The Thermodynamics of Proton Dissociation of Adenine

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The thermodynamic quantities associated with ionization of the \( N_1 \) and \( N_9 \) protons of adenine have been calorimetrically determined as a function of temperature. The \( \Delta H \) values for proton dissociation of these groups, with \( pK \) values of 4.19 and 9.92, were found to be 5.1 and 9.1 kcal/mole, respectively, at \( 25^\circ C, \mu = 0.025 \). The \( \Delta C_p \) values for proton dissociation of these groups were estimated to be \(-11\) and \(-17\) cal/mole-deg. These results indicate that the large heat capacity changes observed during conformational transitions of polynucleotides are not the result of ionization of the bases.

KEY WORDS: Adenine; nucleic acid; ionization; enthalpy; heat capacity; calorimetry.

1. INTRODUCTION

There is a great deal of current interest in the thermodynamic characterization of polynucleotide conformation. This interest has been the impetus for a number of studies ranging from calorimetric studies of the denaturation of deoxyribonucleic acid(1) to equilibrium studies of self-association of simple nucleic acids in a variety of solvent systems.(2,3,4,5) Still, the amount of information related to the simplest model systems is sparse. For example, except for the work of Christensen, Izatt, and coworkers,(6,7,8,9) little complete thermodynamic data on the ionization of nucleic acid components are available. The importance of such information in the interpretation of the thermodynamic changes accompanying nucleic acid structural transitions is demonstrated by the following example. Rawitscher et al.(10) suggested that the heat of the coil-to-helix transition for the polyadenylic and polyuridylic acid complex at the melting temperature \( T_m \) is independent of the value of \( T_m \).

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They rationalized that if the heat of transition for DNA were to show a similar independence of $T_m$, the heat capacities associated with proton dissociation for the bases in denatured DNA would have to be approximately $-100$ cal/mole-deg per base pair. The indication that such a large $\Delta C_p$ of ionization existed stemmed from their estimated values of $-80$ cal/mole-deg for proton dissociation of adenine residues in helical polyadenylic acid and of $-110$ cal/mole-deg for adenine as derived from the data of Lewin and Tann. (11) This latter estimate was based on the magnitude of the second derivative of the potentiometrically determined ionization constant of adenine, a number subject to extremely large error. Since these heat-capacity values seemed unusually large for such a process, and since other sources of heat capacity change during polynucleotide transitions exist (interaction of bases with the solvent and variation of the extent of base stacking in the single strand form as a function of temperature), the true thermodynamic changes accompanying ionization of adenine were desired to clarify the issue. The results of such a calorimetric study using a commercially available flow microcalorimeter unequivocally show that the heat-capacity change for ionization of adenine is an order of magnitude too low to account for the heat capacity change observed in double strand–single strand transitions of polynucleotides.

2. EXPERIMENTAL

Adenine was obtained from Mann Research Laboratories and used without further purification. Concentrations were determined from optical-density measurement at 261 nm using a molar extinction coefficient of 13,400. Reagent-grade KCl was used to vary the ionic strength.

Solutions of adenine (4 mM) were made by either dissolving it in freshly boiled, distilled water or in distilled water at room temperature using frequent, vigorous agitation. The solutions were filtered through millipore discs before use. Initial pH values were always $6.70 \pm 0.05$, but gradually rose upon standing to above 7. This change in pH was insignificant, however, if the solutions were used within two days of preparation, which always was the case.

HCl and NaOH solutions were made by dilution of 5 N HCl (Hartman–Leddon) and NaOH (Anachemia Chemicals Ltd.). For basic titrations, all solutions were purged of CO$_2$ by bubbling nitrogen through the liquids and keeping them in a nitrogen atmosphere during experimental runs.

The LKB flow microcalorimeter, model 10711, was used in these experiments, and is based on a design developed by Wadso. (12) The calorimeter was placed in a stainless steel box which was sealed with a Plexiglas top cover on which an access tube was mounted. The box was placed in a water bath whose temperature varied less than $2 \times 10^{-4}$ °C during a 15-min period. The water-bath cover was machined with a special hole to allow the calorimeter