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Stability and thermodynamics of DNA models ☆

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Abstract

Examples of thermodynamic studies using models of different DNA conformations and models of DNA–protein interactions are given. The thermodynamics of the B–Z DNA transition previously studied in our laboratory are discussed in more detail. The application of the rarely used method of "tie calorimetry" is also described and structural interpretation of the results is given.

Keywords: Calorimetry; DNA; Heat capacity; Hydrogen bond; Protein; Stability; Thermodynamics; Tie calorimetry

1. Introduction

The structure of DNA, a compound in which the genetic information of almost all living organisms is encoded, has been the target of numerous studies in the past 15 years. It was found that DNA is polymorphic, i.e. that in addition to the classical double helical forms (A and B) several other DNA conformations exist that may be of considerable biological interest. Furthermore, the double stranded B form itself occurs in different conformations depending mainly on the base composition and sequence. The structure of various complexes of DNA with specific as well as nonspecific ligands, mainly drugs or proteins, have been described. However, papers

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dealing with the thermodynamics of formation of various structures, their conformational transitions and/or complex formation in solution are quite rare.

2. Stability of B-DNA duplexes

For several decades the basic method widely used to characterize the stability of macromolecular DNA duplexes has been the determination of the "melting temperature" T_m. However, with the advent of the studies on specially designed and synthesized oligonucleotide models it became possible to perform a more rigorous analysis of the melting curves to obtain thermodynamically meaningful data for the stability of duplexes and other conformations. The analysis of equilibrium melting curves (UV absorbance as a function of temperature) usually uses the van't Hoff expression to obtain the value of the reaction enthalpy ΔH. This value should be compared whenever possible with data obtained by direct calorimetric measurements, preferably differential scanning calorimetry (DSC). Combination of the methods used for a number of sequences in the B form gave comparatively reliable databases from which thermodynamic libraries were established that characterize all ten Watson–Crick nearest neighbor interactions in the DNA base pairs [1]. These thermodynamic data now provide an empirical base for predicting the stability (ΔG) and temperature melting behavior (ΔH) of any DNA duplex region in the B form by inspection of its nucleotide sequence [1].

Recently the thermodynamics of melting of normal and bent DNA duplexes were compared using suitable oligonucleotide models [2]. A region of a conformational change preceding the DNA melting was detected. A large heat capacity change ΔC_p observed during the melting for the bent DNA may reflect differential hydration of bent versus normal DNA [2].

3. Thermodynamics of the B–Z DNA transition

The transition of the right-handed B-DNA to the left-handed Z-DNA form in some DNA sequences has received much attention. The thermodynamics of this DNA transition appear to be rather complex [3–5]. The equilibrium between these two forms depends on temperature in different ways as a function of ionic conditions, presence of non-aqueous solvents and presence of metal ions and peptide or other ligands. Recently a number of proteins have been described which bind selectively to Z-DNA. In our previous papers [6,7] we used basic peptides as models for such proteins and studied the thermodynamics of the B to Z (or Z to B) transition induced by the peptide binding. Circular dichroism spectroscopy was used for detecting the conformational changes of DNA.

The polynucleotide poly(dG-dC) · poly(dG-dC) in the presence of 65% methanol assumes the Z conformation and can undergo the Z to B transition by increasing the temperature. The van't Hoff enthalpy value calculated from the transition curve is related to the so called cooperative unit of the transition (about 100 base pairs)

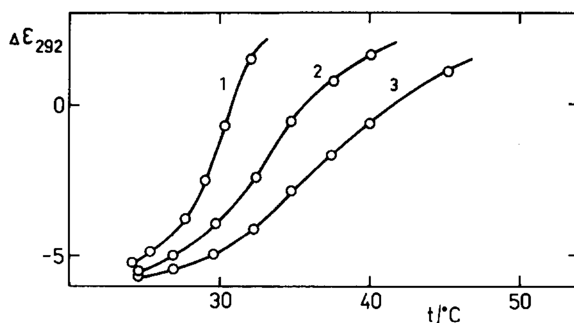

Fig. 1. Temperature dependence of the Z–B transition of poly(dG-dC) · poly(dG-dC) at a low ionic strength in 65% methanol in the presence of $(Lys\text{-}Ala\text{-}Ala)_3$. Curve 1, $r = 0$; curve 2, $r = 0.007$; curve 3, $r = 0.013$. Reprinted with the permission of the copyright holder, Wiley.

$\Delta H = -540$ kJ mol^{-1}. However, more information about the real thermodynamic values can be obtained by inducing the formation of the Z form by the addition of basic oligopeptides of the type $(Lys\text{-}Ala\text{-}Ala)_n$ ($n = 1-5$) or $(Lys\text{-}Leu\text{-}Ala)_n$ ($n = 1-4$).

Fig. 1 shows the temperature dependent Z to B transition in the presence of $(Lys\text{-}Ala\text{-}Ala)_3$ peptide at various peptide/DNA (nucleotide) ratios r. As in the absence of the peptide, the B conformation is preferred at higher temperatures. The addition of peptides results in the increase of the transition midpoint and of the transition width. Qualitatively similar results were also obtained with other peptides studied, i.e. $(Lys\text{-}Ala\text{-}Ala)_n$ ($n = 2, 4, 5, 10$) and $(Lys\text{-}Leu\text{-}Ala)_n$ ($n = 2, 3, 4$). The effect of dimers of both series is lower than that of longer peptides. The effect of the trimer and of the tetramer of $(Lys\text{-}Leu\text{-}Ala)_n$ series is, within limits, identical to that of the trimer and of the tetramer of the $(Lys\text{-}Ala\text{-}Ala)_n$ series. Within both series the values ΔT_0 (the change of the transition midpoint due to the addition of the peptide) and $\Delta\delta T$ (the change of the transition width due to the addition of the peptide) were calculated (Table 1).

According to Lazurkin et al. [8] the results of the temperature dependence of DNA conformational transition in the presence of ligands can be used for the determination of the transition enthalpy ΔH_{B-Z} for one mole of base pair. This

Table 1
Transition enthalpies of the B–Z transitions of poly(dG-dC) · poly(dG-dC) in 65% methanol in the presence of peptides

Peptide	r	ΔT_0/°C	$\Delta\delta T$/°C	$-\Delta H_{B-Z}$/kJ mol^{-1}	$-\Delta H_{B-Z}$/kJ mol^{-1}
$(Lys\text{-}Ala\text{-}Ala)_3$	0.0133	7.0	12.6	5.75	6.42
$(Lys\text{-}Ala\text{-}Ala)_4$	0.01	7.8	13.9	3.87	4.38
$(Lys\text{-}Ala\text{-}Ala)_5$	0.008	7.5	14.0	3.17	3.46
$(Lys\text{-}Ala\text{-}Ala)_{10}$	0.01	6.3	12.2	4.84	4.96
$(Lys\text{-}Leu\text{-}Ala)_3$	0.0133	7.0	12.7	5.75	6.36
$(Lys\text{-}Leu\text{-}Ala)_4$	0.01	7.7	14.0	3.96	4.33

approach, called "tie calorimetry", was used for studying the B–Z transition of poly(dG-dC) · poly(dG-dC) in water–ethanol solutions in the presence of ethidium bromide or polyamines [9]. Under some limiting conditions, which were shown to be fulfilled in our system, the enthalpy values can be calculated using the equations

$$\Delta H_{B-Z} = 2RT_0^2 2r/\Delta T_0$$

$$\Delta H_{B-Z} = 2RT_0^2 2r/\Delta\delta T$$

where ΔT_0 is the shift of the transition midpoint, $\Delta\delta T$ is the widening of the transition curve and $2r$ is the molar ratio of the ligand to DNA base pair. The calculated values are summarized in Table 1.

The average value of ΔH_{B-Z} is -4.8 kJ mol^{-1} of base pairs and can be compared with the van't Hoff enthalpy value of -530 kJ mol^{-1} giving an approximate length of the cooperative unit of the B–Z transition of about 110 base pairs.

Although originally the B–Z transition of poly(dG-dC) · poly(dG-dC) in aqueous salt solution was considered to be entropically driven [3], Chaires and Sturtevant [5] later found in the presence of Mg^{2+} ions a positive calorimetric enthalpy $\Delta H_{B-Z} = 8.4$ kJ mol^{-1} base pair and a cooperative unit of 150 base pairs. The most striking phenomenon is the difference in sign found on comparing the transition enthalpy under different conditions, suggesting that the mechanism triggering the B–Z transition may be very different under different conditions.

In contrast to the unmethylated polymer, the polymer methylated at the 5 position of cytosine poly(dG-m^{5}dC) · poly(dG-m^{5}dC) can undergo a B–Z transition in aqueous solution at low ionic strength in the absence of methanol only due to the binding of the peptide oligomers [7] (Fig. 2a). In this case, however, on increasing the temperature a transition from the B conformation to the Z form was observed (Fig. 2b). Unfortunately only the van't Hoff enthalpies ΔH_{vH} related to the cooperative units could be obtained in this case, since no temperature induced transition occurs in aqueous solution in the absence of peptides and therefore values of ΔT_0 and $\Delta\delta T$ cannot be obtained. The calculated ΔH_{vH} values are obviously positive and for the peptide/nucleotide ratio $r = 0.03$ they are about 130 kJ mol^{-1} for the DNA in the presence of $(Lys\text{-}Ala\text{-}Ala)_2$ and 180 kJ mol^{-1} in the presence of $(Lys\text{-}Leu\text{-}Ala)_2$. Assuming the same cooperative length as for the B–Z transition of the unmethylated polymer, we obtain for the methylated polymer a ΔH_{B-Z} value about 5 to 10 times smaller (in the absolute value) than in the case of the unmethylated polymer. Both the effect of addition of a non-aqueous solvent and the effect of methylation suggest an important influence of hydration and hydrophobic forces in the mechanism driving the B–Z transition.

4. Thermodynamics of protein–DNA interactions

The biologically most important DNA interactions are those with the binding proteins. We have to distinguish between nonspecific and specific interactions. The latter term implicates the interaction of a protein with a specific DNA binding

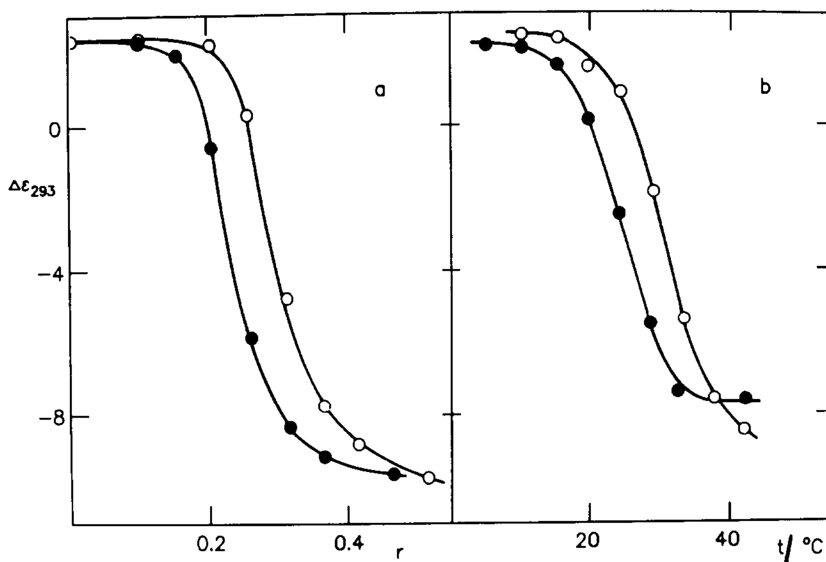

Fig. 2. B–Z transition of poly(dG-m^{5}dC) · poly(dG-m^{5}dC) in aqueous solution at a low ionic strength induced by (Lys-X-Ala) dimers: (●) $(Lys\text{-}Leu\text{-}Ala)_2$, (○) $(Lys\text{-}Ala\text{-}Ala)_2$: (a) as a function of r at $t = 35°C$; (b) as a function of temperature, $r = 0.3$. Reprinted with permission of the copyright holder, Butterworth-Heinemann.

sequence, and the specific interaction differs from the nonspecific one by many orders of magnitude in the equilibrium binding constant (and by a considerable difference in the binding free energy ΔG).

The most significant driving force of the nonspecific binding at low and moderate ionic strength is the polyelectrolyte effect, i.e. the entropic effect of the release of the metal counterions from the double helical DNA on protein binding [10]. From the thermodynamic point of view, the specific DNA–protein interaction is a more complicated process. Large negative ΔC_p values in the process of binding are indicative of protein denaturation and can be interpreted in terms of the release of water molecules from DNA on forming hydrophobic interactions between the DNA and the protein. Recently a more detailed thermodynamic analysis of Cro protein–DNA associations using a highly sensitive pulse flow calorimeter revealed that the heat capacity change ΔC_p is tightly coupled to the binding free energy ΔG_p [11]. The interpretation is that hydrogen bonds, van der Waals and hydrophobic interactions all contribute directly to the heat capacity as well as free energy change. In addition, two different correlations of ΔH and ΔS values with ΔC_p were obtained for a series of slightly modified DNA sequences indicating two different types of complexes probably differing in conformation [11].

References

[1] K.J. Breslauer, R. Frank, H. Blocker and L.A. Marky, Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 3746.

[2] Y.W. Park and K.J. Breslauer, Proc. Natl. Acad. Sci. U.S.A., 88 (1991) 1551.
[3] F.M. Pohl and T.M. Jovin, J. Mol. Biol., 67 (1972) 375.
[4] M.J. Behe, G. Felsenfeld, C.S. Szu and E. Charney, Biopolymers, 24 (1985) 289.
[5] J.B. Chaires and J.M. Sturtevant, Biopolymers, 27 (1988) 1375.
[6] H. Votavová, J. Pírková, V. Gut, J. Šponar and K. Bláha, Biopolymers, 31 (1991) 273.
[7] H. Votavová and J. Šponar, Int. J. Biol. Macromol., 15 (1993) 139.
[8] Yu. S. Lazurkin, M.D. Frank-Kamenetskii and E.N. Trifonov, Biopolymers, 9 (1970) 1253.
[9] K.T. Karapetyan, E.E. Minyat and V.I. Ivanov, FEBS Lett., 173 (1984) 243.
[10] M.T. Record and R.S. Spolar, in A. Revzin (Ed.), Non-Specific DNA–Protein Interactions, CRC Press, Boca Raton, 1990, p. 15.
[11] Y. Takeda, P.D. Ross and C.P. Mudd, Proc. Natl. Acad. Sci. USA, 89 (1992) 8180.