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Denaturation experiments on calf-thymus DNA/polycation complexes in aqueous/organic solvent mixtures

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Abstract

Polyelectrolyte complexes of calf-thymus DNA and polycations, such as PDADMAC, IONENE, and P4VP were formed and analysed with respect to their thermodynamical stability. The UV-VIS spectrographic melting curves yield two denaturation temperatures, $T_{m,1}$ and $T_{m,2}$. This is explained as follows: DNA molecules contain two kinds of repeat unit sequences. There are sequences that are not complexed, they denature at $T_{m,1}$; the other DNA repeat units are complexed with polycation repeat units, they denature at $T_{m,2}$. Thereby, $T_{m,2}$ is much larger than $T_{m,1}$. That is, polycation binding stabilises calf-thymus DNA. Surprisingly, the absolute value of $T_{m,1}$ depends neither on the degree of complexation nor on the polycation type. $T_{m,1}$ agrees quite well with the denaturation temperature of the pure, uncomplexed DNA. Also $T_{m,2}$ does depend not on the degree of polycation complexation, but on the polycation type. One observes $T_{m,2}(\mathrm{PDADMAC}) > T_{m,2}(\mathrm{P4VP}) > T_{m,2}(\mathrm{IONENE})$. The probable reason for this series is the polycation molar mass. $T_{m,2}$ increases slightly as M_w is increased. While $T_{m,1}$ depends on the concentration of the added NaCl-salt, $T_{m,2}$ does not. This is explained using the fact that the complexed DNA repeat units are electrically neutral, so that there are no salt/charge interactions. Measurements in aqueous/organic solvent mixtures show that both $T_{m,1}$ as well $T_{m,2}$ decrease continually as the content of the organic solvent is increased. Very marked is this effect for water/*N*-methylformamide. At $w_{\mathrm{NMF}} \approx 40\%$, $T_{m,1}$ and $T_{m,2}$ are nearly half as large as in pure water. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: DNA-denaturation temperature; Degree of DNA/polycation complexation; Polycation type

1. Introduction

The equilibrium between the helical and randomly coiled conformation of desoxyribonucleic acid (DNA) in solution has been intensively studied, both experimentally [1–5] as well theoretically [6–8], for many decades. Much valuable information has been obtained, in particular, on the forces maintaining the characteristic ordered DNA secondary structure.

In water, at room temperature, moderate ionic strength, and neutral pH, a DNA double helix is stabile. Extremes of pH or ionic strength and high temperature cause DNA-denaturation, i.e. separation into two random strands. This process is called helix-coil transition and can be easily followed by changes of quantities such as viscosity, specific rotation, UV–VIS absorption, or sedimentation coefficient. Many reviews [9–15] are available on this topic.

There are also investigations on nonaqueous DNA solutions [16–18]. Additives such as alcohols, formamide, formaldehyde, or urea destabilise DNA. The denaturation temperature, T_m, at which half of the DNA base pairs broke up decreases as the weight fraction of the organic solvent in the mixture is increased. This is caused, among other factors, by changes in the solvation energy of associated and unassociated groups, in the reduction of electrostatic and dispersion forces, and in entropy changes of mixing the native and the denaturated DNA with solvent. According to Sinanoglu et al. [18–20] the dominant effect is the energy needed to create a cavity in a solvent before placing a bulky solute in it. For water this energy is very high because water has a very large cohesive energy density. This makes the surface tension very strong. In addition, water molecules are rather small. A large number of molecules can form a cage while for organic solvents this is not possible.

Previously [21], we have examined the complexation of calf-thymus DNA with polycations such as poly[*N*,*N*-diallyldimethylammonium chloride] (PDADMAC), poly[(dimethylimino) ethylene (dimethylimino)methylene-1,4-phenylenemethylene dichloride] (IONENE), and quaternized poly[vinylpyridine] (P4VP). The most important result was that there exists a critical salt concentration, $c_{s,d}$, at which the DNA/polycation complexes become unstable and dissociates back into their single strands.

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The main focus of this article is the temperature stability of calf-thymus DNA/polycation complexes, i.e. their denaturation behaviour. We specifically address to the following questions:

1. Is there any correlation between the DNA denaturation temperature and the degree of DNA/polycation complexation?
2. does this effect depends on the polycation type? and
3. are there any influences of the solvent?

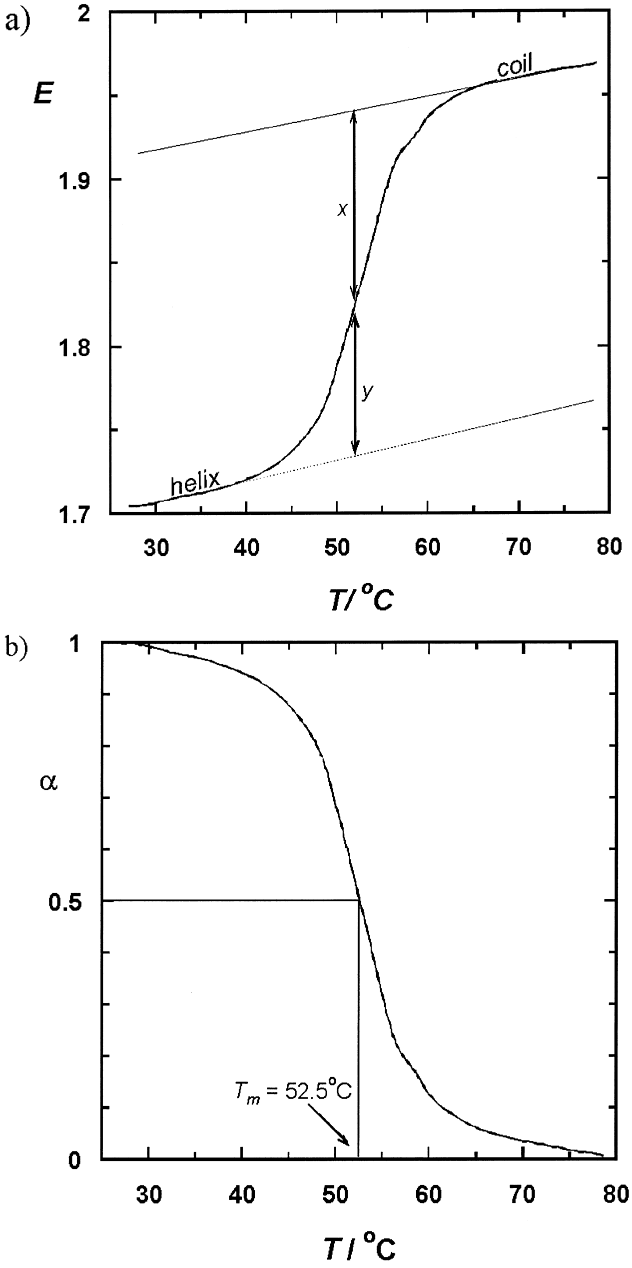

Fig. 1. Typical melting curves of calf-thymus DNA dissolved in water at $c_{\mathrm{NaCl}} = 1\cdot10^{-3}\mathrm{mol/dm}^3$ and $c_{\mathrm{DNA}} = 1.08\cdot10^{-4}\mathrm{mol/dm}^3$: (a) extinction, E, versus temperature; and (b) $\alpha = x/(x+y)$ versus T.

2. Experimental procedure

2.1. Materials

Calf-thymus DNA was purchased as a Na-salt from Sigma. Its weight average molar mass was $M_w = 4.5 \times 10^6$ g/mol. This corresponds to nearly 6500 base pairs per macromolecule. The polycation poly(*N*,*N*-diallyldimethylammonium chloride) (PDADMAC) was obtained from Aldrich in two charges. According to the manufacturer their molar masses were 2.2×10^5 and 4.5×10^5 g/mol. This was confirmed by GPC and light scattering measurements, where M_w/M_n was found to be smaller than 2. The IONENEs were poly[(dimethylimino) ethylene (dimethylimino) methylene-1,4-phenylenemethylene dichloride]. They were synthesised by Beyer [22] following the method of Tsuchida [23,24]. The IONENE molar mass was fractionated between 3000 and 1.2×10^5 g/mol, where M_w/M_n was smaller than 3. Quaternized poly[vinylpyridine] (P4VP) was purchased from Polyscience. Its molar mass was determined as $M_w = 4.0 \times 10^4$ g/mol and M_w/M_n was 2.5.

All samples were plentifully purified by dialysis and centrifugation. The pH was adjusted to 7.6 in a 10^{-3} mol/l HEPES buffer. EDTA was added, in an amount of 10^{-4} mol/l, to neutralise Mg-ions.

2.2. Methods

The degree of DNA/polycation complexation was determined by conductometry and verified by UV–VIS spectroscopy. The details were discussed intensively in Ref. [21]. The apparatus used was the conductivity meter PW 9501 from Philips equipped with the LTA/S cell from WTW. To achieve a constant temperature, a water bath was thermostatted at $T = 25 \pm 0.1$°C. Calibration was made daily, so that the experimental error was lower than 5%.

DNA denaturation was recorded as usual using a Varian Cary 13E UV-spectrometer. The rate of heating was 0.1°C per min and the error in T was smaller than 0.1°C.

2.3. Evaluation of the melting curves

There exists two possibilities to evaluate a DNA denaturation curve. Firstly, a melting curve can be normalised [25,26] as indicated in Fig. 1. There the ratio $\alpha = x/(x+y)$ is the content of the base pairs that are dissociated or denaturated, respectively. These α-values must be determined for each temperature, where the denaturation temperature, T_m, is given then as that temperature at which α is 0.5. For instance, in Fig. 1, T_m is 52.5°C.

Secondly, we can differentiate a melting curve with respect to T. The result is a curve like that presented in Fig. 2 where the maximum gives the position of T_m.

We have combined both methods to derive some important thermodynamic quantities. They are among others, the van't Hoff denaturation enthalpy, ΔH_{vH}, the Gibbs denaturation energy, ΔG_{vH}, the denaturation entropy, ΔS_{vH},

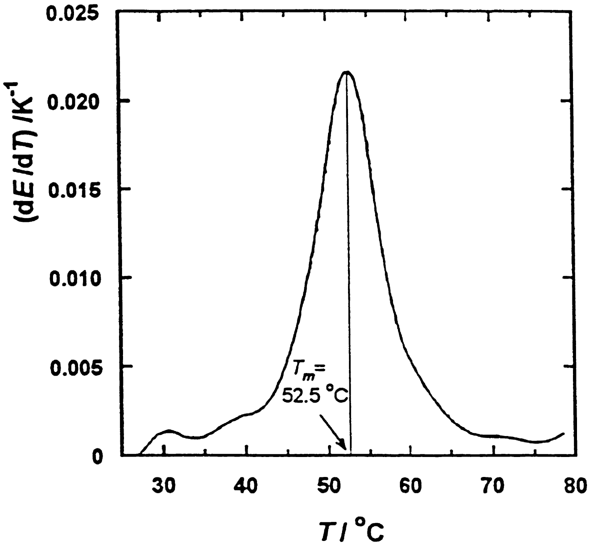

Fig. 2. Differentiation of the melting curve of Fig. 1; the peak presents the position of T_{m}.

and the number, N, of base pairs that dissociates cooperatively. Thereby it is assumed that the helix-coil transition is an all or none process. That is, a DNA repeat unit is either in the helix or in the coil conformation, while no intermediate state is allowed.

To compare the data it is conventional to recalculate them into standard conditions ($T = 298$ K, $p = 1.013 \times 10^5$ Pa). In this aspect, the parameter s defined as

$$s \equiv \frac{\alpha}{(c_{\mathrm{DNA}}/2)(1-\alpha^2)} \tag{1}$$

is the central quantity. Knowing s all other quantities can be easily derived. We have:

$$\Delta H_{\mathrm{vH}} = \mathrm{R}T^2\left(\frac{\mathrm{d}\ln s}{\mathrm{d}T}\right) \tag{2}$$

$$\Delta H^0_{\mathrm{vH}} = \mathrm{R}\ln\left(\frac{s(T_{\mathrm{m}})}{s(T^0)}\right)\frac{T^0 T_{\mathrm{m}}}{T_{\mathrm{m}} - T^0} \tag{3}$$

$$\Delta G^0_{\mathrm{vH}} = \Delta H^0_{\mathrm{vH}}\left(1 - \frac{T^0}{T_{\mathrm{m}}}\right) \tag{4}$$

$$\Delta S^0_{\mathrm{vH}} = \frac{\Delta H^0_{\mathrm{vH}} - \Delta G^0_{\mathrm{vH}}}{T^0} \tag{5}$$

where $T^0 = 298$ K.

It should be pointed out, that ΔH_{vH} is the denaturation enthalpy describing a DNA section that melts cooperatively. The number, N, of base pairs per such a cooperative unit is

$$N = \frac{\Delta H_{\mathrm{vH}}}{\Delta H_0} \tag{6}$$

where ΔH_0 is the transition enthalpy per base pair. The latter can be determined experimentally using a microcalorimeter [27], but it can be calculated theoretically too. A formula that fits the experimental data very well is that of Manning [14]:

$$\Delta H_0 = \frac{1}{2}2.303(\Delta\xi_{h,c})^{-1}\mathrm{R}T^0\left(\frac{\mathrm{d}T_{\mathrm{m}}}{\mathrm{d}\log(c_{\mathrm{NaCl}}/1\,\mathrm{M})}\right) \tag{7}$$

with

$$\Delta\xi_{h,c} = \frac{e^2}{4\pi\varepsilon_0\varepsilon \mathrm{k_B} T^0}\left(\frac{1}{b_{\mathrm{h}}} - \frac{1}{b_{\mathrm{c}}}\right)$$

where b_h and b_c are the distances between two neighbour DNA phosphate groups on the helix and the coil strand, respectively, e the elementary charge, R the gaseous constant, ϵ the dielectric constant of the solvent, $\mathrm{k_B}$ the Boltzmann-constant, and c_{NaCl} the salt concentration. For calf-thymus DNA [14] b_{h} is 1.7×10^{-10} m and $b_{\mathrm{c}} = 4 \times 10^{-10}$ m.

We have no microcalorimeter in our laboratory. Thus, we have used Eq. (7) to calculate ΔH_0. Obviously, this procedure is not exact, but the discrepancy [28,29] found between $\Delta H_{0,\mathrm{experiment}}$ and $\Delta H_{0,\mathrm{theory}}$ is seldom larger than 10%. In addition, we are most interested on general features than on exact thermodynamic values.

3. Results and discussion

A measure for the DNA-stability is the denaturation or melting temperature, T_{m}, respectively. The most comfortable method to determine T_{m} is UV–VIS absorption. There the relative light absorption $E_{\mathrm{rel}} = E(T)/E(T_{\mathrm{ref}})$, with $E(T)$ the absorption at T and $E(T_{\mathrm{ref}})$ the absorption at a reference temperature, T_{ref}, is plotted versus the temperature. Here, we have chosen $T_{\mathrm{ref}} = 40$°C. This is the starting temperature for the experiments.

Some typical results of measurement are presented in Fig. 3(a)–(c). Fig. 3(a) shows the melting curves obtained for the system DNA/PDADMAC; Fig. 3(b) yields the results for the system DNA/IONENE, and Fig. 3(c) presents the results of the system DNA/P4VP. Each figure contains five curves. The upper curve is the melting curve of the pure DNA, while the other curves describe the different degrees of polycation occupation, where $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$ increases from top to bottom, i.e., $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$ takes on the values 0, 0.1, 0.3, 0.5, and 0.7. The total polymer concentration is kept constant at $c_{\mathrm{p}} = 2.1 \times 10^{-4}\,\mathrm{mol/dm^3}$. The solution also contains a certain portion of salt ($c_{\mathrm{NaCl}} = 0.001\,\mathrm{mol/dm^3}$). This is necessary because otherwise the DNA would denature at room temperature itself.

We observe, that the relative absorption, E_{rel}, decreases as the degree of DNA/polycation complexation, $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$, is increased. Only those DNA sections that are UV–VIS active are not complexed with polycations. Thus, E_{rel} becomes zero when $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$ converges to one. Secondly, we define the melting temperature, T_{m}, as that temperature at which the melting curve has its turning point. It can be determined easily by graphically obtaining the derivative. We note that T_m does not depend on the degree of polycation complexation. T_m is of the order of 51°C for uncomplexed DNA and does not change with n_{pc}/n_{DNA}. However, there are significant and interesting differences between the three systems. For the systems DNA/IONENE and DNA/P4VP we observe two melting processes, one at the temperature $T_{m,1}$ and the other at the temperature $T_{m,2}$. At $T_{m,1}$, the uncomplexed DNA sections denature while at $T_{m,2}$, the complexed DNA sections denature. $T_{m,2}$ is significantly larger than $T_{m,1}$, indicating that polycation binding stabilises calf-thymus DNA. Such a behaviour is not unusual. Most biogen binding partners [30] stabilises DNA, although also some destabilising proteins do exist [31]. Here, the stabilisation effect is lowest for IONENE, followed by P4VP and PDADMAC.

We have repeated the above experiments for other salt concentrations leaving c_p and n_{pc}/n_{DNA} unaltered. An overview is given in Table 1. Two effects were observed. First, the $T_{m,1}$ increases as c_{NaCl} is increased. This is a well known effect [32–34]. With increasing salt concentration the DNA phosphate charges become more and more screened electrically so that the coulombic repulsion between them is lowered and finally diminished. The new effect is that $T_{m,2}$ does not depend on c_{NaCl}. The reason is that $T_{m,2}$ corresponds to those DNA sections that are complexed with polycations. These sections are electrically neutral and consequently there are no coulombic interactions at which the salt can act. However, some caution is necessary. At very high salt concentrations DNA–polycation complexes dissociate. Then $T_{m,2}$ becomes dependent on c_{NaCl}, but this effect was discussed already in Ref. [21].

The melting curves can be further analysed. Besides T_m one can derive the van't Hoff melting enthalpy, ΔH_{vH}, the van't Hoff melting entropy $\Delta S_{vH}(T_{m,1})$, or the number, N, of cooperatively acting base pairs. For this propose the curves must be differentiated with respect to T. The result is the gradient $dT_m/d\log(c_{NaCl}/(\text{mol/dm}^3))$. Here we have $dT_m/d\log(c_{NaCl}/(\text{mol/dm}^3)) = 18.3°\text{C}$ which is in good accord with the value found by Record [35]. Inserting this value into Eqs. (1)–(6) we get the data summarised in Tables 2 and 3.

We observe that both van't Hoff enthalpies and entropies depend neither on the degree of DNA/polycation complexation, n_{pc}/n_{DNA}, nor on the polycation type. All values are in quite good accord with those found for uncomplexed DNA [36]. $\Delta H_{vH}(T_{m,1})$ increases linearly as c_{NaCl} is increased. The same effect is observed for $\Delta H_0(T_{m,1})$ and $\Delta S_0(T_{m,1})$. The absolute values of ΔH_0 and ΔS_0 are somewhat smaller than those observed calorimetrically [37–39], but that is not unusual.

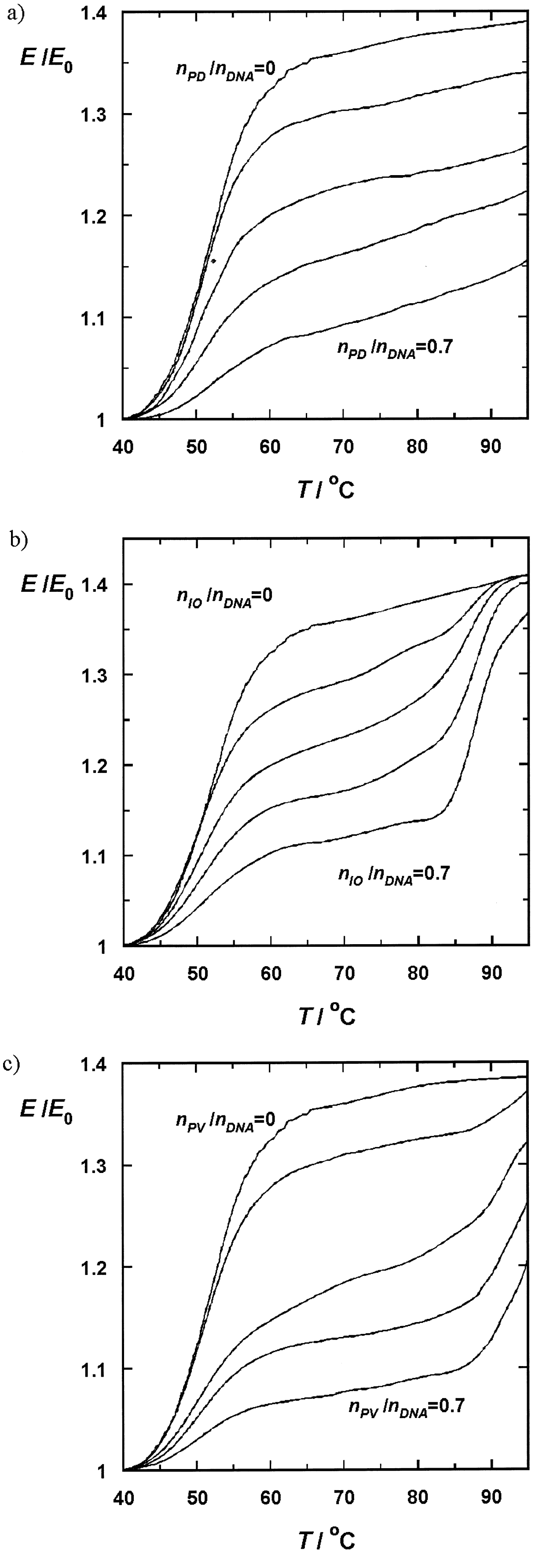

Fig. 3. Melting curves for different degrees of DNA/polycation complexation. The systems are: (a) DNA/PDADMAC; (b) DNA/IONENE; and (c) DNA/P4VP. The parameters are: $n_{pc}/n_{DNA} = 0, 0.1, 0.3, 0.5$ and 0.7, $c_{NaCl} = 0.001\text{mol/dm}^3$ and $c_p = 2.1\cdot10^{-4}\text{mol/dm}^3$.

Table 1
Melting temperature of some DNA/polycation complexes at various degrees of complexation, n_{pc}/n_{DNA}, and two different NaCl-concentrations. c_p is 2.1×10^{-4} mol/dm^3

System	c_{NaCl} (mol/dm^3)	n_{pc}/n_{DNA}	$T_{m,1}$ (°C)	$T_{m,2}$ (°C)
Pure DNA	0.001	—	52.0	—
	0.01	—	64.5	—
DNA/ PDADMAC	0.001	0.3	51.0	> 93.0
		0.5	52.0	> 93.0
		0.7	52.5	> 93.0
	0.01	0.3	65.5	> 93.0
		0.5	65.0	> 93.0
		0.7	65.0	> 93.0
DNA/IONENE	0.001	0.3	51.0	87.0
		0.5	51.0	87.5
		0.7	51.0	87.5
	0.01	0.3	66.0	87.5
		0.5	65.5	88.0
		0.7	65.0	88.0
DNA/P4VP	0.001	0.3	50.5	> 93.0
		0.5	51.0	> 93.0
		0.7	50.5	> 93.0
	0.01	0.3	64.5	> 93.0
		0.5	65.5	> 93.0
		0.7	65.0	> 93.0

The number, N, of base pairs that melt cooperatively increases as c_{NaCl} is increased. This means that the attraction between the base pairs become stronger, while simultaneously the repulsive coloumbic interactions decrease.

Table 3 lists some thermodynamic data for the second denaturation transition at $T_{m,2}$. The system is DNA/IONENE. For the two other systems, DNA/PDADMAC and DNA/P4VP, we cannot present $T_{m,2}$-data because they lie outside the range of temperature measurement.

We see that $\Delta H_{vH}(T_{m,2})$ and $\Delta S_{vH}(T_{m,2})$ are about a factor of 50–100% larger than $\Delta H_{vH}(T_{m,1})$ and $\Delta S(T_{m,1})$. That is, the second helix-coil transition is much more cooperative than the first one. As $T_{m,2}$ depends not on c_{NaCl} it is not possible to calculate ΔH_0, but there is another quantity that can be used as a cooperativity measure. This is the half width temperature, $\Delta T_{1/2}$. The smaller $\Delta T_{1/2}$ the larger is the cooperativity. Here, $\Delta T_{1/2}(T_{m,1})$ is larger than $\Delta T_{1/2}(T_{m,2})$, indicating that the $T_{m,2}$ transition is the more cooperative one. As $\Delta T_{1/2}(T_{m,2})$ is independent on c_{NaCl} this effect is not caused by the salt. The larger cooperativity is solely generated through the presence of the bound polycations.

Another topic is the dependence of the melting temperature on the polycation molar mass. It is imaginable that $T_{m,1}$ and $T_{m,2}$ do depend on the length of a polycation molecule bound to the DNA. For this purpose we have synthesised IONENEs of different molar masses. The masses used were 3000, 12 000, and 16 000 g/mol while the degree of polycation complexation and the salt concentration were kept constant. Some representative results are shown in Table 4.

It is seen that neither $T_{m,1}$ nor $T_{m,2}$ depend on $M_{w,IONENE}$. This is also found for the DNA/PDADMAC system [40], with PDADMAC of two different molar masses. However, this must be examined in more detail, a plan that will be realised in the next article.

Let us finally come to the influences of the solvent composition on $T_{m,1}$ and $T_{m,2}$. According to Levin [41] there is a correlation between the solvent type and the DNA solubility. The solvent quality decides whether DNA is in its native or denaturated state. Solvents in which adenine dissolves quite well but pyrophosphate very badly, should yield very low T_m values. To prove this idea we have performed T_m-measurements in aqueous/organic solvent mixtures, where the organic components were 1,4-dioxane and N-methylformamide (NMF), respectively. These solvents are very interesting because their dielectric constants behave significantly differently. In water/1,4-dioxane ϵ decreases with increasing dioxane content while in water/N-methylformamide ϵ increases as the content of the organic solvent is increased. All other system parameters such as $c_{DNA}, n_{pc}/n_{DNA}$, and c_p were kept constant. Representative results are presented in Table 5.

Before we discuss these results in detail let us present some other results previously recorded by Karge [42]. He

Table 2
Thermodynamic data for the helix-coil transition belonging to those DNA sections that are not complexed with polycations

System	c_{NaCl} (mol/dm^3)	n_{pc}/n_{DNA}	$\Delta H_{vH}(T_{m,1})$ (kJ/mol)	$\Delta S_{vH}(T_{m,1})$ (J/(mol K))	$\Delta H_0(T_{m,1})$ (kJ/mol)	$\Delta S_0(T_{m,1})$ (J/(mol K))	$N(T_{m,1})$
Pure DNA	0.001	0	450	1400	19.5	60.1	23
	0.01	0	570	1700	20.6	61.0	28
DNA/ PDADMAC	0.001	0.1	460	1450	19.5	60.0	24
		0.5	495	1500	19.5	60.1	25
	0.01	0.1	570	1700	20.6	61.1	28
		0.5	530	1600	20.6	61.1	26
DNA/ IONENE	0.001	0.1	420	1300	19.5	60.0	21
		0.5	420	1300	19.5	60.0	21
	0.01	0.1	515	1500	20.6	61.1	25
		0.5	530	1550	20.7	61.1	26
DNA/ P4VP	0.001	0.1	465	1400	19.5	60.0	23
		0.5	420	1300	19.5	60.0	21
	0.01	0.1	490	1450	20.6	61.0	24
		0.5	535	1600	20.7	61.0	26

Table 3
Thermodynamic data belonging to those DNA/IONENE sections that are complexed

System	c_{NaCl} (mol/dm^3)	$n_{\mathrm{pc}}/n_{\mathrm{DNA}}$	$\Delta H_{\mathrm{vH}}(T_{\mathrm{m,2}})$ (kJ/mol)	$\Delta S_{\mathrm{vH}}(T_{\mathrm{m,2}})$ (J/(mol K))	$\Delta T_{1/2}(T_{\mathrm{m,1}})$ (°C)	$\Delta T_{1/2}(T_{\mathrm{m,2}})$ (°C)
DNA/ IONENE	0.001	0.1	820	2300	10.0	6.2
		0.3	820	2300	10.5	7.1
		0.5	840	2350	11.0	6.5
		0.7	800	2200	11.0	6.0
	0.01	0.1	—	—	11.0	—
		0.3	770	2150	12.0	6.3
		0.5	860	2400	11.5	6.5
		0.7	810	2250	12.0	6.0

Table 4
Melting temperature of the system DNA/IONENE as a function of $M_{\mathrm{w,IONENE}}$ at $c_{\mathrm{NaCl}} = 0.001$ M and $n_{\mathrm{pc}}/n_{\mathrm{DNA}} = 0.5$

$M_{\mathrm{w,IONENE}}$ (g/mol)	$T_{\mathrm{m,1}}$ (°C)	$T_{\mathrm{m,2}}$ (°C)
3000	50.5	87.0
12000	50.0	87.0
16000	50.5	88.0

has examined melting curves for pure uncomplexed calf-thymus DNA in several aqueous/organic solvent mixtures. Typical results are shown in Fig. 4 where it is observed that T_{m} decreases continually as the weight fraction, w_{org}, of the organic solvent component is increased. For water/methanol and water/1,4-dioxane the decrease increment is relatively moderate while for water/*N*-methylformamide it is very strong. However, the important point is: independent of the fact that whether ϵ increases or decreases the denaturation temperature decreases. In other words, there is no correlation at all between T_{m} and the dielectric constant.

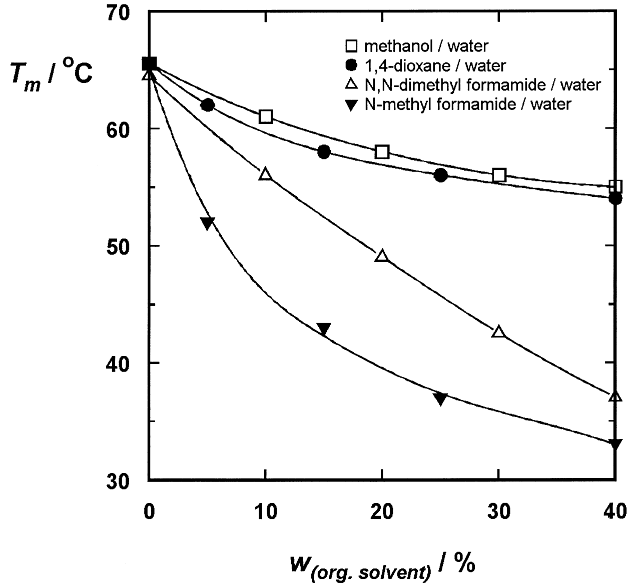

Fig. 4. Denaturation temperatures of calf-thymus DNA in various aqueous/organic solvent mixtures at c_{NaCl} =0.01 mol/dm^3: □ methanol/water, ● 1,4-dioxane/water, △ *N,N*-dimethylformamide/water, and ▼ *N*-methylformamide/water.

The same effect is observed here for complexed DNA. The $T_{\mathrm{m,1}}$ values listed in Table 5 agree nearly exactly with those measured by Karge for uncomplexed CT-DNA, underlining that the degree of polycation complexation, $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$, has no influence on $T_{\mathrm{m,1}}$. $T_{\mathrm{m,1}}$ changes as the solvent composition changes, but there is no correlation between $T_{\mathrm{m,1}}$ and $n_{\mathrm{pc}}/n_{\mathrm{DNA}}$. In addition, the influence of 1,4-dioxane on the other thermodynamic parameters is small. ΔH_{vH} and ΔS_{vH} decrease slightly as w_{diox} increases, but $\Delta T_{1/2}(T_{\mathrm{m,1}})$ remains unaffected. There is also no correlation between 1,4-dioxane and the polycation type.

Similar conclusions can be drawn for $T_{\mathrm{m,2}}$. The melting temperature $T_{\mathrm{m,2}}$ decreases with increasing w_{diox} and also the influence on ΔH_{vH} ($T_{\mathrm{m,2}}$), ΔS_{vH} ($T_{\mathrm{m,2}}$), and $\Delta T_{1/2}(T_{\mathrm{m,1}})$ is marginal (cf. Table 6). However, one significant difference exists between $T_{\mathrm{m,1}}$ and $T_{\mathrm{m,2}}$. While $T_{\mathrm{m,1}}$ decreases non-linearly with w_{diox}, $T_{\mathrm{m,2}}$ decreases linearly. In detail, the following relations hold:

$$T_{\mathrm{m,2}} = [99.4 - 0.526 w_{\mathrm{diox}}(\%)]^{\circ}\mathrm{C} \text{ for DNA/PDADMAC}$$

$$T_{\mathrm{m,2}} = [95.5 - 0.484 w_{\mathrm{diox}}(\%)]^{\circ}\mathrm{C} \text{ for DNA/P4VP}$$

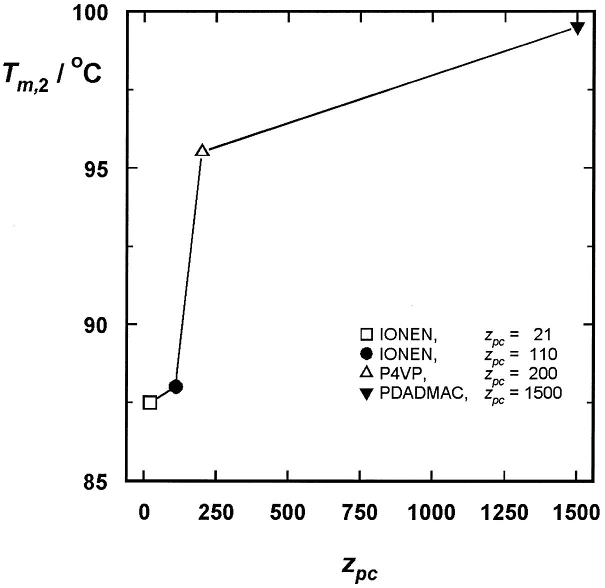

Fig. 5. Plot of $T^0_{\mathrm{m,2}}$ versus the number, z_{pc}, of repeat units per polycation chain.

Table 5
Thermodynamic data of DNA/polycation complexes dissolved in water/1,4-dioxane mixtures at $c_{\mathrm{NaCl}} = 0.01\ \mathrm{mol/dm^3}$, $c_p = 1.5 \times 10^{-4}\ \mathrm{mol/dm^3}$, and $n_{pc}/n_{\mathrm{DNA}} = 0.5$/caption>

System	w_{diox}%	$T_{m,1}$ (°C)	$\Delta H_{vH}(T_{m,1})$ (kJ/mol)	$\Delta S_{vH}(T_{m,1})$ (J/(mol K))	ΔH_0 (kJ/mol)	ΔS_0 (J/(molK))	$N(T_{m,1})$	$\Delta T_{1/2}(T_{m,1})$ (°C)
DNA/	0	65.0	535	1600	20.6	61.1	26	11.5
PDADMAC	5	63.0	535	1600	19.6	58.4	27	11.0
	15	58.5	520	1550	19.6	59.0	27	11.5
	25	56.0	500	1500	19.8	60.1	25	12.0
	40	53.5	470	1450	22.7	69.5	21	11.0
DNA/	0	65.5	530	1550	20.7	61.1	26	11.5
IONENE	5	64.0	575	1700	19.7	58.5	29	10.0
	15	58.5	550	1650	19.6	59.0	28	11.0
	25	54.0	520	1600	19.7	60.1	26	10.0
	40	51.5	490	1500	22.5	69.4	22	11.0
DNA/	0	65.5	535	1600	20.7	61.0	26	11.0
P4VP	5	62.5	530	1600	19.6	58.4	27	11.0
	15	58.0	530	1600	19.5	59.0	27	11.0
	25	56.0	510	1550	19.8	60.2	26	12.0
	40	53.5	485	1500	22.7	69.5	21	12.0

and

$$T_{m,2} = [88.2 - 0.493 \cdot w_{\mathrm{diox}}(\%)]°\mathrm{C}\ \text{for DNA/IONENE}$$

The slopes of these straight lines differ less than 9%. While the slope does not depend on the polycation type, the limit value $T^0_{m,2} = \lim_{w_{\mathrm{diox}} \to 0\%} T_{m,2(w_{\mathrm{diox}})}$does. $T^0_{m,2}$ increases as the number, z_{pc}, of repeat units per polycation chain, is increased (cf. Fig. 5). Interestingly, this curve makes a jump between $z_{pc} = 110$ and $z_{pc} = 200$, just at that position where pure uncomplexed DNA condenses [43,44]. Therefore, it is imaginable that the complexed DNA-sections build torus-like conformations while the uncomplexed sections do not, but this must be proved in more detail.

Finally, we discuss the system water/*N*-methylform amide. Unfortunately, the dielectric constant of this mixture is not known over the complete range of both w_{NMF} and T. Consequently, we can only calculate ΔH_{vH} and ΔS_{vH}, but not ΔH_0 and ΔS_0. An overview gives Tables 7 and 8. The parameters are $n_{pc}/n_{\mathrm{DNA}} = 0.5$, $c_{\mathrm{NaCl}} = 0.01\ \mathrm{mol/dm^3}$, and $c_p = 2.0 \times 10^{-4}\ \mathrm{mol/dm^3}$.

Again plots of $T_{m,2}$ versus w_{NMF} yield a significant decrease of $T_{m,2}$, but a linearity is only observed for $w_{\mathrm{NMF}} \leq 25\%$. In this region ($0 \leq w_{\mathrm{NMF}} \leq 25\%$) we have

$$T_{m,2} = [97.8 - 1.050 \cdot w_{\mathrm{NMF}}(\%)]°\mathrm{C}\ \text{for DNA/PDADMAC}$$

$$T_{m,2} = [93.0 - 1.000 \cdot w_{\mathrm{NMF}}(\%)]°\mathrm{C}\ \text{for DNA/P4VP}$$

and

$$T_{m,2} = [87.1 - 1.054 \cdot w_{\mathrm{NMF}}(\%)]°\mathrm{C}\ \text{for DNA/PDADMAC}$$

The slopes of these curves are nearly equally large again, but their absolute values are about a factor of two larger than those of water/1,4-dioxane. That is, *N*-methyformamide has a much larger effect than dioxane has. This is confirmed by ΔH_{vH}. Now ΔH_{vH} decreases with increasing w_{NMF}. Also $\Delta T_{1/2}(T_{m,2})$ is lower. That is, the helix-coil transition is more cooperative in water/NMF than in water/dioxane.

Table 6
Thermodynamic data belonging to the helix-coil transition of complexed DNA sections

System	w_{diox} (%)	$T_{m,2}$ (°C)	$\Delta H_{vH}(T_{m,2})$ (kJ/mol)	$\Delta S_{vH}(T_{m,2})$ (J/(mol K))	$\Delta T_{1/2}(T_{m,2})$ (°C)
DNA/	5	> 93.0	—	—	—
PDADMAC	15	92.0	800	2200	8.0
	25	87.0	800	2200	8.0
	40	78.0	850	2400	7.5
DNA/	0	88.0	860	2400	6.5
IONENE	5	85.0	750	2100	7.0
	15	80.0	810	2300	6.0
	25	75.0	860	2500	6.0
	40	67.5	850	2500	7.0
DNA/	5	> 93.0	—	—	—
P4VP	15	87.0	720	2000	9.0
	25	83.0	770	2100	7.5
	40	75.0	750	2100	7.0

Table 7
Thermodynamic data for solvent mixtures of water and *N*-methylformamide

System	w_{NMF} (%)	$T_{m,1}$ (°C)	$\Delta H_{vH}(T_{m,1})$ (kJ/mol)	$\Delta S_{vH}(T_{m,1})$ (J/(mol K))	$\Delta T_{1/2}(T_{m,1})$ (°C)
DNA/	15	42.5	560	1750	10.0
PDADMAC	25	38.5	480	1550	9.5
	40	32.0	450	1500	9.5
DNA/	0	65.5	530	1550	11.5
IONENE	5	51.0	530	1650	10.5
	15	43.5	530	1650	10.0
	25	39.0	500	1600	10.0
	40	32.5	480	1550	9.0
DNA/	15	44.0	540	1700	9.5
P4VP	25	39.0	560	1800	9.0
	40	31.5	470	1550	9.0

4. Conclusions

From the evidence presented so far, it is clear that a DNA molecule is stabilised by polycation binding. This is usually associated with a native DNA conformation. Surprisingly, the denaturation temperature does depend neither on the degree of polycation binding nor very sensible on the polycation type.

Undoubtedly, one major source for destabilisation of DNA is the highly charged nature of the two intertwining chains. In fact, in dilute nearly salt free aqueous solution, where the electrostatic repulsions are not screened by low molecular ions, denaturation occurs at or below room temperature. Thus, one could conclude that T_m is lower than stronger are the electrostatic interactions. However, the experiments described here show a much more unambiguous evidence in this point. Complexed DNA segments are more or less electrically neutral, so that $T_{m,2}$ becomes high. This is in accord with the above thesis, but the electrostatic free energy becomes lower also in aqueous/organic solvent mixtures, where $T_{m,1}$ and $T_{m,2}$ decrease as the organic solvent concentration increases. Consequently, electrostatic effects are not the only effects influencing DNA stability.

Interesting and meaningful may be the contribution of hydrogen bonds. Water and also some other solvents such as alcohols from solvent–solute hydrogen bonds with DNA. Therefore, DNA stability depends both on the strength and number of such bonds, as well on the number of solvent–solvent bonds that have to be broken in this process. However, no reliable information is available at present on this point. Moreover, as seen here, solvents such as *N*-methylformamide, dimethylsulfoxide, or dioxane denature DNA although they can utilise only one half of the potential hydrogen bonding sites of the nucleotide residues. Hence additional interactions other than hydrogen bonding must be responsible for a major contribution to the DNA stability in water. These other interactions may be of hydrophobic nature, i.e. attractive forces that cluster together the nonpolar residues of the DNA-macromolecules. In this aspect, it is important to realise that a DNA double helix is a structure in which not solely the maximum number of intramolecular hydrogen bonds is achieved, but it is also a structure with the most intimate clustering of the aromatic

Table 8
Thermodynamic quantities as in Table 7, but the reference temperature is now $T_{m,2}$

System	w_{NMF} (%)	$T_{m,2}$ (°C)	$\Delta H_{vH}(T_{m,2})$ (kJ/mol)	$\Delta S_{vH}(T_{m,2})$ (J/(mol K))	$\Delta T_{1/2}(T_{m,2})$ (°C)
DNA/	5	> 93.0	—	—	—
PDADMAC	15	82.5	580	1650	6.5
	20	76.0	500	1450	6.5
	25	72.0	460	1350	6.5
	40	56.0	440	1350	6.0
DNA/	0	88.0	860	2150	6.5
IONENE	5	81.5	770	2150	6.5
	15	71.0	630	1850	5.5
	25	61.5	600	1800	5.5
	40	47.5	550	1700	5.0
DNA/	5	> 93.0	—	—	—
P4VP	15	78.5	600	1700	6.5
	20	73.0	580	1650	6.5
	25	68.0	550	1600	6.0
	40	53.0	530	1650	6.0

residues of DNA, which is principally the consequence of the tendency to maximise the number of strong solvent–solvent interactions.

A similar situation is given in micelles [45], consisting of several hundreds of detergent molecules, with the hydrocarbon part forming the inner fabric, while the ionic ends distribute on the surface, remaining in contact with the solvent. There, also the maximum number of water–water bonds are formed in the solvent, while the number of solute–solute and solute–solvent hydrogen bonds are rather negligibly small.

The similarities between both systems are also striking in mixed solvents [46–48]. Both DNA as well micelles are less stable in alcoholic solution although electrostatic factors tend to stabilise them. The equivalent conductance in aqueous/organic detergent solutions decreases with increasing organic solvent concentration, indicating that extensive association occurs amongst the charged groups and the counterions. Clearly this must be due to a diminution of the hydrophobic interactions, because in a mixed solvent–solvent interaction energy is smaller than in pure water.

In summary, up to now it is not possible to calculate the different contributions to the stabilisation of DNA. In water electrostatic repulsions destabilises a helical structure while hydrogen bonds and hydrophobic interactions stabilises, where the both latter contributions may play at least an equally important role. In contrast, in mixed solvents the solute–solute, as well as the solvent–solvent interactions become nearly equally strong while electrostatic repulsions become more or less negligible when the organic solvent content becomes significantly high. As a consequence, each molecule interacts with each other in a similar way so that it is no longer necessary to form a DNA-helix or a DNA/polycation complex.

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