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# BASE STACKING IN THE DINUCLEOSIDE PHOSPHATE rApA

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The pH-induced unstacking of rApA has been investigated by batch calorimetry and uv spectroscopy. Equilibrium uv melting curves confirmed that the adenine bases in rApA are stacked at pH 7 but unstacked at pH 1.5. The enthalpy change accompanying this pH-induced unstacking is +2.65 kcal (mole of A-A stack)$^{-1}$ as measured by batch calorimetry. This represents the first direct determination of this important parameter for a dinucleoside phosphate. It is noted that the calorimetrically determined value reported here is considerably lower than published van't Hoff enthalpies but is consistent with values that can be derived from calorimetric data on polymers.

## 1. Introduction

Dinucleoside phosphates have been the subject of intense research as models for single-stranded, nearest neighbor base stacking interactions in nucleic acid molecules. Hyperchromicity studies [1] have allowed us to define the relative stacking capacities of the various bases while optical rotatory dispersion revealed that the bases form a right-handed helix [2]. Further optical studies showed the relative orientation of the bases in di- and trinucleosides to be the same as that found in longer oligonucleotides [3]. Thus, a reasonably good qualitative picture of RNA single stranded base stacking has been obtained.

Considerably less success has resulted from attempts to thermodynamically characterize these stacking interactions. The dinucleoside phosphate rApA provides a particularly relevant and disturbing example. The thermally induced order-disorder transition of this compound has been studied extensively by a number of optical techniques [1,4–7]. The resulting transition curves have been subjected to various statistical treatments in attempts to derive indirectly thermodynamic parameters from the optical data. Unfortunately, due to the assumptions involved in such treatments as well as the very broad, non-cooperative nature of the transition curves, these studies have resulted in enthalpy values that range from 5 to 10 kcal (mole of stack)$^{-1}$. This variation is disturbing considering that such enthalpy data provides the basis for calculations which predict the relative stability of various nucleic acid structures.

In an attempt to alleviate this situation, we have initiated a program in which calorimetric techniques are used to directly determine the enthalpy change accompanying the unstacking of the dinucleoside phosphates. In this paper we describe the method and report the results obtained for the isothermal, pH-induced unstacking of rApA.

## 2. Experimental section

### *2.1. Materials*

ApA and 3′AMP were obtained from Sigma Chemical Co. and used without further purification. Solutions for calorimetry were prepared by dissolving the monomer and the dimer in 0.1 M NaCl and titrating to pH 7 using a Corning Model 12 pH meter equipped with a micrometer driven syringe. Concentrations of these solutions were spectrophotometrically determined by measuring the absorption at $\lambda_{max}$ and using the known extinction coefficients [8].

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Fig. 1. Calorimetric heat burst curve for a 1 millicalorie exothermic reaction.

### 2.2. Equipment

The uv melting curves were determined using an automatic recording spectrophotometer (Perkin Elmer 575) equipped with a programmable, thermoelectrically controlled cell compartment. The temperature was increased at a rate of 0.5 deg C/min.

The batch calorimeter is based upon the design of Prosen and Berger and has been previously described in detail [9]. The apparatus basically consists of a bi-compartment cell surrounded by thermoelectric elements embedded within a massive heat sink. The entire instrument is kept in a temperature-controlled environment. A typical experiment involves filling each compartment of the calorimeter cell with aliquots of the reagents of interest. Reaction is initiated by rotation of the entire apparatus which results in mixing of the reagents. Any heat either liberated or absorbed is quantitatively conducted through the thermopiles to the massive heat sink. The output of the thermopiles, which measures the rate of heat transfer, is amplified and recorded.

The calorimeter was chemically calibrated at 28°C using the heat of neutralization resulting from the reaction between Tris and HCl. These experiments provided an average calibration constant of 8.009 $cm^2$/mcal. The useful sensitivity of the instrument proved to be better than one mcal. Fig. 1 shows a trace of a typical heat burst curve for a 1 mcal exothermic reaction.

### 2.3. The method

The approach described here takes advantage of the fact that the bases in rApA at pH 7 are stacked [10–12] while the protonated bases at pH 1.5 are unstacked due to electrostatic repulsion [13]. This established pH dependence of the base stacking in rApA was reconfirmed for the solution conditions of this study by use of uv melting curves. Inspection of fig. 2 reveals that the melting curve for $rA^+pA^+$ at pH 1.5 exhibits none of the hyperchromicity characteristic of base stacking. In contrast, the hyperchromicity of the pH7 melting curve indicates that stacking exists between the two bases although it is not possible to define the actual extent of stacking relative to the hypothetical "fully stacked" state. Indeed, such a specification would require knowledge of all significantly populated partially stacked states along with their weighted contributions to the temperature-dependent uv absorption. As emphasized by Eigen and Pörschke [14], it would not be proper to simply use the fractional absorbance change to define the extent of stacking. Thus,

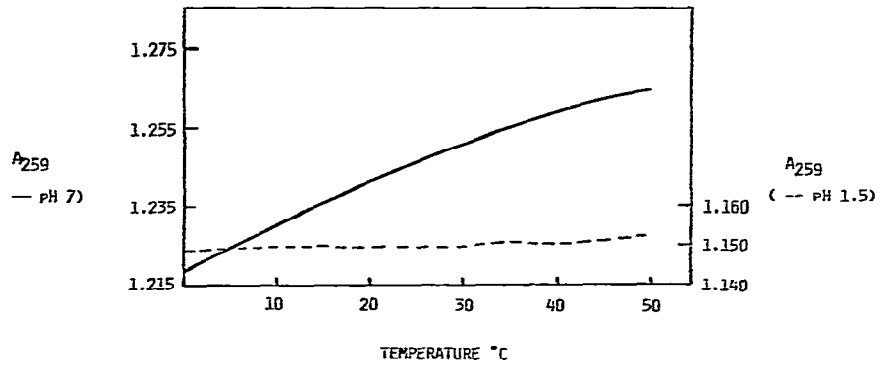
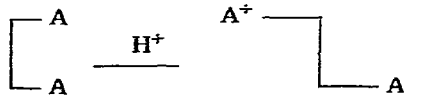



Fig. 2. Absorbance versus temperature curves for rApA at pH 7 (——) and $rA^+pA^+$ at pH 1.5 (– – –).

SCHEME I

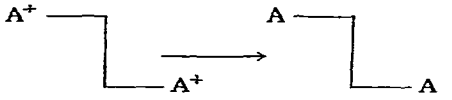
Reaction 1




pH 7 (stacked) pH 2 (unstacked)

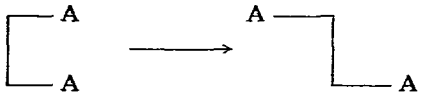
$$\Delta H_1(\text{obs}) = \Delta H_{\text{unst}}^{\text{ApA}} + \Delta H_{\text{prot}}^{\text{ApA}}$$

Reaction 2




$$\Delta H_2(\text{calc}) = -\Delta H_{\text{prot}}^{\text{ApA}} = -2 \times \Delta H_{\text{prot}}^{\text{AMP}}$$

Reaction 3




$$\Delta H_3 = \Delta H_1 + \Delta H_2 = \Delta H_{\text{unst}}^{\text{ApA}}$$

the results reported here correspond to the enthalpy change accompanying the pH-induced disruption of that degree of base stacking which exists in rApA at pH 7 and 28°C.

To determine the enthalpy of unstacking of rApA, a pH-drop experiment was carried out in the batch calorimeter. Typically, 0.2 ml of rApA at pH 7 were mixed with 0.2 ml of HCl to give a final solution of pH 1.5. These experiments yielded a total heat that not only included the pH-induced enthalpy of unstacking of rApA but also the enthalpy of protonation of the two adenine bases. To "correct" for the latter heat effect, we calorimetrically determined the enthalpy of protonation for 3′ AMP and assumed it to be one-half that of rApA. This assumption requires that the enthalpy for addition of the first proton is not significantly influenced by the presence of the adjacent adenine and that the enthalpy for addition of the second proton is not significantly influenced by the presence of the adjacent protonated base. Spectroscopic and potentiometric titration studies on rApA reveal that this is in fact the case [13,15]. Thus, our data can be interpreted in terms of the series of reactions shown in scheme I.

The enthalpy change for reaction 1 [$\Delta H_1$(obs)] is just the calorimetrically measured heat effect accompanying the pH-drop experiment. The enthalpy change for reaction 2 [$\Delta H_2$(calc)] is calculated to be two times the calorimetrically determined enthalpy of deprotonation of 3′AMP. (Use of 5′AMP data provided essentially equivalent results.) The desired enthalpy of unstacking shown for reaction 3 ($\Delta H_3$) is obtained simply by adding the enthalpies of reactions 1 and 2. The data required to carry out this calculation have been calorimetrically determined and are presented in the next section.

## 3. Results and discussion

Tables 1 and 2 summarize the enthalpy data obtained by batch calorimetry for the protonation of 3′ AMP and rApA, respectively. Table 3 shows how these data were used in conjunction with scheme I to calculate the enthalpy of unstacking of rApA at 28°C to be +2.65 kcal mol$^{-1}$.

For the sake of comparison some previously published van't Hoff enthalpies indirectly derived from optical studies on rApA are presented in table 4. The poor agreement between these various van't Hoff enthalpies undoubtedly reflects the difficulties inherent in the model-dependent statistical treatments re-

Table 1
Enthalpy of protonation of 3'AMP in 0.1 M NaCl at 28°C

| Experiment no. | $\Delta H_{prot}^{AMP}$ (kcal mol$^{-1}$) |
|---|---|
| 1 | −2.25 |
| 2 | −2.43 |
| 3 | −2.36 |
| average | −2.35 ± 0.6 |

quired to derive thermodynamic parameters from optical data. The significant observation is that the calorimetrically determined enthalpy value reported here is considerably lower than any of the published van't Hoff values. However, it should be emphasized that the calculated van't Hoff enthalpies listed in table 4 refer to the energy change accompanying the thermally induced transition of rApA over a broad temperature range. In contrast the calorimetrically determined enthalpy change reported here corresponds to the isothermal, pH-induced disruption of that degree of base stacking that exists in rApA at 28°C. Thus, a comparison between the van't Hoff enthalpy data of table 4 and the calorimetric enthalpy data of table 3 may not be justified.

Clearly it would be of interest to compare our results with previously reported calorimetric data. However, since the present study represents the first calorimetric investigation of single-stranded base stacking in a dinucleoside phosphate, only indirect comparisons with published calorimetric data are possible. In making such comparisons one should remember that in the dimer only one-half of the faces of the bases are involved in stacking interactions while in the polymer this ratio approaches unity.

Breslauer and Sturtevant [16] used differential scanning calorimetry to determine the energy change associated with single-stranded base stacking in the

Table 3
Calculated enthalpy of unstacking of rApA in 0.1 M NaCl at 28°C*

| $\Delta H_1$(obs) (kcal mol$^{-1}$) | $-2 \times \Delta H_{prot}^{AMP}$ (kcal mol$^{-1}$) | $\Delta H_{unst}^{ApA}$ (kcal mol$^{-1}$) |
|---|---|---|
| −2.05 | +4.70 | +2.65 ± 0.23 |

* See scheme I for reactions to which enthalpies refer.

Table 2
Enthalpy accompanying a pH drop from 7.0 to 1.5 for rApA in 0.1 M NaCl at 28°C

| Experiment no. | $\Delta H_1$(obs) (kcal mol$^{-1}$) |
|---|---|
| 1 | −1.93 |
| 2 | −2.14 |
| 3 | −1.83 |
| 4 | −2.30 |
| average | −2.05 ± 0.17 |

ribo-oligonucleotide $A_7$. They report an enthalpy change of 3.4 kcal (mole of A-A stack)$^{-1}$ for the thermally induced transition between 0 and 70°C.

Klump (unpublished results) 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 (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 [17] have calorimetrically determined the energy change accompanying the helix to coil transition of the homopolymer poly A · poly U. To correct for the energy contribution due to intramolecular secondary structure in poly A, these investigators examined the transition enthalpies of poly A · poly U at different conversion temperatures between 45 and 75°C (corresponding to different salt

Table 4
Some previously published van't Hoff enthalpies for base stacking in rApA [1].

| Optical property | $\Delta H_{unst}^{ApA}$ (kcal mol$^{-1}$) | Ref. |
|---|---|---|
| Optical rotation | 5.3 | [1] |
| Optical rotation | 6.5 | [5] |
| Circular dichroism | 8 | [4] |
| Hypochroism | 8.5 | [1] |
| Hypochromicity | 9.4 | [6] |
| Hypochromicity | 10 | [7] |

concentrations). From these data Neumann and Ackermann calculated an approximate value of 4.2 ± 2 kcal (mole of adenine)$^{-1}$ for the single stranded base stacking in poly A.

A more direct comparison with the results reported here comes from the work of Rawitscher et al. [18]. These investigators 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 adenine)$^{-1}$ for the complete transition of poly A from the ordered to disordered state at 25°C in 0.1 M KCl.

It is encouraging to note the good agreement between the calorimetric results reported here for rApA and the enthalpies derived from previous calorimetric studies on oligomeric and polymeric systems. One should also note the recent 360 MHz NMR study of Watts, Lee and Tinoco [19] on the base stacking in rApA. These investigators analyzed their temperature dependent NMR spectra by the 3′ endo method and derived a value of 3.5 kcal mol$^{-1}$ for base stacking in rApA. Significantly, this represents the first spectroscopic result that is consistent with the calorimetric data.

## 4. Conclusion

The results reported here provide the first calorimetric determination of the enthalpy change associated with base stacking in a dinucleoside phosphate. Such data is essential for theoretical treatments that calculate the relative stability and conformational flexibility of single-stranded structure. In addition, such data should lead to improved methods of analysis for the extraction of thermodynamic data from RNA melting curves.

These calorimetic studies are currently being extended to other dinucleoside phosphates. The results of these experiments will provide us with nearest neighbor stacking enthalpies as a function of base sequence

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