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Tetramethylammonium does not universally neutralize sequence dependent DNA stability

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ABSTRACT

Effects of different concentrations of Tetramethylammonium on the thermostability of six DNA dumbbells with similar well defined sequences have been investigated. Each molecule has a 16 base pair duplex stem linked on both ends by T_4 single strand loops. Only the sequence of the four central base pairs distinguishes one molecule from the next. The distinguishing central sequences are, [A-T-A-T], [T-A-T-A], [A-A-A-A], [C-G-C-G], [G-C-G-C] and [G-G-G-G] situated between the sequences: 5′-G-T-A-T-C-C-[ ]-G-G-A-T-A-C-3′ which are the same in all molecules. Optical melting curves collected on these molecules as a function of TMA concentration over the range from 0.09 M to 4.5 M revealed there is no single concentration of TMA where all these molecules exhibit the same melting temperature.

INTRODUCTION

For some time it has been believed that at certain 'magical' concentrations of tetramethylammonium (TMA) the effects of sequence heterogeneity, i.e. relative fraction of A·T and G·C base pairs, on duplex DNA stability can be neutralized so that A·T and G·C base pairs melt at the same temperature (1). Recently, with the increasing utilization of hybridization reactions in various assays and amplification protocols (2,3) a resurgence of interest in the solvent conditions under which sequence dependent stability of DNA can be eliminated has emerged. Besides the early studies of von Hippel and co-workers (1) who studied the effects of TMA concentration on long DNAs of different sequence composition, the relationship between local sequence context and dependence of DNA stability as a function of TMA concentration has not been carefully examined. In this paper, we report results of optical melting studies of six DNA dumbbells which contain well-defined sequences of A·T and G·C base pairs in different orders melted over a wide range of different TMA concentrations.

MATERIALS AND METHODS

DNA molecules

The six DNA molecules of this study are shown in Fig 1. These molecules are a subset of the 17 DNA molecules reported in a previous study (4). Preparation of these DNA dumbbells is described there. As seen in Fig 1 these molecules have 16 base pair (bp) duplex stems linked on both ends by T_4 single strand loops. The same six bp sequences adjoin the loops on either end. Only the four central bp's are different for the different molecules. Consequently, each molecule has five unique nearest-neighbor stacking interactions associated with the four unique bps in the centers of the molecules. Thus, only the central four bps and the accompanying five stacking interactions distinguish molecules within the set from one another. The six molecules can be grouped into two sets according to their sequences. Molecules with A-T-A-T, T-A-T-A and T-T-T-T central sequences constitute the first set. Overall, the duplex sequence of these dumbbells is 38% G·C. The second set contains molecules with the central sequences, G-C-G-C, C-G-C-G and G-G-G-G. Overall, the duplex sequence of these molecules is 63% GC. Obviously, within each set only sequence distribution, not content, differentiates one molecule from the next. These well defined molecular features thus provide a sample pool with which to carefully examine effects of local sequence context on melting stability as a function of TMA concentration.

Buffer solutions

Buffered TMA solutions were prepared by dissolving the appropriate amounts of solid TMA (Aldrich, 98% purity) in a solution of 1 mM EDTA, 10 mM sodium phosphate, pH 7.5. TMA solutions were prepared at concentrations of 0.09 M, 1.9 M, 2.9 M, 3.9 M, and 4.5 M. The pH remained unchanged over the entire concentration range of TMA. Buffer solutions were filtered to remove large flocculent aggregates visibly present in freshly prepared TMA solutions. Conductivity readings measured on the buffered TMA solutions before and after filtering were identical, indicating the flocculent matter did not correspond to TMA aggregates.

Melting experiments

To prepare them for melting curve measurements samples were dialyzed versus double distilled H_2O (ddH_2O) at 4°C for one week and then separated into aliquots containing approximately 0.4 OD units determined from the absorbance at 260 nm, and vacuum dried. At the outset, approximately, 50 ml of TMA buffer solution was passed through a 0.45 μm filter. Then 1 ml of filtered

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T T GTATCC ATAT GGATAC T T
T T CATAGG TATA CCTATG T T

T T GTATCC TATA GGATAC T T
T T CATAGG ATAT CCTATG T T

T T GTATCC TTTT GGATAC T T
T T CATAGG AAAA CCTATG T T

T T GTATCC GCGC GGATAC T T
T T CATAGG CGCG CCTATG T T

T T GTATCC CGCG GGATAC T T
T T CATAGG GCGC CCTATG T T

T T GTATCC GGGG GGATAC T T
T T CATAGG CCCC CCTATG T T

Figure 1. The six DNA dumbbells of this study. The duplex sequences all contain 16 base pairs linked on both ends by T_4 single strand loops. Only the central four base pairs (in bold) are unique and distinguish one molecule from the next.

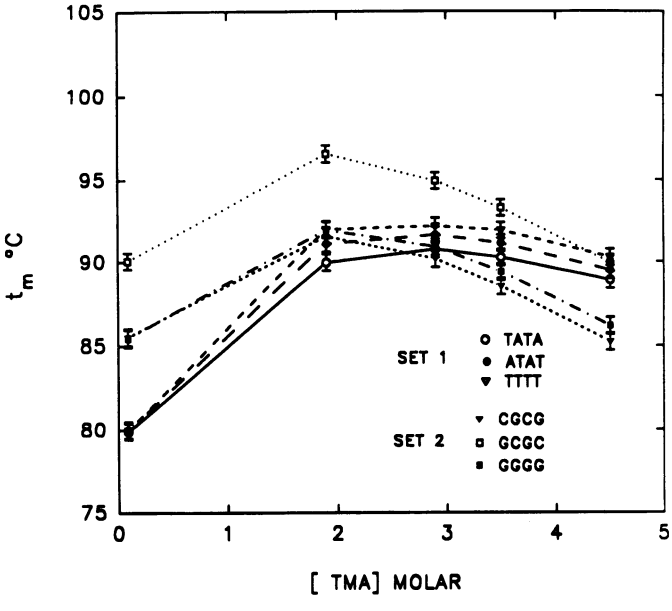

Figure 2. Transition temperatures, t_m, of the DNA molecules in Fig 1 as function of tetramethylammonium (TMA) concentration. The molecule have been grouped into two sets. Set 1 includes the dumbbells with TATA, ATAT and TTTT central sequences. Set 2 is comprised of the dumbbells with CGCG, GCGC and GGGG central sequences. Note, the qualitatively different behavior of the set 1 versus the set 2 molecules. In particular the GCGC molecule of set 2 has singularly significantly higher stability.

buffer was passed through a 0.45 μm filter and added directly to the dried DNA sample. The solution was then gently vortexed for ten minutes, incubated at 37°C for five minutes and then incubated at 20°C. Another 2 ml was passed through a separate 0.45 μm nylon filter and added directly to the reference and

Table I. Melting temperature of the DNA dumbbells as a function of tetramethylammonium (TMA) concentration

	t_m (°C)*					
	Dumbbell Central Sequence					
[TMA], M	TATA	ATAT	TTTT	GCGC	GGGG	CGCG
.09	79.9	80.0	80.0	90.1	85.4	88.1
1.9	90.0	91.1	92.0	96.6	92.0	91.6
2.9	90.8	91.6	92.2	94.9	90.9	90.2
3.5	90.3	91.1	91.9	93.3	89.4	88.5
4.5	88.9	89.5	90.3	89.9	86.2	85.2

* ±0.5 °C

sample cuvettes. After matching the cuvettes containing buffer, the buffer in the sample cuvette was removed and the DNA solution was filtered directly into the cuvette through a 0.45 μm filter. Absorbance readings at 260 nm indicated the DNA concentration was unaffected by this series of filtration steps. After placement in cuvettes, the sample and reference were bubbled with helium gas for at least 20 min. Three drops of white mineral oil were then added to the surface and the cuvettes were tightly sealed with teflon stoppers and wrapped with teflon tape.

Melting experiments were collected on a Hewlett-Packard diode array 8450-A double-beam spectrophotometer at a wavelength of 268 nm. For each DNA sample, (absorbance, temperature) points were collected over the temperature range from 20°C to 105°C. Sample temperatures were determined from the cell-holder temperature. Absorbance measurements were also collected while the sample was cooled back to 20°C. At least two forward and reverse melting curves were collected for all samples. All curves were reversible at the heating-cooling rate of 60°C/hr used for all experiments. Absorbance versus temperature curves were normalized to upper and lower baselines, and converted to θ_B, fraction of broken base pairs, vs temperature (T) curves. From these curves, differential melting curves, $d\theta_B/dT$ vs T curves were constructed. The transition temperature, t_m, was determined as the temperature of the maximum peak height, $(d\theta_B/dT)_{max}$, of the differential melting curve. Reported t_m values are the average of at least two independent experiments.

RESULTS

Effects of TMA on DNA stability are sequence dependent

The transition parameters obtained from optical melting curves (not shown) collected on the six dumbbells of Fig 1 as a function of TMA concentration are summarized in Table I. The effect of TMA concentration on the stabilities of the six DNA dumbbells is clearly seen in Fig 2 where t_m is plotted versus TMA concentration for the six molecules. Clearly the set 1 dumbbells with central sequences T-A-T-A, A-T-A-T and T-T-T-T and set 2 dumbbells with G-C-G-C, C-G-C-G and G-G-G-G central sequences display qualitatively different behavior. In the lowest TMA concentration buffer (.09 M), the t_m's of the set 1 molecules are all essentially equal at ~80°C. In 1.9 M, the t_m's all increase to about 91°C and they remain constant at 2.9 and 3.9 M TMA. The t_m's of the set 1 molecules then decrease to about 90°C in 4.5 M TMA. Although the molecules in set 2 all behave the same over the entire TMA concentration range, they behave differently in response to TMA concentration than the molecules of set 1. In 0.09 M, the molecules with C-G-C-G and

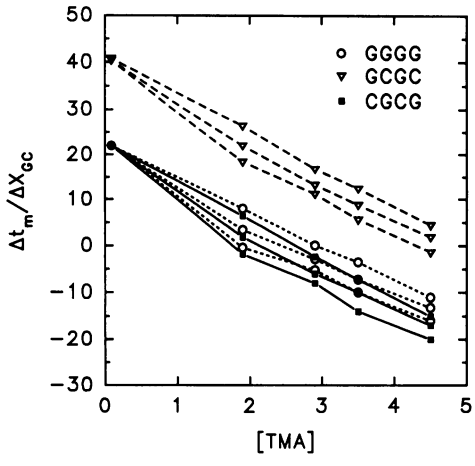

Figure 3. Plots of the ratio $\Delta t_m/\Delta\chi_{GC}$ versus TMA for the Dumbbells in Fig 1. χ_{GC} is the difference in mole fraction of G·C for two different dumbbells. Δt_m is the difference in the t_m's of the two molecules. The ratio is only defined when the denominator is non-zero, i.e. for molecules with different mole fractions of G·C. For example, the dashed curves with inverted triangles are the curves for the dumbbell with a GCGC central sequence compared to the dumbbells of set 1 with TATA, ATAT and TTTT central sequences. Since three curves result for each comparison of a molecule of set 1 with the molecules of set 2 nine different curves result (as shown).

G-G-G-G central sequences have the same t_m = 85.5°C, which is 5°C lower than that of the G-C-G-C central sequence (90°C). From 0.09 M to 1.9 M TMA the t_m's of the set 2 dumbbells increase by approximately 5°C. In contrast to the behavior of the molecules in set 1, at 2.9 M, 3.9 M and 4.5 M TMA the t_m's of the set 2 molecules continually decrease. The t_m's of the set 1 molecules and the C-G-C-G and G-G-G-G central sequences are equivalent within 4°C at 1.9 and 2.9 M TMA. The t_m of the G-C-G-C molecule is 4°C to 6°C higher. At 4.5 M the t_m's of the set 1 molecules agree with that of the G-C-G-C molecule, but the t_m's of the other set 2 molecules are approximately 5°C lower. Apparently, for these molecules there is no single concentration of TMA at which all molecules display the same stability! This observation appears to be directly in contrast to results found for melting of long DNAs where it was shown over the 2−3 M TMA range differential effects of A·T and G·C content are neutralized (1). Thus, in short molecules sequence distribution is the primary determining factor that responds to increased TMA concentration. As suggested in Fig 2 by the behavior of the dumbbell with the G-C-G-C central sequence, some sequences may never be entirely neutralized at the same TMA concentration where other molecules are neutralized.

In previous studies of the effect of TMA on DNA as a function of sequence content, plots like those shown in Figure 3 were reported (1). In Fig 3 the ratios $\Delta t_m/\Delta\chi_{GC}$ are plotted versus TMA concentration. χ_{GC} is the difference in mole fraction of G·C for two different dumbbells. Δt_m is the difference in the t_m's of the two molecules. Obviously, the ratio is only defined when the denominator is non-zero, i.e. for molecules with different mole fractions of G·C. The nine possible curves, one for each unique comparison of a molecule of set 1 with a molecule of set 2 are shown. Fig 3 again shows for all but one molecule, the G-C-G-C sequence, the behavior of $\Delta t_m/\Delta\chi_{GC}$ is essentially the same. Notice, the six lower curves cross zero between 2 and 3 M TMA revealing even though they have different χ_{GC}'s their t_m's are essentially equivalent. Thus, the most significant effect of TMA on DNA sequence dependent stability is to increase and maintain the stability of A·T sequences. The curve of the G-C-G-C molecule never crosses zero meaning, at no TMA concentration is its t_m equivalent to that of the more A·T rich molecules in set 1.

DISCUSSION

These results for a series of dumbbells with well defined sequence have revealed a definite dependence of sequence content and sequence order on the melting behavior as a function of TMA concentration. Because of the well defined similarities and differences of these dumbbell sequences we can identify the nearest-neighbor sequences responsible for the abnormal response of the G-C-G-C molecule. It is precisely this type of detailed local comparison afforded by dumbbells that initially motivated their construction (5). We now compare the unique stacking interactions in the set 2 molecules. The unique stacks in the dumbbell with a G-C-G-C central sequence are 3(C_pG) + 2(G_pC) (p = phosphate). The dumbbell with a C-G-C-G central sequence has 2(C_pC (or G_pG) + C_pG) + (G_pC) stacks. The G-G-G-G central sequence has (C_pG) + 4(G_pG or C_pC) stacks. Differences in free-energy due to each of these stacks in 115 mM Na^+ can be determined from the values recently reported (4). This calculation reveals the differences between the stacking free-energies of the G-C-G-C dumbbell and the C-G-C-G and G-G-G-G dumbbells is −745 and −1660 cal/mol respectively. Surely, these differences in stacking free-energy alone do not account for the differences in TMA dependent melting behavior. If this were the case, then the behavior of the C-G-C-G sequence which is 916 cal/mol lower (more stable) than the G-G-G-G sequence would be expected to respond differently than the G-G-G-G molecule, i.e. on the order of the difference between the G-C-G-C and C-G-C-G molecules. This is not the case. Of course the above analysis is predicated on the assumption that all the dumbbells of this study adopt the same basic type of secondary structure for which the energetic parameters are valid. Although the sequences of the molecules of set 2 are similar, different types of stacks are present. The G-C-G-C sequence does not contain any G_pG (C_pC) stacks while the other two molecules contain at least two. Perhaps the G_pG (C_pC) stacks allow preferential access of TMA to DNA grooves and thereby TMA is able to affect their stability to a greater extent than in the G-C-G-C molecule which does not containing any G_pG (C_pC) central stacks. Perhaps the G-C-G-C molecule adopts a significantly different secondary structure at higher TMA concentrations, one that is more thermally stable than the other molecules. Structural studies of these molecules will be required to explore this latter possibility. Whatever the origins, the G-C-G-C dumbbell is more stable than the other molecules examined and this increased stability is not entirely reduced at TMA concentrations from .09 to 4.5 M.

The sequence dependent features of DNA in response to TMA that have been elucidated in this study of dumbbells could be generally operative in similar sequences of longer DNAs. However, in long DNAs such subtle effects might be averaged out and not so clearly distinguished. At least the utility and unique advantages of using DNA dumbbells for model studies of ligand dependent stability has been demonstrated.

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REFERENCES

1. Melchoir, W.B., Jr. and von Hippel, P.H. (1973) Proc. Natl. Acad. Sci. (USA) 70, 298–302.
2. Wood, W.I., Gitschier, J., Lasky, L.A., Lawn, R. M. (1985) Proc. Natl. Acad. Sci. (USA) 82, 1585–1588.
3. Jacobs, K.A., Ruderdorf, R., Neill, S.D., Dougherty, J.P., Brown, E.L. and Fritch, E.F. (1988) Nucleic Acids Res. 16, 4637–4650.
4. Doktycz, M.J., Goldstein, R.F., Paner, T.M., Gallo, F.J. and Benight, A.S. (1992) Biopolymers 32, 849–864.
5. Wemmer, D.E. and Benight, A.S. (1985) Nucleic Acids Res. 13, 8611–8621.