2 kcal(mole of single strand stack)$^{-1}$ for oligomers of the form A_nU_n where $n = 5$ and 7.

4. Conclusions

It has been shown that van 't Hoff analyses of optical melting curves on oligo and polyadenylic acids consistently result in enthalpy values for single stranded base stacking that are in considerable disagreement with each other and with calorimetrically determined values. This disparity is serious when one considers that such experimental enthalpy data form the foundation of many theories involved with evaluating the relative stability and conformation of single stranded structures. In contrast, the calorimetric experiments described here provide a direct, model-independent determination of the enthalpy change.

These calorimetric studies are being extended to shorter and longer oligomers of adenylic acid in order to investigate the possibility of a chainlength dependence. The results of these experiments should allow us to conclude whether or not the terminal base stacks in single stranded oligoadenylic acids are less stable than the interior base stacks.

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