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Alteration of the Relative Stability of dA·dT and dG·dC Base Pairs in DNA

(solvent additives/tetraalkylammonium ions/melting transitions/circular dichroism/cooperativity)

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ABSTRACT **Several small alkylammonium ions can eliminate, or even reverse, the usual dependence of the DNA transition temperature on base composition. For example, in 3 M tetramethylammonium chloride, or 2.4 M tetraethylammonium chloride, DNAs of different base compositions all melt at a common temperature, and with a greatly decreased breadth of transition reflecting only the sequence-independent components of melting cooperativity. At still higher concentrations of such additives, dG·dC-rich DNAs melt at lower temperatures than dA·dT-rich molecules. Circular dichroism spectra show that these additives alter the structure of the DNA double helix very little at room temperature. This differential (base-specific) effect on helix stability is investigated with several small additives related to the tetraalkylammonium ions. Additives larger than tetraethylammonium ion have little differential effect on helix stability. Preferential binding of ions to dA·dT base pairs, requiring fit into a "groove" of DNA, is consistent with these data and with equilibrium binding studies. These differential effects can be distinguished from general destabilizing effects, which are independent of specific features of macromolecular conformation or chemistry. Possible experimental uses of this ability to alter the base-composition-dependent components of the stability of the DNA helix are discussed, as well as the insight this phenomenon provides into the molecular basis for the differential stability of dA·dT and dG·dC base pairs.**

The differential stability of dA·dT and dG·dC base pairs and, thus, of segments of native DNA differing in base composition, may be critical to several of its biological functions, i.e., DNA–protein recognition mechanisms in processes such as transcription, recombination, etc. (for a recent review see ref. 1). These stability differences have also had practical consequences for biochemists and molecular biologists, who have used differences in melting temperature (T_m) as a measure of DNA base composition (2). They have used preferential melting of dA·dT-rich sequences to physically map bacteriophage and bacterial chromosomes (3) and to fractionate DNA molecules by thermal elution chromatography (4). On the other hand, base sequence heterogeneity has complicated the theoretical interpretation of melting transitions of nucleic acids and the analysis of other aspects of nucleic acid structure in solution (5), in particular making it very difficult to separate sequence-dependent effects from intrinsic (sequence-independent) structural contributions to the cooperativity of melting of the DNA helix.

For the design of experiments in which differential stability of base sequences can be manipulated as an experimental variable, it is necessary to establish aqueous solvent systems in which the differential stability of dA·dT and dG·dC base pairs can be altered with a minimum of disturbance of the other properties of the native DNA helix. Certain solvent additives, such as methanol (6) and several inorganic salts (7), decrease the dependence of T_m on DNA base composition (dT_m/dX_{GC}). However, these additives also alter the stability and structure of DNA at room temperature (6–8).

The immediate impetus for the present work came from the observation (9) that tetramethylammonium chloride (TMA-Cl) and tetraethylammonium chloride (TEA-Cl) bind preferentially to dA·dT-rich DNA. If this binding were preferential for double-stranded DNA, it should stabilize dA·dT-rich regions, decreasing dT_m/dX_{GC}. This paper reports preliminary findings with several aqueous solvent systems containing small alkylammonium ions, some of which can reduce dT_m/dX_{GC} to zero or to negative values with little or no effect on over-all DNA structure, and, in some cases, without appreciably altering the "general" stability of the native DNA helix.

MATERIALS AND METHODS

DNA was purchased from Worthington (calf thymus and *Clostridium perfringens*) or from Miles (*Micrococcus luteus*). The DNA was dissolved in and dialyzed against 0.1 mM Na-EDTA (pH 7) and then into 0.1 mM Na-phosphate (pH 7). All samples had s_{20} = 17–19 S in 0.1 M salt.

Ammonium Salts. TMA-Cl was decolorized with Norit-A and twice recrystallized from 2-propanol. TEA-Cl was recrystallized from tetrahydrofuran:2-propanol. Tetrapropylammonium bromide (TPA-Br), quinuclidine hydrochloride, and triethylamine hydrochloride were used without purification. Piperidine hydrochloride was prepared by slow addition of concentrated HCl to cold (0°) piperidine, followed by precipitation from 2-propanol with ether and recrystallization from 2-propanol. 5-Azoniaspiro[4.4]nonane bromide was prepared by method (*a*) of Roufogalis and Thomas (10). 8.35 ml of pyrrolidine and 6 ml of 1,4-dibromobutane were refluxed for 6 hr in 500 ml of $CHCl_3$. Evaporation, addition of NaOH (aq.), extraction with CH_2Cl_2, and precipitation with petroleum ether gave about 5 g of white crystals. Analysis. Calculated for $C_8H_{16}NBr$: C, 46.61; H, 7.82; N, 6.80. Found: C, 47.71; H, 8.78; N, 6.79. 1,1,4,4-Tetramethylpiperazinium iodide was made by slow addition of 8.6 g of piperazine to 100 ml of cold (0°) methyl iodide and then refluxing for 9 hr. After evaporation, recrystallization from H_2O yielded about 13 g of faintly yellow crystals. Analysis. Calculated for $C_8H_{20}N_2I_2$: C, 24.14; H, 5.06; N, 7.04. Found: C, 23.87;

Abbreviations: TMA^+, TEA^+, and TPA^+ represent tetramethyl-, tetraethyl-, and tetrapropylammonium ion, respectively; T_m, melting temperature; CD, circular dichroism.

H, 5.11; N, 7.16. For use, an appropriate amount was dissolved in water, treated with Norit-A, filtered, and converted to the chloride with a column of AG 2-X8 resin. The solution was evaporated and dissolved in phosphate buffer. Final concentrations were checked by argentometry. All salt stock solutions were passed through Millipore 0.45-μm MF filters, or, for all organic additives and all solutions greater than 1 M, 0.5-μm Solvinert filters.

Spectroscopy. Melting curves were measured on two spectrophotometers. One was a Gilford model 2000 multiple sample absorbance recorder with a Beckman DUR monochromator, an automatic slit control, and a thermostatted sample compartment with a thermistor temperature sensor. The other was a Cary 14 spectrophotometer connected to a VDM 620/i computer through a Varian Spectrosystem 100 spectrophotometer interface. Temperature was measured by a thermocouple in a cuvet in the sample changer. Helium was bubbled through the samples for about 1 min before the cuvets were sealed with Teflon stoppers and General Electric RTV-88 silicone rubber. Heating rates were 0.5–1.4°/min, with the transitions monitored at 260 nm. All data were corrected for thermal expansion of water. Lines drawn through the linear data above and below the melting transition were extrapolated through the melting region, and lines $^1/_2$, $^1/_4$, and $^3/_4$ between these limiting lines were used to measure T_m and $\Delta T_{(3/4-1/4)}$.

Circular dichroism (CD) was measured at 27° on a modified Cary 60 spectropolarimeter with an accessory 620/L computer and Spectrosystem 100 interface.

RESULTS

Melting profiles for three DNAs of different base composition, measured in several different aqueous salt solutions, are shown in Fig. 1, which compares native DNAs from *Clostridium perfringens*, calf thymus, and *Micrococcus luteus* in 0.02 M NaCl with the same samples in 3 M TMA-Cl and 2.4 M TEA-Cl. The usual base-composition-dependent differences in transition temperature are seen for the various DNAs in 0.02 M NaCl. In 3 M TMA-Cl, the melting temperatures of all DNAs are increased as a consequence of the general electrostatic stabilization induced by high salt concentrations. However, the DNAs are stabilized to different extents, so that under these particular salt conditions all the

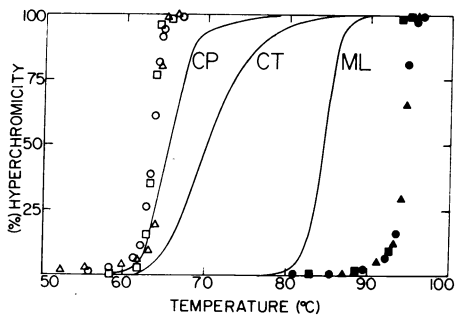


FIG. 1. Typical melting curves of DNAs from *Cl. perfringens* (CP), calf thymus (CT), and *M. luteus* (ML) in various solvents. Corrected for initial and terminal slopes of A_{260} and normalized. *Lines* are results obtained in 0.02 M NaCl. *Open symbols* are in 2.4 M TEA-Cl; *closed symbols* in 3.3 M TMA-Cl. DNA samples: CP (△,▲); CT (○,●); ML (□,■).

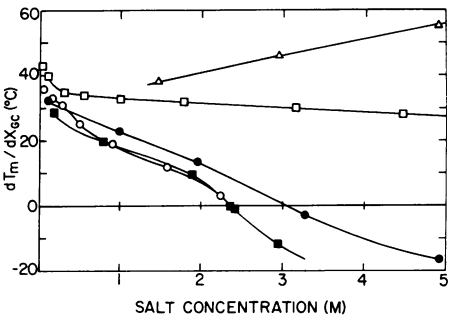
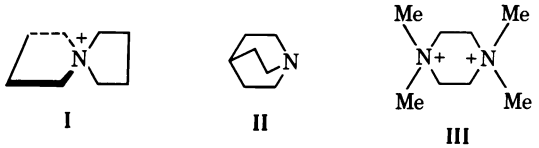


FIG. 2. dT_m/dX_{GC} as a function of concentration of solvent additive. Data from Table 1: TMA-Cl(●); TEA-Cl(■); $NaClO_4$ (△). Data from Gruenwedel *et al.* (ref. 7*a*): NaCl(□); Cs_2SO_4(○).

DNA melting profiles are superimposed. In addition, the transitions are about 5-fold sharper. Similar effects are seen in 2.4 M TEA-Cl, which similarly superimposes and sharpens the transitions and, further, has a general destabilizing effect, decreasing the overall stabilty to thermal disruption of the DNA helix. Thus 3 M TMA-Cl and 2.4 M TEA-Cl represent solvents in which dT_m/dX_{GC} has been reduced to zero. The sharpening of the transition results because this equalization of relative base-pair stability also eliminates the differences in stability between dA·dT-rich and dG·dC-rich sequences within single DNA samples, which are responsible for most of the breadth of the transition.

More complete data illustrating these phenomena for different aqueous salt systems are presented in Table 1 and Figs. 2 and 3. Fig. 2 shows that the dependence of T_m on base composition increases with increasing $NaClO_4$ concentration, is relatively independent of NaCl concentration, and decreases with salt concentration for TMA-Cl, TEA-Cl, and Cs_2SO_4. At high concentrations of the ammonium salts, dT_m/dX_{GC} is negative, i.e., dA·dT base pairs are more stable than dG·dC base pairs. The T_m values in Table 1 demonstrate that, while TMA-Cl has only a strong differential effect, TEA-Cl is also a general destabilizer.

To investigate the size, shape, and charge requirements for the differential destabilizing effect of these additives, we examined several related compounds (Table 1). TPA-Br strongly destabilizes DNA, but has little effect on dT_m/dX_{GC}. This result is consistent with the finding (9) that, unlike TMA^+ and TEA^+, tetrabutylammonium ion is not preferentially bound by dA·dT-rich DNA. All of the other ions in Table 1 are smaller than TPA^+. 5-Azoniaspiro[4.4]nonane (**I**, also called 1,1′-spirobipyrrolidinium); quinuclidine (**II**), a tertiary amine of high pK (>11) with the nitrogen on the outside of the molecule; triethylammonium; and 1,1,4,4-tetramethylpiperazinium (**III**) are closely related structures.



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All these compounds decrease dT_m/dX_{GC} with a molar effectiveness comparable to TMA^+ and TEA^+ (Table 1). *t*-Butyl alcohol, of comparable size and shape to TMA^+ and TEA^+, shows little effect on dT_m/dX_{GC}.

TABLE 1. *Effects of solvent additives on melting parameters**

Additive	Concentration (M)	CP DNA†		CT DNA†		ML DNA†		
		T_m	ΔT	T_m	ΔT	T_m	ΔT	dT_m/dX_{GC}‡
NaCl	0.02	65.5	3.3	69.9	5.4	84.3	2.4	46.3§
	1.96	90.4	1.9	(92.6)	(4.1)			(20)§,¶
	3.51	89.1	1.8	92.9	3.4	(102)		(30)§
$NaClO_4$	1.47			84.2	5.4	95.6	4.2	38.0
	2.95	68.2	3.8	73.8	6.3	87.2	2.5	46.0
	4.91	51.8	5.4	58.0	8.2	74.5	3.1	55.3
Sodium phosphate (pH 7)	1.96‖	95.2	1.4	96.9	2.9	(103.8)		(21.4)
TMA-Cl	0.098	73.5	1.9	76.3	3.7	86.7	1.7	32.7
	0.98			91.7	2.1	98.6	1.1	23.0
	1.96			94.3	1.4	98.5	0.7	13.3
	2.95	94.3	0.6	93.9	1.8			− 4¶
	3.28			94.1	1.0	93.2	0.8	− 3.0
	4.92			89.3	2.6	84.4	2.4	−16.3
TEA-Cl	0.18	70.3	1.7	73.8	3.2	82.2	1.8	28.8
	0.79	75.6	1.1	77.6	2.3	83.7	2.3	19.9
	1.89			70.9	1.0	73.8	1.0	9.7
	2.36	64.1	0.4	63.0	1.2	63.6	0.8	− 0.1
	2.42			61.2	(1.5)	60.9	0.7	− 1.0
	2.95			56.2	(1.2)	52.7	(0.8)	−11.7
TPA-Br	1.96			33.3	3.2	41.6	1.9	27.7
Triethylamine hydrochloride	2.95			62.3		62.6		0.8
Piperidine hydrochloride	1.96			74.8	2.5	81.8	0.9	23.3
Quinuclidine hydrochloride	1.96			69.1	1.5	72.6	0.9	11.7
1,1′-Spirobipyrrolidinium bromide	1.98			74.9	1.3	77.2	1.0	7.7
Tetramethylpiperazinium chloride	0.82			85.9	3.2	93.4	2.0	25.0
t-Butyl alcohol	2.95**	57.4	4.1	62.8	6.1	79.0	5.0	53.0

* For multiple experiments under the same conditions, average values are presented. dT_m/dX_{GC} was calculated by a linear least-squares treatment of all available data. All values are in °C. ΔT means $\Delta T_{(3/4-1/4)}$. Values in parentheses are approximate.

† *Cl. perfringens* DNA, 31% GC; calf-thymus DNA, 42% GC; *M. luteus* DNA, 72% GC (2).

‡ °C/mol fraction dG·dC.

§ The closest corresponding values for NaCl from the work of Gruenwedel, Hsu, and Wu (7*a*) are: 10^{-2} M, 44°; 1.78 M, 32°; 3.16 M, 30°.

¶ Uncertainty in this value is inherently greater than in others, due to the relatively small composition difference between *Cl. perfringens* and calf-thymus DNAs.

‖ Phosphate concentration. Na^+ concentration is 3.16 M.

** With Na phosphate, 10 mM (pH 7).

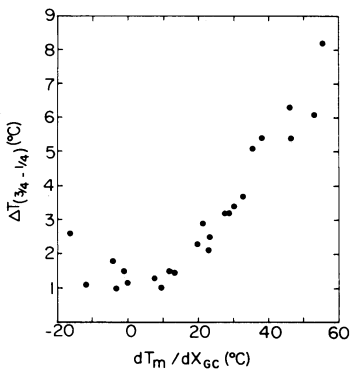

FIG. 3. Relation between breadth of melting transition ($\Delta T_{(3/4-1/4)}$) and dT_m/dX_{GC} for calf-thymus DNA. Data from Table 1.

As expected, all solvent additives that decrease dT_m/dX_{GC} also decrease the breadth ($\Delta T_{(3/4-1/4)}$) of the melting transition, while additives that increase dT_m/dX_{GC} (e.g., $NaClO_4$) also increase $\Delta T_{(3/4-1/4)}$. This correlation is demonstrated graphically for calf-thymus DNA in Fig. 3, showing that $\Delta T_{(3/4-1/4)}$ passes through a minimum of about 1.0° for calf-thymus DNA (and of about 0.5° for bacterial DNAs; data not shown) at $dT_m/dX_{GC} = 0$.

Fig. 4 shows the CD spectra for DNA in several salt solutions. As previously observed by others (8), high concentrations of NaCl appreciably alter the conservative CD spectrum characteristic of the B form of DNA in dilute salt solution. 5 M $NaClO_4$ causes even more change; and 2.5 M Cs_2SO_4, predicted from Fig. 2 to have $dT_m/dX_{GC} \simeq 0$, gives a spectrum essentially indistinguishable from that of a dehydrated film of lithium DNA (8*b*). In contrast, the CD

spectrum of DNA is altered very little at high TEA-Cl concentrations, and even less in elevated TMA-Cl. Since CD is particularly sensitive to conformational effects, such as base tilt, that characterize the conversion of the B form of DNA to other structures (see ref. 8), we may conclude that TMA^+ and TEA^+ cause their differential destabilizing effects without inducing appreciable changes in native DNA conformation.

DISCUSSION

Possible mechanisms of the differential stabilization of dA·dT and dG·dC base pairs by alkylammonium compounds

The fact that the T_m in TEA-Cl is about 30° lower than in TMA-Cl at concentrations for which dT_m/dX_{GC} is zero shows a general destabilization mechanism that is independent of the composition-dependent effects reflected in altered values of dT_m/dX_{GC}. Table 1 shows that increasing the length of the alkyl side-chain of the tetraalkylammonium ion increases its molar effectiveness as a general destabilizer. Similar observations have been made on the effects of these ions on the thermal stability of the globular protein ribonuclease (11). Several experiments have shown that these general destabilizing effects are independent of the details of macromolecular chemistry and conformation (for reviews see ref. 12), but depend on solvent-additive-induced changes in the free energy of transfer of the monomer components from a nonaqueous to an aqueous milieu. Thus, the relative effectiveness of these agents as destabilizers of the DNA helix is roughly paralleled by their effectiveness in increasing the solubility of the individual nucleotide bases in aqueous solution. As expected from the base-composition-independent nature of the effect, the magnitude of these increases in solubility is essentially independent of base type (ref. 13; unpublished data from this laboratory).

The mechanisms whereby these additives alter the *relative* stability of the dA·dT and dG·dC base pairs is evidently quite different. Thus, both $NaClO_4$ and TEA-Cl are general destabilizers; but $NaClO_4$ *increases* dT_m/dX_{GC}, while TEA-Cl *decreases* this parameter. The "equalization" effect of the smaller alkylammonium compounds could be attributed to preferential binding, either to native dA·dT-rich sequences or to denatured (unstacked, single-stranded) regions rich in dG and dC. Both the results of Shapiro *et al.* (9), who showed preferential binding of TMA^+ and TEA^+ to dA·dT-rich native DNAs, and the size criterion mentioned above, i.e., the abrupt loss of the effect (between TEA^+ and TPA^+) with increasing ion size, support the former interpretation and suggest preferential binding of these compounds in the grooves of DNA at dA·dT base pairs. Comparison of Corey–Pauling–Koltun models of the various additives with a model of double-helical DNA suggests that TMA^+ and TEA^+ fit well into the grooves of the DNA structure, while TPA^+ is too large to be easily accommodated. Piperidinium ion, which is not very effective in stability equalization, fits into the grooves easily, but is too small to make much contact with the DNA. All the other effective compounds are smaller than TPA^+. Compounds with structural similarities [e.g., 1,1′-bipyrrolidinium ion (I) and TEA^+, quinuclidine (II) and triethylamine] show quantitatively similar molar effectiveness in reducing dT_m/dX_{GC}. As yet, however, little can be said about the stereochemical features of the dA·dT pair responsible for binding. Shapiro *et al.* (9) have demonstrated

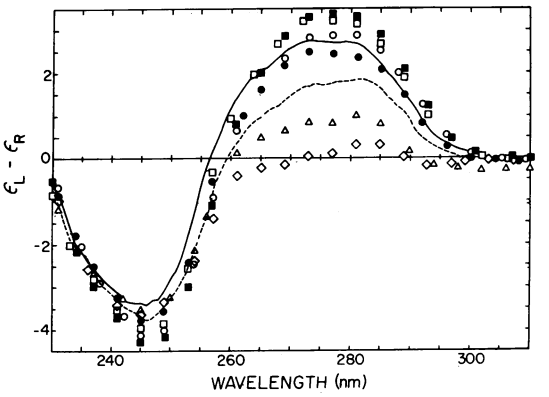

FIG. 4. CD spectra of calf-thymus DNA in various solvents. For clarity, only part of data is shown. Solvents: 0.1 M NaCl (——); 2 M NaCl (– – –); 3 M TMA-Cl(●); 5 M TMA-Cl(○); 1 M TEA-Cl(□); 2.4 M TEA-Cl(■); 5 M $NaClO_4$(△); 2.5 M Cs_2SO_4(◇).

that substitution of dU for dT in native DNA (i.e., loss of the methyl group on the 5-position of dT) has little effect on the preferential binding of TMA^+ or TEA^+.

We have attempted to induce equalization using uncharged molecules of structure comparable to the effective ions, but so far without success. Thus, *t*-butyl alcohol, similar in size and shape to TMA^+, is ineffective. Perhaps positively charged species are required to create a sufficiently high local concentration of the additive around the negatively charged DNA molecule, or a charge–charge interaction with a phosphate may be needed to properly orient the binding molecule in the groove. However, tetramethylpiperazinium ion, though doubly charged, has about the same effect as TMA-Cl, showing that a greater positive charge does not necessarily increase effectiveness as a stability equalizer.

Differences in degree of hydration of the dA·dT and the dG·dC base pairs may play a role in the differential destabilization effect. Gruenwedel *et al.* (7*a*) have rationalized the dT_m/dX_{GC}-depressing effects of high concentrations of Cs_2SO_4 and Na_2SO_4 in terms of effects of these ions on water activity. Tunis and Hearst (8*c*) have suggested that dA·dT pairs are more highly hydrated than dG·dC pairs. If so, this excess water of hydration may participate in forming a suitable binding site for one or more of the nonpolar arms of the preferentially bound alkylammonium ions.

Applications

In order to apply these base-pair equalization methods to problems of biological and structural interest, it is necessary to demonstrate that the additives used do not, in themselves, alter the solution conformation of the DNA helix. The CD spectra of Fig. 4 suggest that structural alteration induced by TMA^+ or TEA^+ is minimal.

We have shown in this study that dT_m/dX_{GC} is independent of DNA base composition for any particular concentration of TMA^+ or TEA^+ (Table 1). This finding demonstrates that the preferential interaction of these ions with DNA is at the level of a single base pair, since a requirement for longer sequences would lead to a nonlinear dependence of T_m on base composition. Furthermore, since dT_m/dX_{GC} is independent of base composition, it is also independent of temperature. This result is important since, in biological applications, one

would want to know that base-pair stabilities have been equalized at physiological temperatures, as well as at T_m.

The dramatic decreases in the transition breadths of compositionally heterogeneous DNAs (Figs. 1 and 3; Table 1) as dT_m/dX_{GC} goes to zero are of considerable theoretical and structural interest, since under conditions of total equilization the breadth of the transition represents only sequence-independent aspects of cooperativity. In principle, this result should make it possible to introduce sequence-dependent and sequence-independent parameters separately into helix-coil calculations, with a great increase in tractability and consequent molecular insight. (These aspects will be considered elsewhere; Melchior and von Hippel, in preparation.)

Solvent systems capable of altering the dependence of DNA stability on base composition not only find application in the study of DNA structure, but also present intriguing possibilities for the study and manipulation of the behavior of DNA in areas ranging from denaturation mapping of genes by electron microscopy (3) to interactions with proteins of biological interest (1). However, the solvent systems reported in this work may not be ideal for all such purposes. Even though the conformation of the DNA helix is not appreciably altered, these solvent systems are quite concentrated and viscous, and have high ionic strength. Efforts are being continued to find perturbants that will minimize some of these problems.

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