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Simultaneous effect of cadaverine and osmolytes on ct-DNA thermal stability

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Abstract

In the real cellular conditions, the biological macromolecules, as DNA and proteins, live in high concentrations of biomolecules, salts and so on. In particular organisms and cellular systems, which have adapted to extreme environmental conditions, accumulate particular organic solutes, known as osmolytes, at moderate to high concentrations to raise the osmotic pressure in the cytoplasm. This molecular crowding affects stability and activity of biological macromolecules. The goal of this work is to study the simultaneous effect of a natural osmolyte and polyamine on the stability of DNA. Particularly, we report a calorimetric study of the dependence of the thermal stability of a calf-thymus DNA (ct-DNA) on changing the osmolyte concentration in the presence of a constant concentration of a 1,5-diaminopentane, commonly known as cadaverine, which belongs to the class of natural polyamines. The osmolytes utilised were betaine, D-arabitol, L-arabitol and D-sorbitol. The effect on the DNA thermal stability of cadaverine and of each osmolyte separately was also reported for comparison. Our data show that cadaverine does not lose its own ability to protect DNA by the destabilising action of high concentration of osmolyte molecules synthesised in conditions of environmental stress.
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Keywords: Thermal stability; Calf-thymus DNA; Osmolytes; Polyamine; Differential scanning calorimetry

1. Introduction

Many aquatic organisms experience some form of environmental stress, like high or fluctuating salinity, desiccation or freezing. The organisms that are found living from fresh water to saturated brines (6 M NaCl) are confronted with potential problems of water gain or loss and their intracellular osmotic pressures may fluctuate greatly [1]. It would be very interesting to understand what kind of cellular mechanisms have been developed to let some organisms survive in very hostile habitats. It is well known that organisms and cellular systems, which have adapted to extreme environmental conditions, accumulate particular organic solutes, known as osmolytes, at moderate to high concentrations to raise the osmotic pressure in the cytoplasm [2]. Chemically, the naturally occurring osmolytes can be grouped into three major classes of low molecular weight compounds: polyols (glycerol, mannitol, arabitol), free amino acids and their derivatives (taurine, ß-alanine) and urea and methylamines (trimethylamine-*N*-oxide TMAO, betaine, sarcosine). Polyols occur in many unicellular algae, certain plants and many insects exposed to freezing temperature. Amino acids and their derivatives are the dominant solutes in salt-tolerant bacteria, halophytes, marine invertebrates and hagfishes. TMAO and methylglycines, instead, occur in some marine organisms in which urea is accumulated as an important product of nitrogen metabolism [1]. The natural capability of these substances to give protection to proteic and enzymatic systems without interfering with the metabolic functions would appear to provide an important and general selective advantage for organisms to adapt to environmental stresses [3]. This selective advantage would greatly reduce the necessity to genetically modify organism's proteins in order to face these extreme conditions [1,4]. On the other hand, these substances greatly contribute to increase the molecular crowding and it was recently studied the consequence of the molecular crowding on DNA triplex

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[5] and quadruplex structures [6]. Furthermore, in the real cellular conditions, DNA is not a naked molecule, but there are strong interactions between DNA and different classes of proteins, like histones, positively charged, which may link to the double helix to form a supramolecular structures. In spite of this molecular complexity, almost all DNA/biomolecules interactions have been investigated through experiments performed on a naked DNA and in solutions containing low concentrations of biomolecules.

The goal of this work is to study the simultaneous effect of a natural osmolyte and polyamine on the stability of DNA. Particularly, we report a calorimetric study of the dependence of the thermal stability of the calf-thymus DNA (ct-DNA) on changing the osmolyte concentration in the presence of a constant concentration of a 1,5-diaminopentane, commonly known as cadaverine, which belongs to the class of natural polyamines. The osmolytes utilised were betaine, D-arabitol, L-arabitol and D-sorbitol. Polyamines, like histones, are positively charged at physiological conditions, and increase DNA stability by means of electrostatic interactions [7]. The effect of cadaverine alone and of each osmolyte disjointedly on the stability of DNA was also reported for comparison. Our data are particularly useful to gain more insight into mechanisms of interaction of the different osmolytes with DNA and suggest that the natural polycationic molecules (like cadaverine) are able to stabilise DNA also in presence of high concentrations of osmolyte molecules synthesised in conditions of environmental stress.

2. Experimental

2.1. Materials

Calf-thymus DNA sodium salt, with 42% dG–dC base pairs [8], was purchased from Sigma and used without further purifications.

DNA stock solutions were prepared as follows: lyophilised DNA samples (ca. 3 mg ml^{-1}) were dissolved in 1.0 mmol l^{-1} Tris buffer, 10 mmol l^{-1} NaCl and 0.2 mmol l^{-1} Na_2EDTA at pH 7.2 for 48 h at 4 °C, then dialysed exhaustively against the same buffer solution. The DNA concentration was determined spectrophotometrically at 260 nm by using a molar absorption coefficient of 13200 dm^3 mol^{-1} cm^{-1} (expressed in molarity of base pairs). The mean molecular weight of a base pair was assumed to be 660 g mol^{-1}.

Betaine, L-arabitol, D-arabitol and D-sorbitol were purchased from Sigma and used without further purification. 1,5-Diamminopentane was purchased from Fluka and used without further purification. The osmolytes were dried over P_2O_5 in a vacuum dessicator for 12 h prior to use.

We prepared fresh stock solutions at known concentration of osmolyte and cadaverine before each experiment in the same buffer as DNA. Samples for calorimetric measurements were prepared by mixing appropriate volumes of DNA, cadaverine and osmolyte solutions at known concentration and diluting with the buffer solution to a standard volume (usually 3.0 ml). DNA concentration was kept constant at 2 mmol l^{-1} (molarity of base pair) for all experiments. Particularly in the DNA/cadaverine system, the cadaverine concentration was varied in order to obtain solutions with [cadaverine]/[ct-DNA] base pair concentration ratios R in 0.1–2 range. In the DNA/cadverine/osmolyte systems, R was kept constant at 0.5 and the concentration of osmolyte was varied.

2.2. Differential scanning calorimetry (DSC)

Differential scanning microcalorimetry is a powerful investigation methodology for thermal denaturation. It allows a direct measurement of the enthalpy changes associated with all the conformational changes undergone by the macromolecular system. Calorimetric measurements were carried out on a second-generation Setaram Micro-DSC apparatus, suitable for working on dilute solutions of biological macromolecules in the temperature range 0–100 °C. The calorimeter was interfaced to an IBM PC computer for automatic data collection and analysis using a software previously described [9]. The apparent molar heat capacity versus temperature profiles were obtained by subtracting buffer versus buffer curves from the sample versus buffer curves. The data were normalised with regard to the concentration, sample volume and scan rate. The excess heat capacity function $\langle \Delta C_p \rangle$ was obtained after baseline subtraction, assuming that the baseline is given by the linear temperature dependence of the native state heat capacity [10,11].

The denaturation enthalpies, ΔH_d, were obtained by integrating the area under the heat capacity versus temperature curves. Each value represents the average of at least three experiments. The determination of melting temperature in all the experiments shows little deviation (±0.2 °C) from the mean value even though different samples were used. The relative uncertainty of the value of the denaturation enthalpy is below 5%. The van't Hoff enthalpy was obtained by the commonly used formula:

$$\Delta H_{\text{v.H.}} = \frac{\sigma R T_{\max}^2 \langle \Delta C_p \rangle_{\max}}{\Delta H_d}$$

where $\langle \Delta C_p \rangle_{\max}$ is the maximum height of the observable excess molar heat capacity, $T_{\max}$ the melting temperature corresponding with $\langle \Delta C_p \rangle_{\max}$, R the gas constant and $\sigma = 6$ in this case referring to the double-stranded nature of the native system [12,13].

3. Results

3.1. DNA/cadaverine interaction

In the Fig. 1 (curve a), the characteristic melting profile of calf-thymus DNA is shown. Heat capacity as a function of

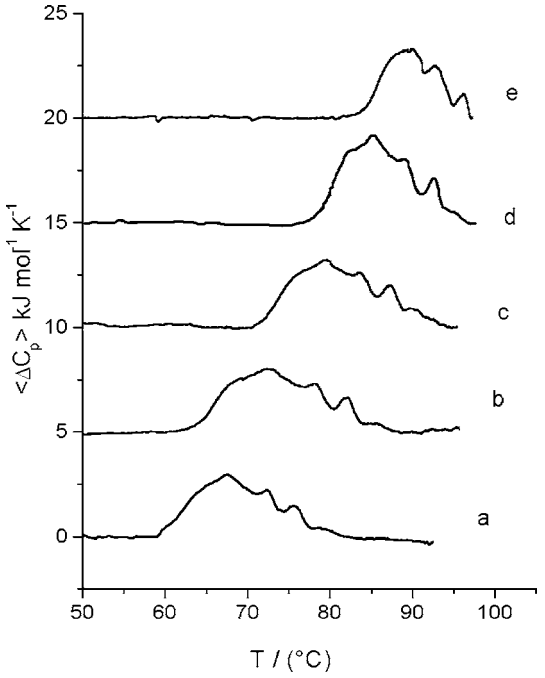

Fig. 1. DSC profiles of calf-thymus DNA in the absence or in the presence of different amounts of cadaverine. The amount of cadaverine is indicated as R corresponding to the ratio between cadaverine and DNA concentrations: (a) $R = 0$; (b) $R = 0.2$; (c) $R = 0.5$; (d) $R = 1.0$ and (e) $R = 2.0$. Excess capacity values have been shifted along the y-axis for ease presentation.

temperature is unique for a DNA sequence according to its base composition. The melting profile of calf-thymus DNA near the principal maximum, centred at 67.5 °C, shows three further "satellite" peaks at higher temperatures. The ct-DNA melting pattern is reproducible and identical to that obtained by Klump [14]. Both experimental and theoretical approaches have attributed such "satellites" to the presence in the genome of highly repetitive short base pair sequences with higher content of dG–dC [14–17]. The portion of highly, moderately, or poorly repetitive sequences varies from the DNA of one species to those of others [18,19].

The denaturation of DNA is completely irreversible because the denaturation profile disappears after the first heating and the subsequent cooling at room temperature. However, this criterion for irreversibility could be too restrictive [20]. In fact, the irreversibility arises essentially from the kinetic control on the renaturation step due to the enormous number of base pair recombination. Homopolynucleotides, as poly(dA–dT) or small oligonucleotides, show reversible reconstitution of the double helix [18].

In Fig. 1 are shown the melting profiles of the ct-DNA in presence of cadaverine on changing [cadaverine]/[ct-DNA] ratio (R). The stabilisation of the double helix of DNA by cadaverine is clearly shown by the increase of temperature of principal maximum on increasing the ratio R. In fact a shift toward higher temperatures of the whole broad thermal denaturation peak is observed, furthermore its asymmetry is only slightly altered, while the fine structure is still evident.

Table 1
Thermodynamic parameters for the denaturation process of calf-thymus DNA at different [cadaverine]/[ct-DNA] ratios (R)

R	T_{max} (°C)[a]	ΔH_d[b] (kJ mol^{-1})	$\langle m \rangle$
0	67.5	34.0	16
0.1	70.7	34.6	13
0.2	72.6	38.4	13
0.3	76.8	36.2	13
0.5	81.3	36.5	13
0.7	83.1	37.1	13
0.8	83.6	46.3	13
1.0	85.3	43.2	14
1.4	88.3	–	–
1.5	89.0	–	–
2.0	90.0	–	–

[a] The error in T_{max} does not exceed 0.2 °C.
[b] The estimated (relative) uncertainties on ΔH_d are below 5% of reported values. The ct-DNA concentration (expressed as molarity of base pair) is 2×10^{-3} mol l^{-1}.

As shown in Fig. 2, at R values ranging from 0 up to 2, T_{max} varies from 67.5 to 90 °C: the temperature rapidly increases at lower R values and reaches a plateau at higher R values.

Table 1 summarises the thermodynamic parameters that characterise the thermal denaturation process of the ct-DNA in the presence of different concentrations of cadaverine. The calorimetric enthalpy increases with an increase in the cadaverine concentration. It is not possible to obtain ΔH_d values at R higher than 1.0 because denaturation profiles occur outside the temperature range experimentally accessible. A quantitative comparison of the calorimetric enthalpy with the van't Hoff enthalpy gives information on the cooperativity of the transition. The $\Delta H_{v.H.}/\Delta H_d$ ratio, indicated with the parameter $\langle m \rangle$ in the Table 1, is correlated with the mean number of nucleotides that melt as a single thermodynamic entity, it is an index of the mean cooperative unit size [13,17]. The $\langle m \rangle$ parameter slightly decreases with respect to the DNA alone, but there is no large alteration of the size of the cooperative unit for different R values.

3.2. DNA/betaine interaction

In Fig. 3 the DSC profiles of the ct-DNA/cadaverine/betaine system are compared with those of system ct-DNA/betaine previously reported [21]. At increasing concentration of betaine, the DNA broad thermal denaturation profile becomes sharper and the fine structure becomes less evident. In fact, there is a progressive shift of melting temperatures of satellite peaks, until they merge all together to a common temperature value: $T_{max} = 49.1$ and 64 °C in absence and in presence of cadaverine, respectively. This situation occurs at 5 mol l^{-1} betaine in both systems.

Table 2 summarises the thermodynamic parameters that characterise the thermal denaturation process of the calf-thymus DNA in presence of different betaine concentration (expressed in mol l^{-1}) and of a fixed cadaverine concentration ([cadaverine]/[ct-DNA] = 0.5). The thermodynamic

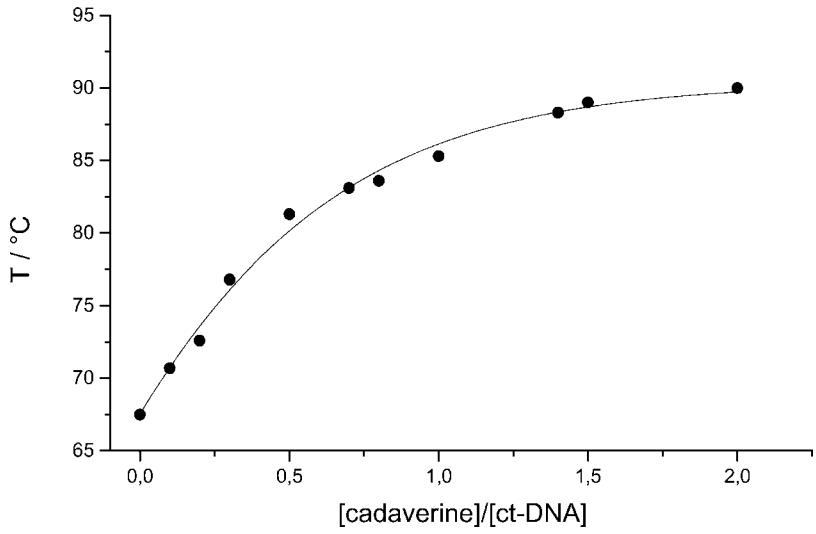

Fig. 2. Melting temperatures for calf-thymus DNA as a function of the ratio (R) between the cadaverine and DNA concentrations.

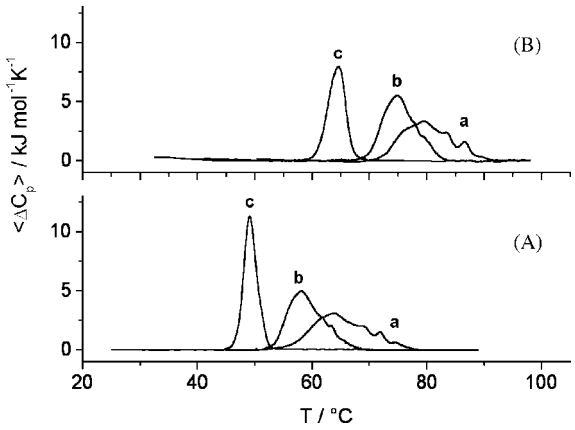

Fig. 3. Comparison between the DSC profiles of calf-thymus DNA in presence of different amount of betaine and in absence (A) or in presence (B) of a fixed cadaverine concentration ([cadaverine]/[ct-DNA] = 0.5). The amount of betaine concentration are: (a) 1 mol l^{-1}; (b) 3 mol l^{-1} and (c) 5 mol l^{-1}.

parameters of the corresponding ct-DNA/betaine systems are also reported for comparison. From this Table it can be seen that the decrease of T_{max} on increasing the concentration of betaine is the same for both systems: increasing the betaine concentration, T_{max} decreases almost linearly. Particularly, the increase of betaine concentration from 1 to 5 mol l^{-1} leads to a shift in T_{max} of about 14.5 °C. The values of the parameter $\langle m \rangle$ strongly rise on increasing concentration of betaine, as shown in Table 2. This corresponds to a progressive increase of the cooperativity of the denaturation process. Since the calorimetric enthalpy is practically constant, the significant T_{max} variation must be due to entropic effects, as the increase of parameter $\langle m \rangle$ suggests.

3.3. DNA/polyols interaction

In Fig. 4 are shown the DSC profiles for the ct-DNA/cadaverine/D-sorbitol on increasing D-sorbitol concentration compared with the DSC profile of ct-DNA alone. A shift of the whole denaturation profile toward lower temperatures is observed, but the shape of DSC profile is not altered. This

Table 2
Thermodynamic parameters for the denaturation process of calf-thymus DNA in the presence of different concentrations of betaine and in absence or in presence of a fixed concentration of cadaverine ([cadaverine]/[ct-DNA] = 0.5)

[Betaine] (mol l^{-1})	ct-DNA/betaine[a]			ct-DNA/cadaverine/betaine		
	T_{max} (°C)[b]	ΔH_d (kJ mol^{-1})[c]	$\langle m \rangle$	T_{max} (°C)[b]	ΔH_d (kJ mol^{-1})[c]	$\langle m \rangle$
0	67.5	34.0	16	81.3	36.5	16
1	63.9	34.3	16	78.4	35.5	16
2	61.3	35.1	21	75.6	37.6	20
3	58.0	34.3	26	72.8	37.4	24
4	54.0	34.9	35	68.8	36.0	37
5	49.1	34.7	55	64.0	35.4	51

[a] Data from ref. [21].
[b] The error in T_{max} does not exceed 0.2 °C.
[c] The estimated (relative) uncertainties on ΔH_d are below 5% of reported values. The ct-DNA concentration (expressed as molarity of base pair) is 2×10^{-3} mol l^{-1}.

Table 3
Thermodynamic parameters for the denaturation process of calf-thymus DNA in the presence of different concentrations of polyol and in absence or in presence of a fixed concentration of cadaverine ([cadaverine]/[ct-DNA] = 0.5)

[Osmolyte] (mol l^{-1})	T_{max} (°C)[a]	ΔH_d (kJ mol^{-1})[b]	⟨m⟩	T_{max} (°C)[a]	ΔH_d (kJ mol^{-1})[b]	⟨m⟩
		ct-DNA			ct-DNA/cadaverine	
0	67.5	34.0	16	81.3	36.5	16
		ct-DNA/D-arabitol[c]			ct-DNA/cadaverine/D-arabitol	
0.5	65.5	32.2	15	80.6	35.3	15
1	64.2	31.1	15	80.0	32.3	17
		ct-DNA/L-arabitol			ct-DNA/cadaverine/L-arabitol	
0.5	65.8	32.4	15	80.2	35.1	17
1	64.5	32.0	15	79.0	33.8	17
		ct-DNA/D-sorbitol[c]			ct-DNA/cadaverine/D-sorbitol	
0.5	65.4	34.7	15	79.8	36.7	15
1	63.1	30.2	14	78.4	32.6	17

[a] The error in T_{max} does not exceed 0.2 °C.
[b] The estimated (relative) uncertainties on ΔH_d are below 5% of reported values. The ct-DNA concentration (expressed as molarity of base pair) is 2×10^{-3} mol l^{-1}.
[c] Data from ref. [22].

suggests that the D-sorbitol does not distinguish the different composition in base pairs, consequently the ct-DNA retains the typical pattern of satellite peaks. A similar behaviour was also observed for D- and L-arabitol.

Table 3 summarises the thermodynamic parameters characterising the thermal denaturation process of ct-DNA in the presence of polyols at two different concentrations (0.5 or 1 mol l^{-1}) and at fixed cadaverine concentration. The thermodynamic parameters of the corresponding ct-DNA/polyol systems are also reported for comparison. Polyols concentrations higher than 1 mol l^{-1} could not be tested because of the low solubility of each polyol in the buffer solution containing DNA.

The results show a thermal destabilisation of the DNA in the solutions containing polyols; in fact both the denaturation temperatures and enthalpies decrease.

In the ct-DNA/cadaverine/polyol systems we can observe that the destabilising effect of the polyols is not greater than 3 °C, whereas ΔH_d does not significantly change. In absence of cadaverine the destabilising effect is 1–2 °C higher than in the corresponding ct-DNA/cadaverine/polyol system. Furthermore, no difference was observed in the destabilising effect between D- and L-arabitol. This observation indicates that the destabilisation mechanism is not influenced by stereochemistry. The ⟨m⟩ parameter is almost the same of the DNA itself, indicating no large alteration of the size of the cooperative unit for different R values.

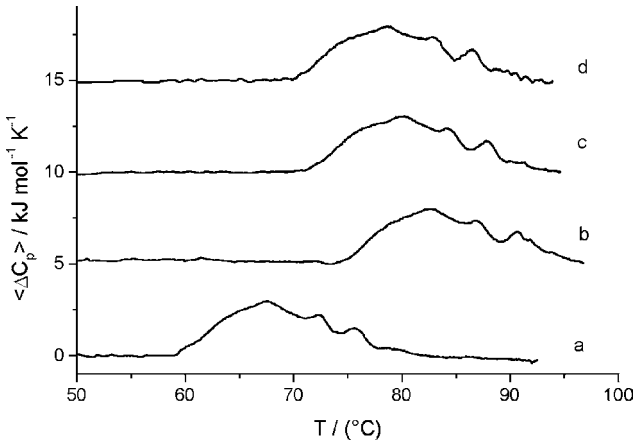

Fig. 4. DSC profiles of calf-thymus DNA alone (a) and in presence of a fixed concentration of cadaverine ([cadaverine]/[ct-DNA] = 0.5) and of different amount of D-sorbitol; (b) no polyol; (c) 0.5 mol l^{-1} and (d) 1.0 mol l^{-1}. Excess capacity values have been shifted along the y-axis for ease presentation.

4. Discussion

This work was devoted to the study of the simultaneous effect of osmolytes and of a natural polyamine (cadaverine) on the stability of ct-DNA. The osmolytes used were betaine, L-arabitol, D-arabitol and D-sorbitol. We firstly discuss the results obtained utilising betaine that bring out two different independent phenomena: the isostabilising effect and the destabilising effect on ct-DNA. These phenomena occur either in presence or in absence of cadaverine.

The isostabilising effect of betaine on DNA has already been studied in previous works [21,22]. To explain it, the hypothesis proposed by Melchior and von Hippel [23] was followed. They suggested that hydrophobic solutes are attracted into the macromolecular domain more strongly on increasing their concentration. Many of these compounds of suitable size can be accomodate in the major groove of the B-DNA double helix and give rise to preferential weak and non-cooperative hydrophobic interactions with the methyl group of the thymines exposed in the major groove. This increases the local stability of dA–dT base pairs, probably because the removal of the water makes the interbase hydrogen bonds stronger. Similar preferential interactions are impossible for dG–dC base pairs because of the lack of methyl groups. This yields a decrease of the stability difference between dA–dT and dG–dC base pairs.

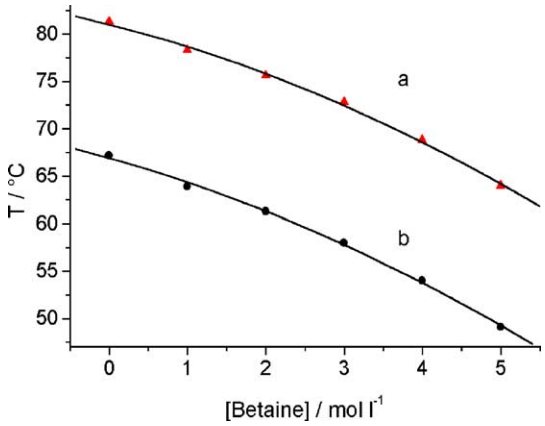

Fig. 5. Melting temperatures for calf-thymus DNA as a function of betaine concentration in presence (a) and in absence (b) of a fixed concentration of cadaverine ([cadaverine]/[ct-DNA] = 0.5).

The osmolyte concentration at which the stability difference between dA–dT and dG–dC completely disappears is termed the "isostabilisation concentration" [22]. From our experimental results it is evident that the effect is the same in the ct-DNA/cadaverine/betaine system. In other words, cadaverine has not consequence on this effect. This observation reinforces the previous hypothesis that "isostabilising effect" is due to a binding of betaine to DNA major groove. In fact, the polyamines are a minor groove ligands, as demonstrated by Liquori et al. [24], and, consequently cannot affect the binding of betaine in the major groove.

Regard to the destabilising effect of betaine, the Fig. 5 clearly shows that the amount of destabilisation on ct-DNA is the same in absence or in presence of cadaverine. Indeed, the absolute values of ΔT are the same for each concentration of betaine independently from the presence of cadaverine. In order to understand this result, it must be considered how betaine affects DNA stability. In DNAs, the purinic and pyrimidinic bases are largely "stacked" in the interior of the macromolecule, with most of their surfaces removed from contact with the solvent, whereas, in the denatured state, these groups are more solvated. Consequently, the denaturation of DNA is favoured by every change that increases the solvent hydrofobicity. This mechanism is invoked to explain the destabilising effect of betaine on DNA double helix [21]. On the other hand, cadaverine stabilises DNA by direct binding to the double helix. We can conclude that betaine and cadaverine affect, independently each other, DNA stability with different mechanisms: betaine acts on solvent properties whereas cadaverine acts directly on DNA structure.

In the case of polyols, the presence of the hydroxyl groups, makes them capable to destabilise DNA by forming hydrogen bonds with the DNA bases, both in the minor and major grooves of DNA, increasing dehydration of DNA [25]. This hypothesis is consistent with the observation that the destabilising capability of these compounds is related to the molecular size and thus to the number of hydroxyl groups on each polyol molecule. In fact, sorbitol which has six hydroxyl groups has a destabilising effect higher than arabitol, which has five hydroxyl groups. Polyol molecules can compete with cadaverine molecules in the minor groove of DNA. This explains because the polyols destabilising effect is slightly reduced in the presence of cadaverine also in the case of relatively high concentrations of polyols.

In conclusion, our data are particularly useful to quantify the simultaneous effect of cadaverine and osmolytes on DNA stability and suggest that the natural polycationic molecules (like cadaverine) are able to stabilise DNA also in the presence of high concentrations of osmolyte molecules synthesised in conditions of environmental stress.

Acknowledgements

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