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# EFFECT OF TETRAMETHYLAMMONIUM IONS ON CONFORMATIONAL CHANGES OF DNA IN THE PREMELTING TEMPERATURE RANGE

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The reversible conformational change of DNAs and polydeoxyribonucleotides occurring before melting was followed by circular dichroism. $\Delta\theta/\Delta T$, the rate of change of ellipticity $\theta$ with temperature, was used mainly as a measure of this premelting phenomenon. If sodium ions were replaced by tetramethylammonium ions $\Delta\theta/\Delta T$ decreased for poly (dA) poly (dT) and poly (dA.dT) poly (dT.dA), but increased for poly (dG.dC) poly (dC.dG). DNAs of different base composition showed no more premelting ($\Delta\theta/\Delta T \sim 0$) even at low molarities of TMACl provided the Na/TMA ratio was very small. For all cases studied the $\theta$ values at 0°C and at a given ionic strength were smaller in NaCl than in TMACl. When studying the series of ammonium ions from $NH_4^+$ to $(C_2H_5)_4N^+$, the $\Delta\theta/\Delta T$ values first decreased, going through zero with $TMA^+$ ions, and then increased again. A tentative and qualitative explanation of our results can be given: (a) Hydration of the polymers increases in presence of TMA ions and their average stability decreases; locally, however, (AT) pairs are preferentially stabilized by TMA ions owing to a specific interaction at the level of $O_2$ of thymine. (b) In order to explain the different behaviour of (AT) polymers and DNA, it is assumed that only the B structure is able to accommodate TMA ions in the small groove of the double stranded helix.

## 1. Introduction

Before its cooperative thermal denaturation the DNA molecule experiences conformational changes for which the word "premelting" was coined, since they occur in a temperature range lying largely before the melting temperature $T_m$. An extensive bibliography of this phenomenon will be found in a comprehensive review recently published by Palecek [1]. As pointed out in this review, there are as many explanations of the premelting phenomenon as there are methods used for its detection. One of the main difficulties lies in the comparison of physical properties which are not related to the same structural features of the DNA molecule. There is, however, general agreement about the following characteristics of the process:

a) Premelting is a reversible and non-cooperative process, affecting only double-stranded structures of DNA and polydeoxyribonucleotides. It is definitely distinct from melting [2–4].

b) The conformational change observed during premelting cannot be simply related to a transition between any one of the main A, B, C or D structures [4–8].

c) As thoroughly documented and discussed in Palecek's review [1], premelting appears to be more likely a local process affecting only a limited number of base pairs. However, any explanation of premelting in terms of localized defects (loops, hair pins, single strand breaks) seems to be ruled out.

The above statements can be considered as a "negative" definition of premelting and do not offer any explanation of the process even from a qualitative point of view.

In the present study we have tried to present some new experimental evidence by comparing CD premelting in NaCl and in alkylammonium salt, especially tetramethylammonium chloride (TMACl).

Tetraalkylammonium salts were shown to be preferentially bound to (AT) base pairs [9] and could therefore be used as a probe of their conformational change. On the other hand the thermal stability of DNA is affected by high molarities of tetraalkylammonium ions, particularly tetramethylammonium ($TMA^+$) and tetraethylammonium ($TEA^+$) [10].

a) There is a general destabilizing effect dependent

on the alkyl chain length. It is higher with TEACl than with TMACl.

b) A differential effect on (AT) and (GC) pairs is also present, since $dT_m/dX_{GC}$ was reduced to zero in 3 M TMACl or 2.4 M TEACl. This latter effect is likely due to preferential binding to (AT) pairs, the corresponding stabilization being a function of the size and the charge of the cation. In this paper $TMA^+$ will be shown to be so far the only cation able to prevent any premelting conformational change of the DNA.

## 2. Experimental

### 2.1. Materials

Purified calf thymus DNA (CT DNA) and E. coli DNA (EC DNA) were prepared according to the method of Kay et al. [11]. These preparations had a protein content of less than 0.5%, as determined by amino acid analysis, and a sedimentation coefficient of about 20 S in 0.1 M NaCl. M. Lysodeikticus DNA (ML DNA) was purchased from Miles, and polydeoxyribonucleotides: poly (dA.dT) poly (dT.dA), poly (dA). poly (dT), poly (dG.dC) poly (dC.dG) from P.L. Biochemicals Inc. Analytical reagents were obtained from Merck. Tetramethylammonium chloride (TMACl) was found to contain traces of $Ca^{++}$ and $Mg^{++}$ by atomic absorption spectroscopy, which were removed by chromatography of a 1 M TMACl solution through a column of Dowex A-1 chelating-resin (Chelex). Constant ionic strength buffer (citrate-phosphate buffer) was prepared at different pH according to the procedure of Elving et al. [12]. All samples of DNA were solubilized in 1 mM phosphate buffer (pH 7), 0.2 mM EDTA, and then exhaustively dialyzed against the appropriate solvent containing in each case 0.2 mM EDTA to remove divalent ions. In some cases, nonbuffered solutions of TMACl were used in order to minimize the concentration of $Na^+$ ions.

### 2.2. Spectroscopy

*DNA concentrations* were determined from absorbance $A_{260}$ per nucleotide residue of 6600 $M^{-1}$ $cm^{-1}$ for EC DNA and CT DNA, and 7800 $M^{-1}cm^{-1}$ for ML DNA. The $A_{260}$ used for polydeoxyribonucleotides were taken from Allen et al. [13].

*Absorbance melting curves* were recorded with a system described in a preceding paper [14]. The degree of denaturation of (A + T) and (G + C) base pairs as a function of temperature was determined by multiple wavelength analysis [15].

*Circular dichroism* spectra were recorded with a Roussel-Jouan 185 dichrograph equiped with a 150 W Xenon lamp or with a Roussel-Jouan DC III dichrograph. The CD signal at a given wavelength versus temperature was registered as previously described [16]. The results are presented in terms of molar ellipticities in degree $\cdot cm^2 \cdot decimole^{-1}$, based on the molar nucleotide concentration. In each case, absorbance and CD melting curves were recorded on the same sample of DNA in order to control the hyperchromicity and the $T_m$ of the sample. On the premelting range, quasi linear variations of CD signal with temperature at a given wavelength $\lambda$, were represented by the average slope $\Delta\theta_\lambda/\Delta T$ in degree $cm^2$ $dmole^{-1}$ $°C^{-1}$.

## 3. Results

### 3.1. Behaviour in NaCl

Many data have already been published [1–4,7,17', 18] and our results are in agreement with previous experiments. They are briefly summarized in table 1. The highest slope, $\Delta\theta_\lambda/\Delta T$, is found with (AT) rich polymers. However, at a low molarity, poly (dG.dC) poly (dC.dG) presents also an important premelting effect.

### 3.2. Behaviour in TMACl

*Absorbance melting curves*. At a given value of the ionic strength the $T_m$ of DNA is lower in TMACl than in NaCl even at low molarities (table 2B). The destabilization is about 10°C in the range of ionic strength of 0.01 M to 1 M. When $TMA^+$ is not the unique cation, the $T_m$ takes an intermediate value between that measured in NaCl solutions and in TMACl solutions. These results are consistent with the existence of a competition between $TMA^+$ and other cations such as $Na^+$.

The melting parameters of polynucleotides and DNAs of various sources in 0.1 M NaCl and 0.1 M TMACl are compared in table 2A. The richter the DNA is in (AT) pairs, the less is its $T_m$ affected. It

Table 1
CD premelting parameters of DNA from different sources and deoxypolynucleotides in sodium salt

| Deoxypolynucleotide | %(A T) | Ions strength [a] (M) | $T_m$ (°C) | λ [b] (nm) | $\theta_\lambda^{25°C}$ | $\Delta\theta_\lambda/\Delta T$ |
|---|---|---|---|---|---|---|
| Poly (dA) poly (dT) | 100 | 0.1 | 65 ± 0.5 | 282 | 5400 ± 200 | 167 |
| Poly (dA.dT) poly (dT.dA) | 100 | 0.1 | 57 – | 262 | 7900 – | 100 |
| CT DNA | 58 | 0.1 | 83 – | 280 | 8100 – | 32 |
| ECDNA | 50 | 0.1 | 87.5 | 280 | 8250 – | 13 |
| ML DNA | 28 | 0.1 | 97 – | 280 | 9300 – | 11 |
| Poly (dG.dC) poly (dC.dG) | 0 | 0.003 | 85 – | 280 | 6700 – | 46 |

[a] Solvent was NaCl 0.1 M, phosphate buffer 0.001 M pH 7, EDTA 0.0002 M. [b] Wavelength at which the CD melting curve was recorded.

should be pointed out that poly (dA.dT) poly (dT.dA) represents the only case of a higher $T_m$ in 0.1 M TMACl than in 0.1 M NaCl.

This property of $TMA^+$ is well demonstrated in fig. 1, which shows the separate melting curves of (AT) and (GC) pairs of CT DNA in NaCl and TMACl solvents, according to the method of Felsenfeld and Sandeen [15]. As expected, there is a general decrease of the $T_m$ and a reversal of the thermal stability of (AT) and (GC) pairs with respect to the same experiment in the presence of NaCl, at least in the first half of the absorbance melting curve. Again we notice that the shift of $T_m$ is smaller for (AT) pairs than for (GC) pairs. Consequently there is a narrowing of the breadth of the helix-coil transition and the $T_m$'s of (AT) and (GC) pairs tend to be equal.

*Circular dichroism.* The CD behaviour of CT DNA in TMACl at room temperature is also very peculiar, if compared to the CD spectra in NaCl. The variation of the molar ellipticity at 280 nm versus the logarithm of the ionic strength are plotted in fig. 2. Contrasting with the strong effect of NaCl, high concentrations of TMACl affect the positive CD band of DNA only slightly. Fig. 3 shows the CD melting curves of CT DNA at various concentrations of TMACl in the presence of 1 mM phosphate buffer (pH 7) and 0.2 mM EDTA. Under these conditions we can observe that CD premelting disappears in the presence of 1 M TMACl. If the phosphate buffer is actually omitted and thus the $Na^+$ concentration minimized to that due to 0.2 mM EDTA, any premelting disappears in the presence of 0.01 M TMACl (data not shown).

A relation between the size of the ion and its effectiveness in reducing $dT_m/dX_{GC}$ was suggested by Melchior and Von Hippel [10], since there was a sudden loss of the effect when shifting from $TEA^+$ (tetraethylammonium) to $TPA^+$ (tetrapolyammonium). It

Table 2
Melting temperatures of DNAs and polynucleotides in NaCl and TMACl solutions

Table 2A – $T_m$ values (°C) in 0.1 M

| Deoxypolynucleotide | %(A T) | NaCl 0.1 M [a] | TMACl 0.1 M [a] | $\Delta T_m$ (NaCl-TMACl) |
|---|---|---|---|---|
| Poly (dA) poly (dT) | 100 | 65 ± 0.5 | 64 ± 0.5 | 1 |
| Poly (dA.dT) poly (dT.dA) | 100 | 59 | 61 | –2 |
| CT DNA | 58 | 83 | 77 | 6 |
| EC DNA | 50 | 87.5 | 82 | 5.5 |
| ML DNA | 28 | 97 | 87 | 10 |
| Poly (dG.dC) poly (dC.dG) | 0 | 115 [b] | 102 | 13 |

Table 2B – Influence of ionic strength on CT DNA melting

| | NaCl | TMACl [a] | TMACl [c] |
|---|---|---|---|
| $10^{-2}$ M | 65 ± 0.5 | 62 ± 0.5 | 54 ± 0.5 |
| $10^{-1}$ M | 83 | 77 | 71 |
| 1 M | 96 | 93.5 | 86 |

[a] In the presence of phosphate buffer 1 mM, pH 7, EDTA 0.2 mM. [b] Extrapolated data. [c] Non-buffered TMACl solutions. In this case heating of solutions is accompanied by a small pH change which does not affect the structure of DNA.

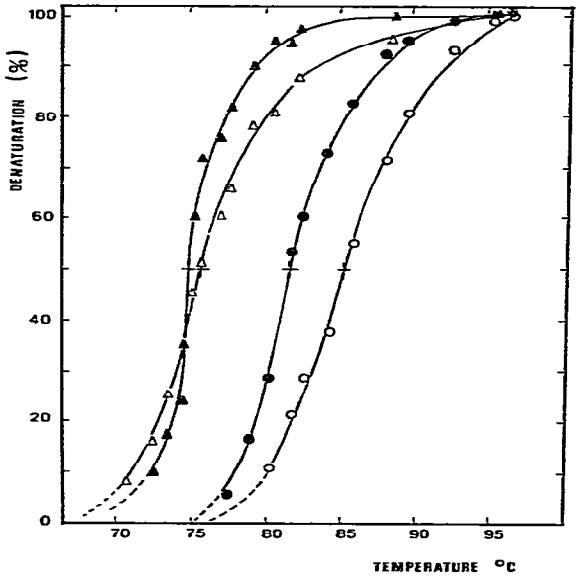


Fig. 1. Differential melting profiles of (AT) and (GC) base pairs in NaCl and TMACl for CT DNA: • (AT), ○ (GC) in NaCl 0.1 M, 1 mM phosphate buffer, pH 7, 0.2 mM EDTA. ▲ (AT), △ (GC) in TMACl 0.1 M, 1 mM phosphate buffer, pH 7, 0.2 mM EDTA.

was therefore interesting to investigate the CD behaviour of DNA in the presence of these ammonium salts by using the same 1 M concentration in order to work in a buffered solution with negligible interference of $Na^+$ ions. The results are displayed in table 3: when going from ammonium chloride (ACl) to tetraethylammonium chloride (TEACl) the $\theta$ values (at 280 nm and 25°C) increased regularly from 4300 to 9200 degrees $cm^2$ $dmole^{-1}$. On the other hand, the slope $\Delta\theta/\Delta T$ first decreased to a zero value in TMACl solution and then increased again in TEACl solution.

Table 3 shows some additional properties:

a) The replacement of $Na^+$ ions by $TMA^+$ ions at a given ionic strength (0.1 M) was found to induce an increase of CD with (GC) polymers and a decrease with (AT) polymers.

b) Upon heating, $\Delta\theta/\Delta T$ is greater in TMACl than in NaCl for poly (dG.dC) poly (dC.dG), but much lower for (AT) polymers.

c) From the values of $\theta_{280}$ at 25°C and assuming a constant rate of change $\Delta\theta/\Delta T$ in the range 0–60°C, it is easy to show that $\theta_{280}$ values in TMACl at 0°C are always higher than those in NACl at the same temperature, but smaller than the final $\theta_{280}$ values at the end of the premelting process in NaCl.

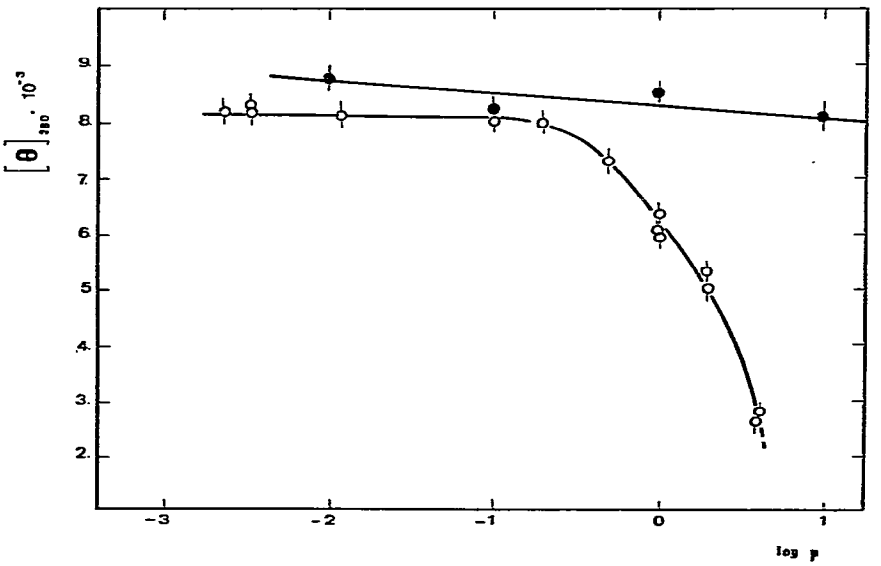


Fig. 2. Ionic strength dependence of the molar ellipticity at 280 nm for CT DNA in: ○ NaCl, • TMACl. Same conditions as in fig. 1.

## 4. Discussion

It is not easy to correlate premelting with a given structural modification of the double helix, since the magnitude of the CD signal depends upon both the nature and the relative geometry of the base pairs. Instead of looking for a precise relationship between ellipticity and any geometrical parameter, let us as a first approach, interpret premelting as simply reflecting the change of stability of a double-stranded structure. In this regard, as was already pointed out [17,19], there is a striking correlation of the heat of hydration of the cation and the magnitude of the premelting as expressed by $\Delta\theta/\Delta T$.

These results point to a possible role of water in premelting. According to Manning [20] the change of stability of DNA upon binding of different alkaline cations cannot be explained in terms of any specific site binding to phosphate groups, as was originally assumed [19]. Actually this differential stabilization reflects the cation hydration. Water can be viewed of as a destabilizer of the helical structure of DNA by competing with base pairing for hydrogen binding. The properties of water around the condensed counterions (hydration shell) would thus affect helix hydration and modify its stability accordingly. In line with this interpretation, the destabilizing effect of $TMA^+$ ion would be due to its small hydration as compared to that of alkaline ions. Premelting could be interpret-

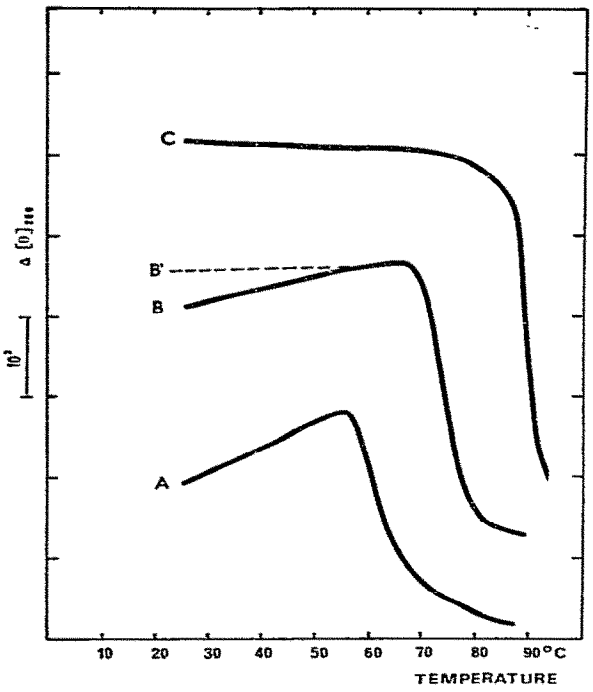


Fig. 3. Temperature dependence of the molar ellipticity at 280 nm of CT DNA in different concentrations of TMACl and in the presence of 1 mM phosphate buffer pH 7, 0.2 mM EDTA: A (0.01 M); B (0.1 M); B' (0.1 M unbuffered); C (1 M). For the sake of clarity, CD melting profils have been arbitrarily translated along the $\theta$ axis. The scale on the ordinate represents a $\Delta\theta$ value of $10^3$ degree $cm^2$ $dmole^{-1}$.

Table 3
Effect of tetraalkylammonium salts on CD parameters of DNA's and deoxypolynucleotides

| Deoxypolynucleotide | Solvent [a] | λ (nm) | $\theta_\lambda^{25°C}$ | $\Delta\theta_\lambda/\Delta T$ |
|---|---|---|---|---|
| CT DNA | TMACl 0.01 M | 280 | 8600 ± 200 [e] | 26 |
| | TMACl 0.1 M | 280 | 8400 | 12 |
| | TMACl 1 M | 280 | 8600 | 0 |
| | TMACl 10 molal | 280 | 8200 | < 0 |
| | [b] ACl 1 M | 280 | 4300 | 32 |
| | [c] MACl 1 M | 280 | 7600 | 13 |
| | [d] TEACl 1 M | 280 | 9200 | 44 |
| Poly (dA) poly (dT) | TMACl 0.1 M | 282 | 4200 | 83 |
| Poly (dA.dT) poly (dT.dA) | | 262 | 7700 | 33 |
| EC DNA | | 280 | 8700 | 4 |
| ML DNA | | 280 | 9600 | 4 |
| Poly (dG.dC) poly (dC.dG) | | 280 | 7600 | 65 |

[a] In the presence of phosphate buffer 1 mM pH 7, EDTA 0.2 mM. [b] Ammonium chloride. [c] Methylammonium chloride. [d] Tetraethylammonium chloride. [e] The incertitude in $\theta$ measurements is about the same in each case.

ed as a conformational change toward a less stable helical structure, triggered by a temperature induced modification of the hydration shell of the counter-ion.

Such a simple scheme has to be revised in view of the results obtained with $TMA^+$ ions, which, in comparison with $Na^+$ ions, present two conflicting effects: (i) a destabilization of the helical structure due to the small hydration of $TMA^+$ ions, which affects any base pair, (ii) a specific stabilization of AT pairs which is related to the preferential binding of $TMA^+$ ions [9]. $O_2$ of thymine, which in crystalline ApU and TpT is coordinated to $Na^+$ ions [21,22], could be a good candidate as a preferential binding site to $TMA^+$ ions in solution [23]. With DNA one would expect some balance between these two antagonistic effects at the level of (AT) and (GC) pairs. When using the $\Delta T_m$, recorded in table 2, for (AT) and (GC) polymers, the melting behaviour of the three DNAs can indeed be satisfactorily predicted on the basis of their (AT) content.

However, such a prediction cannot be made with $\theta$ and $\Delta\theta/\Delta T$ values which must strongly depend on base sequence.

A relationship between CD values and the rotation per residue angle was already proposed [7,8,18], but has not so far been correlated with any theoretical study. However, winding or unwinding processes reflect the stability of the double stranded structure, since the value of the winding angle depends on the respective intensities of stacking forces between base pairs and electrostatic repulsion between phosphate groups. Any decrease of stacking forces and/or increase of phosphate repulsion destabilizes the molecule and would generally trigger a decrease of the winding angle and thus an increase of the positive ellipticity $\theta_{280}$.

Such effects are encountered upon decreasing the ionic strength, raising the temperature, or upon interaction with small molecules. Of course, the reverse process (increase of winding angle and decrease of $\theta_{280}$) depends on the opposite effects: increased ionic strength, lower temperature and interaction with positively charged molecules. If such a qualitative interpretation of CD values is assumed, then premelting can be considered as a reversible, partial unwinding of the double helical structure of the DNA. In TMACl, the process still occurs with synthetic polydeoxyribonucleotides, but with DNA it is absent. This striking difference of behaviour can be tentatively explained on a structural basis.

Poly (dA) poly (dT) was shown to have a B' structure slightly different from the B form [24]. Poly (dA.dT) poly (dT.dA) can take either a B or a D structure [25] and even a C structure was recently postulated (7) to be present at low temperature, giving progressively rise to a B form during premelting.

A common feature to all of these structures B', C or D is the width of the small groove, the minimum value of which is 1.02, 1.08 and 0.7 nm respectively, which is smaller than the corresponding parameter (1.2 nm) in the B form. On the other hand, (AT) rich DNA [26] as well as satellite DNA with repetitious base sequences [27] reveal only a classical B structure.

If $O_2$ of thymine is a preferential binding site for $TMA^+$ ions, its accessibility depends strongly on the size of the small groove. Hindering of the rotational movement of (AT) pairs would therefore be totally efficient to prevent premelting only in the case of a B structure, i.e. with DNA.

Finally two remarks can be made:

a) If the assumption made is valid, the recognition process between (AT) pairs and $TMA^+$ ion appears to be very sensitive to the local geometry of the nucleotidic chain. Such a model would also be able to take into account the role of the ionic radius in premelting when going from ammonium ion to tetraethylammonium ion. Further experiments are needed to check this geometrical interpretation.

b) In any case the premelting of DNA is prevented when a specific interaction occurs at the level of AT pairs. The process can thus be viewed as a local effect initiated in AT rich regions and then extending to the rest of the molecule. Recent results of our laboratory (to be published) on dye-DNA interaction are in favour of this latter interpretation.

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