Thermodynamics of left-handed helix formation

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The thermodynamics of right- and left-handed helix formation by poly[d(G-C)]·poly[d(G-C)] and by poly-(dG-m⁵dC)·poly(dG-m⁵dC) were measured spectrophotometrically and calorimetrically. From the spectrophotometric measurements the thermal stabilities of the alternative helical conformations were evaluated as a function of counterion concentration. From the calorimetric measurements the enthalpies of either right-handed or left-handed helix formation were determined. The corresponding experimental ΔH values are -8.6 and -11.2 kcal/mol base pairs for the two conformations in poly[dG-C)]·poly[d(G-C)], and -9.0 and -12.7 kcal/mol base pairs, respectively, for poly(dG-m⁵dC)·poly(dG-m⁵dC).

Enthalpy   Helix formation   Left-handed helix   Calorimetry   Entropy

1. INTRODUCTION

The desire for thermodynamic data on nucleic acids or their polynucleotide analogues reflects the basic interest in defining the nature of the intramolecular and intermolecular forces that contribute to the stability of the various double-helical conformations of DNAs. This interest intensified when it was shown that besides the canonical right-handed double helix, a reversed left-handed helix could exist [1]. Prerequisites for the occurrence of the left-handed structure are either a moderate to high counterion concentration in the solution (>2.5 M Na$^+$) or a mixture of alcohol and aqueous buffer as a solvent [2]. The methylation of the 5'-position in the cytosine ring facilitates the occurrence of the left-handed conformation. Unfortunately, the thermal stability of the ordered secondary structure of polynucleotides that contain exclusively GC base pairs is so pronounced that they can only be denatured at $T_m > 100°C$ at millimolar sodium concentration. In a recent paper the authors demonstrated [4] how to overcome these restrictions for spectroscopic measurements. In the meantime this experimental restriction has also been eliminated to a certain extent for calorimetry [3]. Thus, it will be demonstrated here that the thermodynamics of left-handed helix for-

mation is accessible today, and a comparison with the thermodynamic parameters for the analogous right-handed double-helical conformation is at hand.

2. MATERIALS AND METHODS

Poly(dG-m⁵dC)·poly(dG-m⁵dC) and poly[d(G-C)]·poly[d(G-C)] were purchased from Pharmacia P-L Biochemicals, Freiburg. The samples were dissolved in double-distilled water in the cold and dialyzed against several changes of a suitable buffer solution. Thermal transition curves at 260 nm were recorded with the help of a Pye Unicam model 1800 spectrophotometer, modified to record at 981 kPa [4]. Calorimetric experiments were run on a differential adiabatic scanning calorimeter (model DASM-1M or DASM-4 from Techmashexport, Bergisch Gladbach, FRG). The instruments were assembled by the workshop of the Soviet Academy of Sciences, Institute of Protein Research, Poustchino, Moscow Region, following the design of Privalov [5]. The advantage of the DASM-4 is that on account of a modification of the shape of the cells it is possible to apply 350 kPa extra pressure to the surface of solutions thus shifting the boiling point of water to about 140°C. The instrument allows one to record the heat

capacity automatically to 133°C. The average heating rate was 1°C/min for all experiments. The instrument allows scanning in the heating and cooling modes. To facilitate data processing and computation of the desired parameters, as well as subtracting the instrumental baseline, the calorimeter was interfaced to a PC model Commodore 64, equipped with a data storage device and a plotter. Computer print-outs are shown in figs 1 and 2. To test the reproducibility of the results the same solution was re-run after cooling overnight. To test for reversibility of the polymer system Z-DNA helix formation was recorded immediately after recording the temperature-induced helix-coil transition of the left-handed conformation. Parallel to the data acquisition by the computer, absorption spectra and absorbance vs temperature profiles were measured to ensure, on the one hand, that the polynucleotide is present in the desired conformation and, on the other, to compare the results from the UV melting curves with the parameters obtained by microcalorimetry. The concentrations of the polymers in solution were determined spectrophotomerically by measuring the absorbance at 260 nm. For poly[d(G-C)] we used 17.9 A_{260} units per mg, and for poly(dG-m^{5}dC) 18.3 A_{260} units per mg as reference values. The average calorimetric solution contained about 400 μg per ml. The volume of the cell amounts to 0.460 ml.

3. RESULTS

A typical heat capacity vs temperature curve for poly[d(G-C)] · poly[d(G-C)] in 4 M Na$^+$, where it is present in the left-handed conformation, is shown as plotted by the computer in fig.1 (solid line). The left-handed ordinate gives the compensating electric power to keep the temperature difference between the two cells as small as possible (abscissa: temperature in °C). This is reasonable since the instrument works with a constant heating rate, i.e. the temperature changes linearly with time. The right-hand ordinate gives the degree of transition to scale the transition curve (dashed line), i.e. the fraction of the polynucleotide in the coiled state. The area under the peak represents the total transition enthalpy Δh. The actual value is determined by comparing the peak area to an electrically set calibration mark of known energy content. The
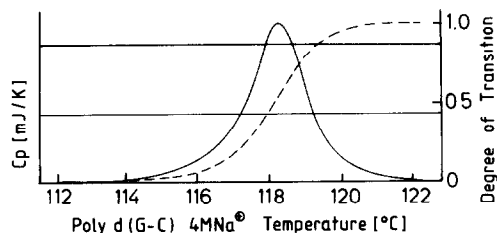

Fig.1. Electrical power (fed back to sample cell to maintain it at the same temperature as the reference cell) vs temperature. Tracing is of recorder output from Commodore 64 PC. (——) Transition of poly[d(G-C)] (~200 μg) in 4 M Na$^+$. (– – –) Degree of transition as a function of temperature.

molar transition enthalpy ΔH is calculated from the total enthalpy change Δh, due to the order-disorder reaction, by division by the actual amount of subunits (base pairs) in moles, determined by either phosphorus analysis or the UV absorbance at 260 nm in a standard 1 cm cuvette (ΔH = 11.2 kcal/MBP). The temperature at the peak maximum is taken as the calorimetric transition temperature T_m (T_m = 118.3°C). This is reasonable since the peak is almost symmetric. By partially integrating the peak area the degree of transition at any given temperature can be calculated. The course of the degree of transition of the actual system as function of temperature is included in fig.1 (dashed line). From the slope of this curve at $T = T_m$ where θ equals 0.5, the van 't Hoff enthalpy of transition ΔH_{vH} can be calculated following standard thermodynamic relations [6] (ΔH_{vH} = 734.9 kcal). From the ratio of ΔH_{vH} and ΔH of an individual base pair the mean cooperative length $\langle m \rangle$ can be calculated ($\langle m \rangle$ = 65.6) [11]. This refers to the number of base pairs changing conformation simultaneously at the transition temperature T_m. This value is a little smaller than that estimated on theoretical grounds possibly due to some hairpin structures as intermediates [12]. The left-handed helix is thermally quite stable, the cooperativity of the transition to the random coil is high, but the transition enthalpy per base pair is within the range of the enthalpy change of the right-handed conformations. When the same sample is re-run to test for reproducibility of the experimental parameters, it turns out that the shape of the curve changes slightly, and the transition enthalpy, maximum temperature and transition in-

terval remain almost unchanged. The instrument also allows one to test for reversibility of the polymer system simply by changing the direction of the temperature change. The results of two corresponding scans are shown in fig.2. The general shape of the transition curves is preserved upon cooling. The width of the transition interval and the tailing of the area at the low-temperature side are in good agreement. The peak area, representing the formation of the helix, is slightly decreased during the cooling and the maximum temperature is decreased from 118 to 115°C. This small hysteresis effect may be due to the fast cooling rate, which apparently leads to some intermediate looped structures similar to the structures in poly[d(A-T)] as described by Inman and Baldwin [7], which heal up during further cooling. Since the instrument does not allow for different cooling rates it is not possible to elucidate the differences further. As mentioned above, allowing the solution to stand overnight and scanning the same sample again lead to a completely reproducible result. Fig.3 (upper) gives the original calorimetric transition curve from the strip-card recorder (cf. fig.1) and the corresponding absorbance vs temperature curve (fig.3, lower) for poly[d(G-C)] · poly[d(G-C)] in 4 M Na$^+$. There are no detectable discrepancies between the results from the spectrophotometer and calorimeter; T_m, the transition interval and the slope of the transition curve at $T = T_m$ are identical. Fig.4 shows the corresponding results for the left-handed helix-coil transition of poly(dG-m^{5}dC) in 2 mM Mg^{2+}. Again the striking correspondence of the two complementary methods is worth noting. Since the emergency shut down of the DASM-4 calorimeter does not allow scanning
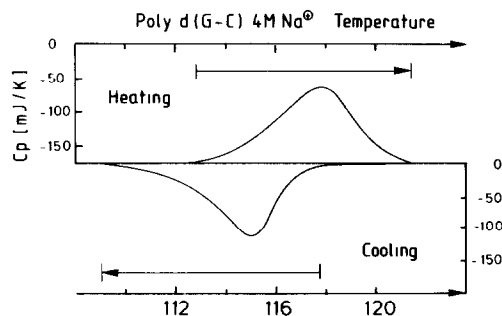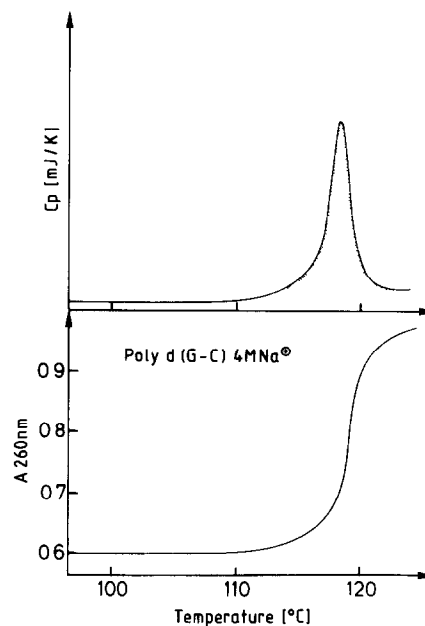
Fig.3. Absorbance vs temperature curve for poly[d(G-C)] in 4 M Na$^+$ (lower) as compared to the strip card recorder tracing of the same process, plotted direct from the DASM-4 calorimeter (upper).

beyond 132.5°C the peak was restored to its final shape by completing the small missing part according to the shape of the same peaks at lower temperature. It is reasonable to assume that this procedure is valid. Fig.5 presents the transition enthalpy per base pair for poly[d(G-C)] as a function of the sodium concentration (on a logarithmic scale). Open circles represent the values from [4], completed in the mean time (unpublished) and the results from oligo(GC) in 1 M Na$^+$ as taken from [6]. The increasing straight line, which fits the data at low N$^+$ concentration, levels off at about 200 mM and starts to decrease with further increasing sodium concentration. The behavior of this system resembles the results obtained by Gruenwedel [8] for poly[d(A-T)]. The conformation of the polymer to this cation concentration is right-handed. The linear extrapolation (dashed line) fits the value obtained for the transition of the left-handed conformation of poly[d(G-C)] as measured in 4 M Na$^+$. The linear dependency of the thermodynamic parameters ΔH (full symbols) and ΔS (open symbols) from the transition temperature is finally shown for poly[d(G-C)] (circles) and for poly(dG-m^{5}dC) (squares) in fig.6.

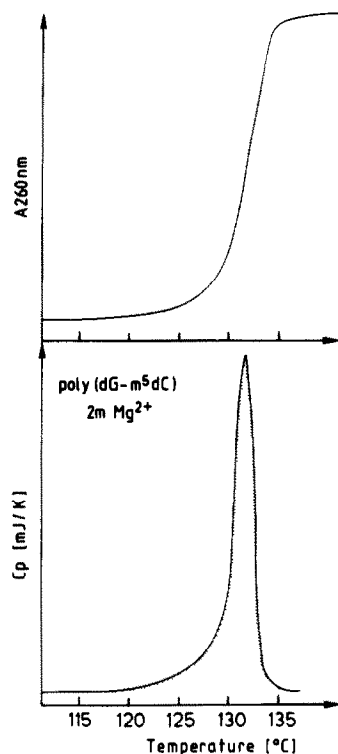Fig.2. Computer print-outs of heat capacity vs temperature curves as in fig.1 in the heating and cooling mode.

Fig.4. Absorbance vs temperature curve for poly(dG-m^{5}dC) in 2 mM Mg^{2+} (upper) as compared to the direct heat capacity vs temperature curve from the calorimeter (lower).
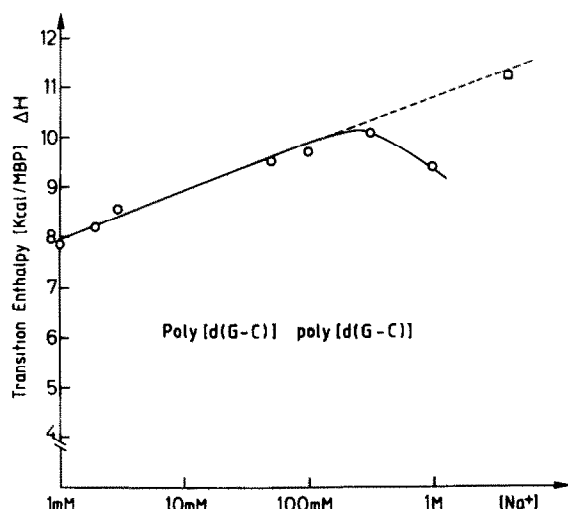

Fig.5. Transition enthalpy per mol base pairs for poly[d(G-C)] as a function of log [Na$^+$]. ($\square$) Z-DNA structure.
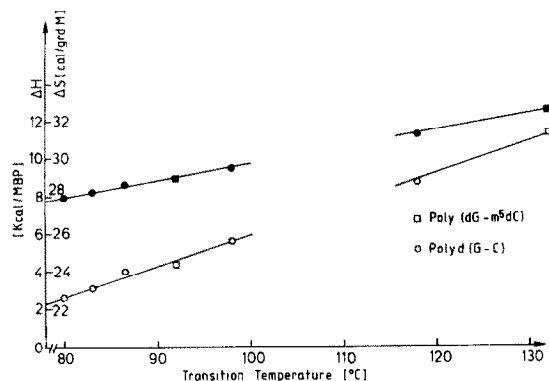

Fig.6. Transition enthalpy and transition entropy as a function of the transition temperature T_m for poly[d(G-C)] (circles) and poly(dG-m^{5}dC) (squares).

ΔS is calculated according to the Gibbs equation as the ratio of ΔH and T_m for a given set of experimental conditions. The left-hand part of the plot presents the results obtained for the polymers at a mean ionic strength below 20 mM Na$^+$, conditions under which both ΔH and T_m, and hence $\Delta H/T_m = \Delta S$ depend linearly on log [Na$^+$], and the secondary structure of the polymer is a right-handed helix. Since T_m and ΔH decline with higher cation concentrations that part of the curve presenting these results is omitted (middle part in fig.6). The values obtained for very high T_m for the left-handed helix-coil transition of the strictly alternating sequences fit surprisingly well to a linear extrapolation of the lower part of the graph. However, this fact is of an obvious nature and follows from the independence of the B→Z conversion of the temperature in aqueous solution.

4. DISCUSSION

Any systematic investigation of the thermal stability of polynucleotides, which exclusively consist of GC or modified GC base pairs such as dG-m^{5}dC, was severely handicapped by extremely high transition temperatures, which exceed the boiling temperature of the solvent water at atmospheric pressure. Since we have modified the instrumental set up [4] to increase the outside pressure sufficiently to raise the boiling point of water beyond the transition temperature of the polynucleotides, the restriction has been overcome, and the experimental approach yields the desired thermodynamic quantities.

Hence, this paper presents the first direct calorimetric measurements of the enthalpies and entropies of double-helix formation of the left-handed Z-DNA structure. By checking for reproducibility and reversibility (cf. fig.2) of the system we can substantiate our claim that the calorimetric values obtained for the double helix-coil transition also apply to the enthalpy of formation of the ordered secondary structure (with respect to the correct sign). Comparison of the denaturation curves recorded by either calorimetry or absorption changes (cf. figs 3 and 4) shows a surprisingly good agreement of the observed parameters; i.e. both methods record the same molecular process, the helix-random coil transition with rising temperature and left-handed helix formation with decreasing temperature. Since we are now certain as to which molecular process the enthalpy and entropy values correspond, we can discuss the present results in more detail. A significant observation is the extraordinarily 'regular' behavior of the thermodynamic parameters corresponding to the left-handed helix formation. The experimental values corresponding to the structural transition of the right-handed conformation at low sodium concentration and those corresponding to the transition of the left-handed conformation at high sodium concentration can be fitted by the same straight line (cf. fig.6). This agreement suggests that the energetics of base stacking are not influenced by the helical screw sense in the first place but rather depend on the identity of the bases participating in the stacking interactions. Consequently, stability differences between the right-handed and left-handed duplexes containing identical sequences result from differential entropy effects. As demonstrated previously [9], ΔS is unaffected by any sequence variation in DNAs and reflects purely changes in the conformational freedom of backbone structures. An approximate maximum conformational entropy decrease would correspond to all the bonds being completely rigid in the double strands and three orientations occurring in the single strands. This would give for the 6 bonds per nucleotide in each strand: $\Delta S = -2 \, (6R\ln 3) = -26$ cal/degree per mol base pairs. Inspection (fig.6) of the entropy vs temperature plot for the B-DNA conformation (left part) shows that the agreement of this estimation [10] with the highest experimental value obtained is quite good.

It is possible that there is some restriction in torsional oscillations about the equilibrium bond angles when a double helix is formed. The sugar ring conformation is also affected by the left-handed Z-DNA formation. Both would give an additional entropy loss on helix formation. Finally, we should consider the solvent entropy change to explain the estimated figures and the measured values of -29 to -31 cal/degree per mol base pairs for the entropy change for the Z-DNA formation, but it is beyond the capability of our experimental techniques to separate the different contributions to the entropy change.

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REFERENCES

[1] Breslauer, K. (1985) in: Chemical Thermodynamic Data for the Biological Sciences (Hinz, H.-J. ed.) Reidel, London.

[2] Hall, K. and Maestre, M. (1984) Biopolymers 23, 2127–2139.

[3] Privalov, P. and Filimonov, V. (1978) J. Mol. Biol. 122, 447–464.

[4] Klump, H. and Löffler, R. (1985) Biol. Chem. Hoppe-Seyler 366, 345–353.

[5] Privalov, P. (1974) Biochim. Biophys. Acta, suppl. 40,

[6] De Prisco Albergo, D., Marky, L., Breslauer, K. and Turner, D. (1981) Biochemistry 20, 1409–1413.

[7] Inman, R. and Baldwin, R. (1962) J. Mol. Biol. 5, 172–195.

[8] Klump, H. (1985) Thermochim. Acta 85, 457–463.

[9] Sundaralingam, M. (1969) Biopolymers 7, 821–860.

[10] De Voe, H. and Tinoco, I. jr (1962) J. Mol. Biol. 4, 500–517.

[11] Engel, J. and Schwarz, G. (1970) Angew. Chem. Int. Edn 9, 389–399.

[12] Scheffler, I., Elson, E. and Baldwin, R. (1968) J. Mol. Biol. 36, 291–304.