Stability of a Short DNA Duplex as a Function of Temperature: the Effect of $\Delta C_p$ and Added Salt Concentration$^\dagger$

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$^\dagger$Dedicated to Prof. Dr. Jože Škerjanc on the occasion of his 70$^{th}$ birthday

Abstract

Recently, it has been shown that $\Delta C_p$ effects accompanying the helix-to-coil transitions of DNA duplexes are not negligible. To find out if this is the case also with a short model (5'-CGAATTCG-3'), duplex we studied its thermal unfolding by high sensitivity differential scanning calorimetry (DSC) at 0, 0.1, 0.3 and 1 M added NaCl. We successfully described the measured DSC thermograms by the model function that is based on the two-state approximation of the unfolding process and contains three adjustable parameters: $T_{1/2}$ – the melting temperature, $\Delta H_{T_{1/2}}$ – enthalpy of unfolding at $T_{1/2}$ and $\Delta C_p$ – the change of heat capacity upon DNA unfolding. These $\Delta H_{T_{1/2}}$ and $\Delta C_p$ values are in good agreement with those obtained by the straightforward integration of the DSC thermograms. From the available experimental data a $\Delta H_{T_{1/2}}$ versus $T_{1/2}$ plot was constructed and $\Delta C_p$ was obtained as a slope of the observed linear relationship. We believe that excellent agreement between this $\Delta C_p$ and the one obtained as adjustable parameter from fitting the experimental DSC thermograms strongly supports our suggestion that $\Delta C_p$ accompanying unfolding of DNA is independent of temperature an added salt concentration and may be accurately determined from the described fitting procedure. Analysis of our results shows that even for short DNA duplexes the errors in the nearest-neighbor estimates of $\Delta G(T)$, $\Delta H(T)$ and $\Delta S(T)$ of unfolding based on the $\Delta C_p = 0$ assumption may be significant. It also shows that the experimental $T_{1/2}$ versus $\ln[\text{Na}^+]$ slope depends on the salt concentration and agrees reasonably well with the one predicted by the electrostatic polyelectrolyte theory.

Keywords: DNA, thermodynamics, differential scanning calorimetry, heat capacity, stability.

1. Introduction

Temperature induced melting of DNA duplexes is the process of separating the two strands wound in a double helix into two single strands. Characteristically, such phase transitions are accompanied by a relatively small transition free energy, $\Delta G(T)$, that results from the compensation of a large transition enthalpy, $\Delta H(T)$, and large transition entropy, $\Delta S(T)$.

In such transition studies the heat capacity change, $\Delta C_p$, that also accompanies the melting processes has been typically overlooked. Only recently,\textsuperscript{5,9} with the employment of the last generation of high-sensitivity differential scanning calorimetry (DSC) instruments in DNA melting studies, it has become increasingly clear that DNA thermal transitions may be associated with a significant change in $\Delta C_p$. This means that quantitative knowledge of $\Delta C_p$ is required to compare $\Delta G(T)$, $\Delta H(T)$, and $\Delta S(T)$ of transition of nucleic acids at some common reference temperature. Empirically, $\Delta C_p(T)$ is thought to arise mainly from solvent effects that accompany the exposure of non-polar\textsuperscript{10–13} and/or aromatic surfaces upon unfolding. Since net base stacking is greater in the folded duplex than in the unfolded single strands\textsuperscript{14} one would expect duplex unfolding to be accompanied by a $\Delta C_p(T)$ that corresponds to increased amount of unstacked bases and consequently to increased hydration of aromatic surfaces. Despite the potential for such “hydration” heat capacity to provide a key to understanding solute–solvent interactions there is as yet no general model for $\Delta C_p(T)$ that can be applied to macromolecular transitions. Currently, the only successful models that describe $\Delta C_p(T)$ resulting from molecular conformational transitions are those for proteins and are based on the accompanying changes in solvent-accessible surface areas. These models employ...
empirical formulas that have been parameterized using model compound (peptides, amino acid fragments) transfer and protein unfolding thermodynamic data.\textsuperscript{10–13,15} Unfortunately, there are no data for corresponding nucleic acid model compounds i.e. bases, sugars and fragments of phosphate backbone. Thus the only approach used to date for obtaining reliable $\Delta c_p(T)$ for nucleic acid unfolding has been its direct measurement by DSC.

The polyelectrolyte nature of DNA adds further complexity to its behavior in solution. A dense atmosphere of condensed counterions around the polyanionic DNA backbone has a major impact on the thermodynamics of unfolding. Experimental and theoretical studies show that the temperature dependence of the corresponding transition quantities, the temperature at the midpoint of the thermally induced duplex-to-single-strands transitions (melting temperature, $T_m$) strongly depends on the concentrations and charges of cations in the solutions.\textsuperscript{5,6,8,9,16,17} They also show that upon unfolding a substantial amount of the condensed counterions is released because the extent of counterion binding to DNA in the duplex form is larger than in the single-stranded form.\textsuperscript{18–21} By contrast, recent analysis of $\Delta c_p(T)$ of DNA melting suggests,\textsuperscript{4} the electrostatic effects arising from the DNA’s polyelectrolyte nature cannot account significantly for the observed $\Delta c_p(T)$ values. This further means that $\Delta c_p(T)$ is not expected to depend on the added salt concentration.

The primary goal of our study was to investigate whether the $\Delta c_p(T)$ effects accompanying the helix-to-coil transitions of short DNA duplexes are as important as those observed with longer double helical oligonucleotides.\textsuperscript{7} To see to what extent these $\Delta c_p(T)$ values affect the temperature dependence of the corresponding transition quantities $\Delta G(T)$, $\Delta H(T)$ and $\Delta S(T)$ we studied the thermally induced unfolding of the $(5’-\text{CGAATTCG}-3’)_2$ duplex at different concentrations of NaCl by employing DSC. Using these data we also tried to find out whether the observed $\Delta c_p(T)$ exhibits any significant dependence on temperature or added salt concentration.

### 2. Experimental

**Materials.** Oligonucleotide $(5’-\text{CGAATTCG}-3’)_2$ was purchased from Invitrogen Co., Germany. Its concentrations in buffer solutions were determined at 25 °C spectrophotometrically in the Cary Bio 100 UV-spectrophotometer. For the extinction coefficient of its duplex form we used the value $\varepsilon_{260} = 143600 \text{ M}^{-1} \text{ cm}^{-1}$ that was determined from the nearest-neighbor data of Cantor et al.\textsuperscript{22} The buffer solutions used in all experiments consisted of 10 mM phosphate buffer, 1 mM Na$_2$EDTA and 0, 0.1, 0.3 or 1 M NaCl. All solutions were adjusted to pH = 7.0.

**Differential scanning calorimetry (DSC).** DSC measurements were performed with a Nano-II DSC calorimeter (CSC, UT). Thermal denaturation of the $(5’-\text{CGAATTCG}-3’)_2$ duplex in buffer solutions with different added NaCl concentrations was monitored between 5 and 95 °C. A heating rate of 1 °C min$^{-1}$ was used. Essentially the same results were obtained also at the heating rate of 0.25 °C min$^{-1}$. The unfolding of DNA was monitored in terms of $(\bar{c}_{p2(T)} - \bar{c}_{pA1(T)})$ versus $T$ thermograms in which the differences between the raw signal, corrected for the buffer contribution, $\bar{c}_{p2(T)}$ and the corresponding heat capacity of the native duplex state, $\bar{c}_{pA1(T)}$, are normalized for the duplex concentration (Fig 3a).

### 3. Analysis of DSC Data

The thermally induced duplex to single strands transition that appears to be an all-or-none process

$$A_2 \xrightarrow{K(T)} 2A; \quad K(T) = \frac{4\alpha^2(T)c_{sd}}{1 - \alpha(T)}$$

may be described in terms of the total duplex concentration, $c_{tot}$, and the fraction of duplex molecules that undergo transition into single strands, $\alpha(T)$. Experimentally this transition can be followed by DSC. At very low $c_{tot}$ values used in DSC experiments the measured $c_p(T)$ (baseline subtracted) can be equalized with the DNA partial molar heat capacity, $c_p(T)$. Thus, the overall heat effect, $\Delta H(T_1, T_2)$ that accompanies the transition of DNA from its native duplex state at $T_1$ ($\alpha(T_1) = 0$) to its denatured single stranded state at $T_2$ ($\alpha(T_2) = 1$) can be expressed as

$$\Delta H(T_1 \rightarrow T_2) = \int_{T_1}^{T_2} \bar{c}_{p2}(T)dT$$

Since enthalpy is a state function eq. 2 can be expressed as

$$\Delta H(T_1 \rightarrow T_2) = \int_{T_1}^{T_2} \bar{c}_{p2}(T)dT + \Delta H(T_1) + \int_{T_1}^{T_2} 2\bar{c}_{pA1}(T)dT$$

and by choosing $T_{1/2} = T_{1/2}$ where $T_{1/2}$ is the melting temperature ($\alpha = 1/2$) one obtains that the molar enthalpy of transition at $T_{1/2}$, $\Delta H_{cal}^{M}(T_{1/2})$ is

$$\Delta H_{cal}^{M}(T_{1/2}) = \int_{T_1}^{T_2} (\bar{c}_{p2}(T) - \bar{c}_{pA1}(T))dT$$

Evidently, $\Delta H_{cal}^{M}(T_{1/2})$ can be determined from the measured DSC thermogram simply by integration of the experimental $(\bar{c}_{p2}(T) - \bar{c}_{pA1}(T))$ and $(\bar{c}_{p2}(T) - 2\bar{c}_{pA1}(T))$ curves over the corresponding temperature intervals and thus, may be considered as a model-independent quantity. Moreover, the corresponding $\Delta c_p(T)$ at given $T$ is defined as a difference between the post-transitional ($2\bar{c}_{pA1}(T)$) and the pre-transitional ($\bar{c}_{pA1}(T)$) baselines extrapolated to that $T$ (Fig. 3). According to the measured DSC thermogram the $\Delta c_p(T)$ for the $(5’-\text{CGAATTCG}-3’)_2$ duplex appears to be independent of temperature (Fig. 3).
For the temperature dependent equilibrium described by eq. 1 the partial molar enthalpy of DNA, $H_{2(T)}$, is defined as

$$\bar{H}_{2(T)} = \frac{\partial H_{A(T)}}{\partial T} \Bigg|_{n_1, n_2}$$

(5)

where $n_2$ and $n_1$ are the total numbers of moles of the DNA duplex and solvent, respectively. When expressed in terms of the corresponding partial molar enthalpies of the double stranded and single stranded state, $H_{A(T)}$ and $H_{A'(T)}$, $H_{2(T)}$ takes the form:

$$\bar{H}_{2(T)} = (1 - \alpha(T))\bar{H}_{A'(T)} + 2\alpha(T)\bar{H}_{A(T)}$$

(6)

By taking the temperature derivative of eq. 6 at constant $p$, $n_1$ and $n_2$ one obtains

$$\bar{c}_{p(T)} = \bar{c}_{pA(T)} + \alpha(T)\Delta c_{p(T)} + \Delta H_{p(T)} \left( \frac{\partial \alpha(T)}{\partial T} \right)_{n_1, n_2}$$

(7)

where $\Delta c_{p(T)} = (2\bar{c}_{pA(T)} - \bar{c}_{pA'(T)})$, $\Delta H_{p(T)} = (2\bar{H}_{A(T)} - \bar{H}_{A'(T)})$, the subscript 2 denotes DNA at a given thermal equilibrium and the subscripts A and A' refer to its corresponding duplex and single-stranded state, respectively. Since $\Delta c_{p(T)}$ and $\Delta H_{p(T)}$ appear to be independent of the DNA concentration, which means that $\Delta c_{p(T)} = \Delta c_{pA(T)}$ and $\Delta H_{p(T)} = \Delta H_{pA(T)}$, eq. 7 becomes

$$\bar{c}_{p(T)} - \bar{c}_{pA(T)} = \alpha(T)\Delta c_{pA(T)} + \Delta H_{pA(T)} \left( \frac{\partial \alpha(T)}{\partial T} \right)_{n_1, n_2}$$

(8)

and can be considered as the model function for the measured DSC thermogram.

A detailed description of the thermal duplex-to-single-strands equilibrium (eq. 1) includes the following relations

$$\Delta G_{(T)}^o = -RT\ln K_{(T)}$$

$$\Delta G_{(T)}^o = -RT\ln (2\alpha_{eq})$$

$$\alpha(T) = \frac{1}{2} \left( \frac{K_{(T)}}{4\alpha_{eq}} + \left( \frac{K_{(T)}}{4\alpha_{eq}} + \frac{K_{(T)}}{4\alpha_{eq}} \right) \right)$$

$$\left( \frac{\partial \alpha(T)}{\partial T} \right)_{n_1, n_2} = \frac{\Delta H_{pA(T)} \alpha(T)(1 - \alpha(T))}{RT^2}$$

(9)

$$\Delta H_{(T)} = \Delta H_{(T)} + \Delta c_{pA(T)}(T - T_{1/2})$$

$$\Delta G_{(T)} = T \left[ \frac{\Delta G_{(T)}^o}{T_{1/2}} + \Delta H_{(T)} \left( \frac{1}{T} - \frac{1}{T_{1/2}} \right) + \Delta c_{pA(T)} \left(1 - \ln \frac{T}{T_{1/2}} - \frac{T_{1/2}}{T} \right) \right]$$

$$\Delta S_{(T)} = \frac{\Delta H_{(T)} - \Delta G_{(T)}^o}{T}$$

in which the quantities $\Delta G_{(T)}^o$, $\Delta G_{(T)}^o$, $\Delta H_{pA(T)}$, $\Delta H_{(T)}$, and $\Delta c_{pA(T)}$ refer to the duplex-to-single-strands transitions that occur between their standard states at T or $T_{1/2}$ and $\Delta c_{pA(T)}$ is assumed to be independent of temperature. Inspection of eqs 1, 8 and 9 shows that the model function (eq. 8) contains only three adjustable parameters: $T_{1/2}$, $\Delta H_{pA(T)}^o$, and $\Delta c_{pA(T)}$. They can be determined by fitting the model function to the experimental ($\bar{c}_{pA(T)} - \bar{c}_{pA'(T)}$) versus $T$ curve and used to calculate the $\Delta G_{(T)}^o$, $\Delta H_{(T)}^o$, and $\Delta S_{(T)}^o$ values at any other $T$ (eq. 9). To obtain the “best fit” values of the three adjustable parameters at each of the added NaCl concentrations the non-linear Levenberg-Marquardt regression procedure was used. Inspection of these results shows that $T_{1/2}$ increases with increasing added salt concentration. Since the enthalpies of transition appear to be salt-independent quantities (Fig. 2b) $\Delta c_{pA(T)}$ may be calculated simply as the slope of the $\Delta H_{pA(T)}^o$ versus $T_{1/2}$ curve constructed from the “best fit” $\Delta H_{pA(T)}^o$ and $T_{1/2}$ values determined at different NaCl concentrations (Fig 3b). Using these data we also obtained the experimental dependence of $T_{1/2}$ on the concentration of Na+ ions (Fig 4) from which the amount of Na+ ions released upon the unfolding of the DNA duplex at a given salt concentration was estimated.

4. Results and Discussion

The results of DSC melting experiments performed on duplex solutions in phosphate buffer and 0, 0.1, 0.3 and 1 M NaCl are presented in Fig. 1. Good agreement between the model dependent quantities ($\bar{c}_{pA(T)} - \bar{c}_{pA'(T)}$) (eq 8), ($\bar{H}_{2(T)} - \bar{H}_{A(T)}$) (eq 6) and $\alpha(T)$ obtained from the “best fit” model parameters $T_{1/2}$, $\Delta H_{pA(T)}^o$, and $\Delta c_{pA(T)}$ with the corresponding non-model values obtained directly from the measured DSC thermograms (Fig. 1) indicates that the observed transition may be considered as a two state transition. The two-state description of the (5'-CGAATTCG-3') unfolding process may be justified also in the usual, less strict, way by the observed good agreement of the model dependent and model independent $\Delta H_{pA(T)}^o$ values (Table 1). Moreover, the $\Delta H_{pA(T)}^o$ value determined in 1 M NaCl (Fig. 2b) is close to the corresponding transition enthalpy, $\Delta H_{pA(25 °C)}^o$, calculated at 25 °C using the nearest neighbor approach. Since the nearest neighbor data reported at 25 °C were obtained from the experimental data at the corresponding melting temperatures assuming that $\Delta c_{pA(T)} = 0$ the observed agreement, in fact, supports the credibility of our results.

By using the “best fit” values of the adjustable parameters $T_{1/2}$, $\Delta H_{pA(T)}^o$, and $\Delta c_{pA(T)}$ and eq. 9 the characteristic thermodynamic quantities of duplex-to-single-strands transitions $\Delta G_{(T)}^o$, $\Delta H_{(T)}^o$, and $\Delta S_{(T)}^o$ were determined as functions of temperature and salt concentration (Fig. 2). They appear to be moderately temperature-dependent and $\Delta G_{(T)}^o$ versus $T$ curve exhibits a slightly curved shape typi-
cal of the processes that are accompanied by $\Delta c°p > 0.24$–26
In the measured temperature interval the highest duplex stability (the highest $\Delta G°(T)$ value) is observed at the highest salt concentration. The comparison of $\Delta G°(25°C) - \Delta H°(25°C)$ and $\Delta S°(25°C)$ determined in 1M NaCl with the corresponding nearest-neighbor (n.n.) data23 shows that the errors in the nearest-neighbor estimates that result from the $\Delta c°p = 0$ assumption are significant even for short DNA duplexes like (5’-CGAATTCG-3’)2:

$\Delta \Delta G°(25°C) = \Delta G°(25°C)_{exp} - \Delta G°(25°C)_{n.n.} = -1.2$ kcal/mol and the corresponding $\Delta \Delta H°(25°C) = -7.2$ kcal/mol and $\Delta \Delta S°(25°C) = -20.5$ cal/mol K.

As shown in Fig. 2b the enthalpy of transition, $\Delta H°(T)$, appears within the experimental error to be independent of salt concentration. This observation strongly supports the use of the already mentioned alternative method of determining $\Delta c°p$ according to which $\Delta c°p$ is obtained as the ($\Delta \Delta H°(T_{1/2})/\Delta T_{1/2}$) slope of the measured linear dependence of $\Delta H°(T_{1/2})$ on $T_{1/2}$ (Fig. 3b). Inspection of data in Table 1 reveals that the $\Delta c°p$ obtained from both methods is almost identical and agrees rather well with the model independent estimate of $\Delta c°p$ obtained by the extrapolation method (Fig. 3a). Its value of $27 \pm 5$ cal (mol of base pairs)–1 K–1 also agrees well with $\Delta c°p$ values reported by Tihomirova et al.7 for a number of 13-mer duplexes.

We believe that for the observed two state transition the $\Delta c°p$ value obtained from fitting the DSC thermogram is more reliable than the one determined in the most frequent manner, that is by subtracting the pre-transitional DSC baseline from the post-transitional one. Namely, the described fitting procedure involves a large number of experimental points while subtraction of the two baselines is always problematic due to their unsafe extrapolation over the measured temperature interval.

Fig. 4 presents the salt dependence of the melting temperature, $T_{1/2}$, determined from the corresponding DSC thermograms. The values of $T_{1/2}$ increase with increasing salt concentration, a result that reflects the well known salt-effect on the stability of nucleic acids.27 Temperature induced helix-to-coil transitions of DNA in solution, in the absence or presence of added salt, have been described in terms of the counterion condensation polyelectrolyte theory of Manning and Record18,28,29 which takes into account only electrostatic polion-counterion interactions and predicts that

$$\frac{\partial T_{1/2}}{\partial \ln [\text{Na}^+] = 0.9 \cdot \left(\frac{R T^2}{\Delta H°_{\text{Na}^+}}\right) \cdot \Delta n_{\text{Na}^+}}$$

Table 1. Thermodynamic parameters of (5’-CGAATTCG-3’)2 duplex unfolding at [NaCl] = 0.1 M.

<table>
<thead>
<tr>
<th>$T_{1/2}$/°C</th>
<th>$\Delta H°_{\text{Na}^+}$/kcal mol⁻¹</th>
<th>$\Delta c°p$/kcal mol⁻¹ K⁻¹</th>
<th>$\Delta n_{\text{Na}^+}$</th>
<th>$\Delta c°p$/kcal mol⁻¹ K⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>value</td>
<td>cal</td>
<td>fit</td>
<td>slope</td>
<td>cal</td>
</tr>
<tr>
<td>error</td>
<td>±0.2</td>
<td>±1.5</td>
<td>±0.5</td>
<td>±0.06</td>
</tr>
</tbody>
</table>

$^a$Number of released Na+ ions per base pair.
where $\Delta H^o_{T1/2}$ is the enthalpy of the helix-to-coil transition of the (5'-CGAATTCG-3')$_2$ duplex at $T_{1/2}$, the 0.9 is a correction factor that takes care of the conversion of the mean ionic activity to ionic concentration and $\Delta n_{Na^+}$ is the number of Na$^+$ ions released upon unfolding of a single duplex molecule. A fit of a second order polynomial function to the experimental $T_{1/2}$ versus $\ln [Na^+]$ curves has allowed us to estimate the slopes in order to calculate the release of the counterions (eq. 10, Fig. 4). The value of $\Delta n_{Na^+} = 0.2$ counterions/base pair obtained at 0.1 M NaCl (Table 1) is in a reasonably good agreement with the $\Delta n_{Na^+}$ values reported to accompany unfolding of some other oligonucleotide duplexes.}\(^{16,17}\) In the counterion condensation theory the fraction of the counterions bound to a DNA duplex and its single strands can be expressed in terms of their linear charge densities. The theory predicts that for polymeric DNA (B form) the fraction of phosphates neutralized by the monovalent counterions bound to the double-helical DNA and its single strands, $\Psi_H$ and $\Psi_C$, are $\Psi_H = 0.88$ and $\Psi_C = 0.71$. For short DNA oligomers it has been shown, however, that due to the reduced counterion condensation at the ends of the molecules these oligomers exhibit a reduced counterion binding as compared with polymers of the same linear charge density. According to Record and Lohman the average fractions of condensed counterions for 8-mer DNA dou-

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ble- and single-stranded form are $\Psi_{\text{helix}} = 0.72$ and $\Psi_{\text{coil}} = 0.67$, respectively, which means that upon unfolding of the double helix only 0.1 of counterions/base pair will be released.\textsuperscript{17,28} Relatively good agreement of this theoretical value with the one determined from our experimental $T_{1/2}$ versus $\ln[\text{Na}^+]$ slopes (Fig. 4, eq. 10) is consistent with a general observation that the electrostatic counterion condensation polyelectrolyte theory can successfully describe a very limited number of physico-chemical phenomena that occur in solutions containing DNA. These include for example the unfolding of DNA with accompanying $\Delta n_{\text{Na}^+}$ or ligand binding to DNA with accompanying salinity of the binding constant. By contrast, numerous studies that involve energetics of conformational transitions or ligand binding to DNA have shown that electrostatic effects and thus electrostatic polyelectrolyte theories alone cannot account for the observed behavior of thermodynamic quantities.\textsuperscript{27,30,31} This is the case also with the present study which shows that only the release of Na\textsuperscript{+} counterions upon unfolding of the (5'-CGAATTCG-3')\textsubscript{2} duplex can be explained in terms of electrostatics while the stability parameters $\Delta G^{\circ} (T, \gamma)$, $\Delta H^{\circ} (T, \gamma)$, $\Delta S^{\circ} (T, \gamma)$ and $\Delta c_p^{\circ}$ seem to be determined mainly by non-coulombic interactions such as hydrogen bonding, van der Waals interactions and hydrophobic hydration.

5. Acknowledgment

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6. References


![Figure 4](image-url). The helix-to-coil transition of the (5'-CGAATTCG-3')\textsubscript{2} duplex: the dependence of $T_{1/2}$ on the added NaCl concentration.
Povzetek

V zadnjem času se je pokazalo, da efekti $\Delta c_p$, ki spremljajo razvitje dvojnih vijačnic DNA, niso zanemarljivi. Da bi odgovorili na vprašanje ali velja ta ugotovitev tudi za kratke DNA oligomere, smo izvršili toplotno inducirano razvitje dupleksa $(5'-CGAATTCG-3')_2$ v vodnih raztopinah ob dodatku 0, 0,1, 0,3 in 1 M NaCl, pri čemer smo uporabili visoko občutljivo diferencialno dinamično kalorimetrijo (DSC). Izmerjene DSC termograme smo uspešno opisali z modelno funkcijo osnovano na enostopenjskem modelu procesa razvitja, ki vsebuje tri prilagodljive parametre: $T_{1/2}$ – temperaturo polovice prehoda, $\Delta H(T_{1/2})$ – entalpijo razvitja pri $T_{1/2}$ in $\Delta c_p$ – spremembo toplotne kapacitete DNA, ki spremlja njeno razvitje. Tako določene vrednosti $\Delta H(T_{1/2})$ se dobro ujemajo z ustreznimi vrednostmi, ki jih dobimo z neposredno integracijo DSC termogramov. S pomočjo eksperimentalnih podatkov smo konstruirali $\Delta H(T_{1/2})$ – $T_{1/2}$ diagram in določili $\Delta c_p$ kot naklon doblijene linearne odvisnosti. Menimo, da odlično ujemanje obeh $\Delta c_p$ vrednosti potrjuje upravičenost naše postavke, da $\Delta c_p$ razvitja DNA ni odvisna od temperature ali koncentracije dodane soli in jo lahko zanesljivo določimo s pomočjo popisovanja eksperimentalnih DSC termogramov z ustrezno modelno funkcijo. Analiza naših rezultatov pokaže, da ocene $\Delta G(T), \Delta H(T)$ in $\Delta S(T)$ razvitja dvojne vijačnice, osnovane na »nearest-neighbor« približku in predpostavki, da je $\Delta c_p = 0$, lahko celo za kratke oligomere DNA vodijo do znatnih napak. Pokaže tudi, da eksperimentalno določena odvisnost $T_{1/2}$ od ln[Na+] ni linearna, a se kljub temu dokaj dobro ujema z napovedjo elektrostatske teorije raztopin polielektrolitov.