Betaine Can Eliminate the Base Pair Composition Dependence of DNA Melting†

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ABSTRACT: We show that the amino acid analogue betaine shares with small tetraalkylammonium ions [Melchior, W. B., Jr., & von Hippel, P. H. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 298–302] the ability to reduce or even eliminate the base pair composition dependence of DNA thermal melting transitions. The "isostabilizing" concentration of betaine (at which AT and GC base pairs are equally stable) is ~5.2 M. Betaine exerts its isostabilizing effect without appreciably altering the conformation of double-stranded DNA from the B form. The presence of >5 M betaine also does not greatly change the behavior of DNA as a polyelectrolyte; this lack of effect on electrostatic interactions is expected because betaine exists as a zwitterion near neutral pH. Study of DNA melting transitions in high concentrations of betaine thus allows the experimental separation of compositional and polyelectrolyte effects on DNA melting. As a consequence, betaine solutions can also be used to investigate DNA–protein interactions under isostabilizing (or close to isostabilizing) conditions, which has not been possible using isostabilizing salts. This potential is illustrated by examining the highly salt concentration-dependent interaction of ribonuclease A with DNA in concentrated betaine solutions.

The thermodynamic stability of double-stranded nucleic acids has two major determinants. These are the polyelectrolyte effect (Record et al., 1978; Manning, 1978; Le Bret & Zimm, 1984) and the base composition/sequence effect (Breslauer et al., 1986; Freier et al., 1986). Detailed differences between base pair sequences are averaged out for long DNAs from natural sources, and the dependence of the melting temperature (T_m)[1] on salt concentration and base pair composition can be represented by the empirical equation:

$$T_m = -16.6 \log [S] + 41.5(X_{GC}) + 81.5 \qquad (1)$$

where T_m is the melting temperature (in degrees centigrade), [S] is the total monovalent salt concentration in moles per liter, and X_{GC} is the mole fraction of GC base pairs in the DNA (Marmur & Doty, 1962; Schildkraut & Lifson, 1965). Thus, T_m increases linearly with the GC content of the DNA at a fixed salt concentration, and T_m also increases linearly with the logarithm of the total monovalent salt concentration at a fixed base pair composition. It is of basic theoretical (as well as practical) interest to find experimental conditions that allow these two effects to be separated and studied in isolation. The polyelectrolyte effect is strongly influenced by the ionic nature of the solvent, while the base pair composition/sequence effect is strongly influenced by additives that alter the polarity or hydrophobicity of the solvent. Thus, it is logical to evaluate different solvent additives in an attempt to separate the two effects.

An early report (Melchior & von Hippel, 1973) described a class of solvent additives that radically altered the base composition/sequence effect on nucleic acid stability. This class of additives consists of the tetraalkylammonium (TAA) salts and their close relatives. It was found, for DNA, that the dependence of T_m on base pair composition could be eliminated at 3.3 M TMACl[1] or 2.4 M TEACl.[1] The term "isostabilizing" was coined to describe the critical concentration of additive at which AT and GC base pairs display the same melting temperature. All genomic DNAs were shown to melt at a single high temperature in 3.3 M TEACl, and at a single low temperature in 2.4 M TEACl. The stability of short oligonucleotide duplexes can also be rendered independent of base composition in concentrated TAA salt solutions (Jacobs et al., 1988; Marky et al., 1988).

Melchior and von Hippel (1973) showed that the isostabilization induced by TAA salts is not correlated with a change in the helical structure of the DNA, since circular dichroism spectra indicated that the typical B-form conformation of the DNA is retained in these solutions. The effect was interpreted in terms of two components: (i) a general destabilization of the DNA, which increases with TAA chain lengths; and (ii) a noncooperative differential stabilization of the AT base pairs, relative to the GC base pairs, within the DNA.

As expected, all tetraalkylammonium compounds larger than TMA^+ work as general destabilizers (von Hippel & Wong, 1965). However, it was found that only the smallest TAA^+ cations (TMA^+ and TEA^+) exhibit the differential stabilization effect. The general effect reflects a destabilization of the "hydrophobic" base pair stacking interactions of native DNA due to the increasing nonpolar character of the solvent, while the differential effect has been attributed to preferential binding of TAA^+ to AT pairs in the native structure [see Shapiro et al. (1967)]. This might explain why only TMA^+ and TEA^+ exhibit the differential effect, since only these ions are small enough to fit into the major groove of the B-form DNA double helix (Melchior & von Hippel, 1973).

TAA salts are strong electrolytes and dissociate in aqueous solution to yield TAA cations and anions. These ionic species then perturb the polyelectrolyte properties of the nucleic

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[1] Abbreviations: betaine, *N,N,N*-trimethylglycine; TAA, tetraalkylammonium; TMACl, tetramethylammonium chloride; TEACl, tetraethylammonium chloride; T_m, melting temperature; w/w, weight percent.

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acids under study. The fact that TAA salts introduce a direct thermodynamic linkage between the polyelectrolyte properties and the composition/sequence properties of the DNA is unsatisfying from a theoretical point of view, because it complicates analysis of the system. In addition, from a practical standpoint, the need for multimolar concentrations of TAA salts makes it impossible to examine the effects of DNA isostabilization on DNA–protein complexes, since these complexes are invariably destabilized by increasing salt concentrations. For this reason, we have searched for other classes of solvent additives that could alter the sequence/composition-dependent stability of the nucleic acids while leaving their behavior as polyelectrolytes relatively unchanged.

In early experiments, Melchior and von Hippel (unpublished results) observed that the zwitterionic amino acid analogue betaine is such a compound; here we present a thorough experimental study of this observation. Betaine is chemically related to the TAA compounds. However, because it is an amino acid analogue, this molecule carries both a positive and a negative charge at pH values close to neutrality. Thus, it is not expected to engage in counterion condensation on nucleic acid lattices and should have only minimal effects on the electrostatic interactions of DNA with proteins and other charged ligands. Consequently, as shown here, studies in solutions containing various concentrations of betaine permit the resolution of electrostatic and base pair composition/sequence effects on DNA stability. Even high concentrations of betaine should not greatly affect the electrostatic portion of the interaction free energy that stabilizes DNA–protein interactions. Therefore, these complexes may be studied at differing betaine concentrations in which the relative stability of AT and GC base pairs can be manipulated.

MATERIALS AND METHODS

Betaine and Salts. Betaine (*N,N,N*-trimethylglycine) monohydrate was purchased from Sigma and used without further purification. Lot B-2754, used in the majority of the experiments reported here, was shown to be >95% pure by thin-layer chromatography and mass spectrometry (Richard Wielesik, University of Oregon), and >99% pure by elemental analysis (Microanalytics Inc., Corona, NY). This particular lot of betaine showed little yellow coloration, though most of the other commercial lots that we examined contained variable amounts of a UV-absorbing contaminant and appeared yellowish to the eye. A 5.6 M solution of one of our purest lots of betaine had an optical density of 0.5 at 260 nm in a 1-cm path-length cell. This optical density increased linearly with temperature to ~0.9 at 98 °C.

The chemical stability of phosphate-buffered betaine solutions, stored under N_2, was monitored at 20 °C. Over 1–3 weeks, these solutions displayed a progressive increase in yellow color and in pH (+0.1 pH unit/week), indicating a breakdown of the betaine by some undefined reaction. Therefore, betaine solutions were used within 7 days of preparation in all of our experiments.

TMACl (reagent grade, Eastman Kodak Co.) and Cs_2SO_4 (optical grade; Var Lac Oid Chemical Co., Inc., Bergenfield, NJ) were used without further purification. All other salts and organic chemicals were reagent grade or better.

DNA Samples. Escherichia coli, Micrococcus lysodeikticus, and *Clostridium perfringens* DNAs were purchased from Sigma, and calf thymus DNA and poly(dA-dT) were from Pharmacia. All DNAs were received in lyophilized form, with buffer salts present. To prepare DNA stock solutions, lyophilized DNA samples were suspended at concentrations of 1–4 mg/mL in H_2O, dialyzed exhaustively against 5 mM K_2HPO_4 containing 0.1 mM Na_2EDTA (pH 7.5), and stored at 4 °C. For extinction coefficients, we used 6.6×10^3 M^{-1} cm^{-1} at 260 nm for calf thymus DNA (Mahler et al., 1964) and 6.65×10^3 M^{-1} cm^{-1} at 262 nm for poly(dA-dT) (Inman & Baldwin, 1962). These extinction coefficients are defined in terms of molar concentrations of nucleotide residues. The molecular masses of the natural DNAs were found to exceed 5×10^6 daltons on the basis of electrophoretic mobility in nondenaturing agarose gels. The poly(dA-dT) samples had $s^0_{20,w}$ values ranging from 8.1 to 21.3 S, corresponding to molecular masses ranging from 4.7×10^4 to 7.7×10^6 daltons (Studier, 1965).

UV Absorbance Melting Studies. All our spectrophotometric studies were conducted at pH 7.5 in solutions containing 5 mM K_2HPO_4, 0.1 mM Na_2EDTA, and the indicated concentrations of KCl and other additives. Solutions were filtered through a 0.2-mm pore-size filter (Nalgene) before DNA addition. DNA was added to a concentration giving an OD_{260} of ~0.2 in a 1-cm path-length cell, and was incubated overnight at 37 °C before use in thermal denaturation experiments.

DNA melting profiles were monitored at 260 nm on a Hewlett-Packard 8450A spectrophotometer equipped with a Hewlett-Packard 89100A Peltier temperature controller. Teflon-stoppered quartz curvettes (1-cm path length) were employed in these studies (Precision Cells, Inc.). After spectral balancing, the sample and reference cells were heated together at a rate of +0.6 °C/min, and the net absorbance at 260 nm was recorded automatically after every 0.5 °C increase in temperature. No light-scattering corrections were required. The apparent optical densities obtained were not corrected for thermal expansion of the solutions. The T_m of each DNA sample was determined graphically from the transition midpoint of the absorbance versus temperature profile.

Circular Dichroism. CD spectra were measured with a Jasco J-600 spectropolarimeter equipped with a Cary 14 thermostated cell holder. Temperature was held constant at 22 °C with a Peltier device. CD was expressed as molar ellipticity, $[\theta] = 100(\theta/lc)$, where θ is the recorded ellipticity (in degrees centimeter squared per decimole), l is the cell path length (in centimeters), and c is the DNA concentration (decimolar nucleotide residues).

Differential Scanning Calorimetry. DSC studies were performed with a Microcal MC-2 scanning calorimeter (Microcal Inc., Northhampton, MA). Calf thymus DNA was examined at a concentration of 3.0 mM (in nucleotide residues) in the absence of betaine, and at a concentration of 1.65 mM in the presence of 5.2 M betaine. Samples were heated at a rate of +0.33 °C/min.

RESULTS

5.2 M Betaine Eliminates the Composition (and Sequence) Dependence of DNA Melting. AT-rich regions melt at lower temperatures than GC-rich regions in random-sequence DNA, and this differential thermal stability is the primary determinant of the breadth of a DNA melting transition (Melchior & von Hippel, 1973). High concentrations of betaine eliminate this differential stability and shift the melting temperatures of DNAs of all base pair compositions to a single (common) value.

These effects are easily demonstrated in optical melting studies of natural DNAs. In Figure 1A, we show the melting behavior of calf thymus DNA in 5 mM potassium phosphate/0.1 mM EDTA (pH 7.5), in the absence and presence of 5.0

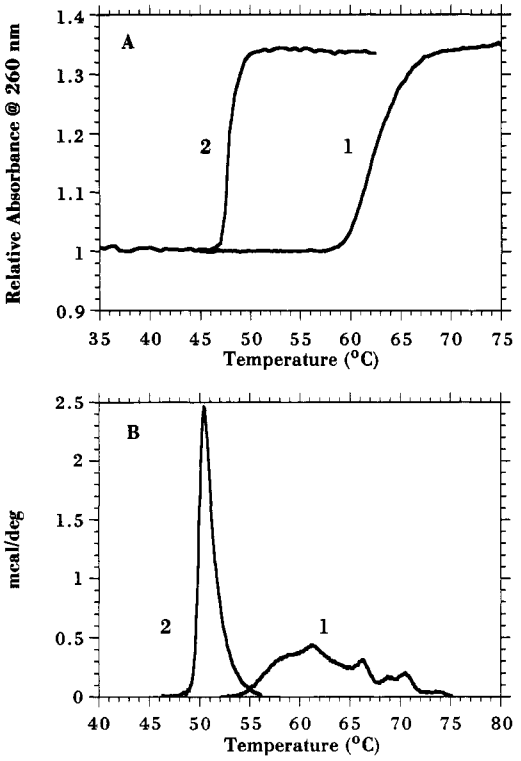

FIGURE 1: Melting of calf thymus DNA in the absence or presence of betaine. The buffer was 5 mM potassium phosphate/0.1 mM EDTA (pH 7.5) in all experiments. (A) Melting monitored by the UV absorbance at 260 nm. The DNA concentration was ~10 μg/mL ($OD_{260} \approx 0.2$ cm^{-1}). The heating rate was 0.5 °C/min. Curve 1, no betaine; curve 2, 5.0 M betaine. (B) Melting monitored by differential scanning calorimetry. The DNA concentration was 3.04 mM (in nucleotide residues) ($OD_{260} = 199.8$) in the absence of betaine and 1.65 mM ($OD_{260} = 10.8$) in 5.2 M betaine. The data are normalized to a DNA concentration of 1.0 mM nucleotide residues. The heating rate was +0.25 °C/min. Curve 1, no betaine; curve 2, 5.2 M betaine.

M betaine. It is apparent that this concentration of betaine greatly sharpens the melting transition and shifts it to a lower temperature. An even more striking demonstration of this effect can be seen with differential scanning calorimetry (DSC). In Figure 1B, we show DSC profiles of calf thymus DNA in the same buffer in the absence or presence of 5.2 M betaine. The sharpening of the melting transition in 5.2 M betaine and its shift to lower temperatures are clearly evident.

These effects of betaine are not limited to calf thymus DNA. In Figure 2, we plot the melting temperatures of a number of natural and synthetic DNAs, measured in 5 mM potassium phosphate/0.1 mM EDTA (pH 7.5), as a function of the concentration of added betaine. As this figure shows, the isostabilizing concentration of betaine for all the natural DNAs that we tested is close to 5.2 M. Isostabilization of the DNA results because GC-rich DNAs are greatly destabilized in betaine, while AT-rich DNAs are destabilized much less.

Figure 2 shows that the same general trend is followed by the synthetic alternating copolymers poly(dA-dT) and poly(dG-dC). However, the isostability point of the synthetic DNAs appears to fall slightly above 5.2 M betaine. This slight difference may reflect the fact that these alternating copolymer polynucleotides differ somewhat in structure from the B-form conformation characteristic of natural DNAs (Klug et al., 1979; Shindo et al., 1979; Patel et al., 1981; Cohen et al., 1981; Eckstein & Jovin, 1983).

The B-Form Conformation of DNA Is Retained in High Betaine Concentrations. We have used circular dichroism

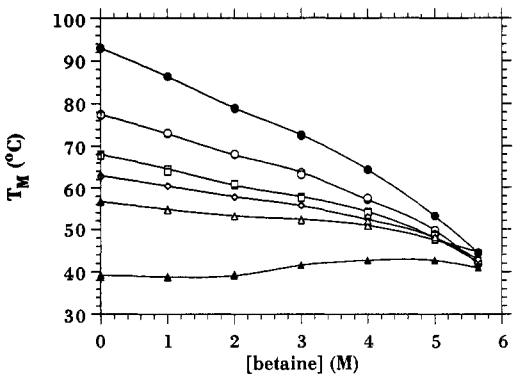

FIGURE 2: Variation of T_m with betaine concentration for DNAs of varying base composition. Melting was monitored by the UV absorbance at 260 nm. Buffers and heating rates were as in Figure 1A. (Open circles) *M. lysodeikticus* DNA (72% GC); (open squares) *E. coli* DNA (50% GC); (open diamonds) calf thymus DNA (42% GC); (open triangles) *Cl. perfringens* DNA (26% GC); (filled triangles) poly(dA-dT); (filled circles) poly(dG-dC).

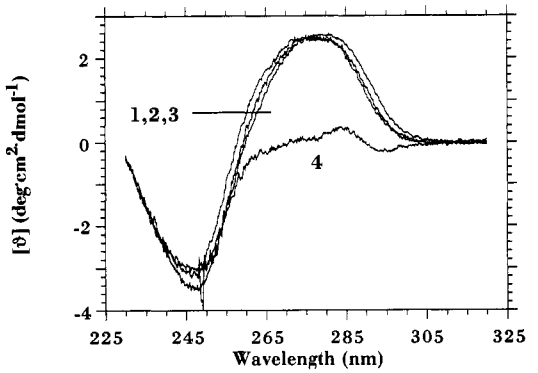

FIGURE 3: Circular dichroism of calf thymus DNA at low salt concentration, in Cs_2SO_4, and in isostabilizing concentrations of betaine and TMA. Curve 1, low-salt buffer (same as in Figure 1A), 0.13 mM DNA (in nucleotide residues); curve 2, 3.0 M TMACl, 0.13 mM DNA; curve 3, 5.6 M betaine, 0.13 mM DNA; curve 4, 2.5 M Cs_2SO_4, 0.10 mM DNA.

spectroscopy to examine the conformation of DNA at high betaine concentrations. In Figure 3, we show CD spectra of calf thymus DNA in several solvent environments. The betaine spectrum was extended down only to about 250 nm, due to the excessive optical density of concentrated betaine solutions at lower wavelengths; the other spectra were only carried down to about 230 nm. Comparison of the spectrum in 5.6 M betaine with the low-salt spectra suggests that the double-stranded DNA remains in the B conformation at high betaine concentrations. For comparative purposes, we also show the CD spectrum of calf thymus DNA in 2.5 M Cs_2SO_4. Here the 280-nm band is severely depressed, indicating that double-stranded DNA in this salt solution has been perturbed from the B conformation, perhaps reflecting a significant change in the twist angle of the DNA base pairs (Ivanov et al., 1973; Anderson & Bauer, 1978). We conclude that natural double-stranded DNAs retain their B-form conformation in high concentrations of betaine, as shown previously for double-stranded DNA in high concentrations of TAA salts (Melchior & von Hippel, 1973).

The Electrostatic Properties of DNA Appear Nearly Unchanged at Isostabilizing Betaine Concentrations. The data of Figure 2 can be replotted to clarify other aspects of the betaine effect. Figure 4 presents some of the data of Figure 2 in the form of a plot of T_m versus X_{GC}, the average mole fraction of GC base pairs in the DNA. Panel A plots T_m values for DNAs of various base pair composition in 5

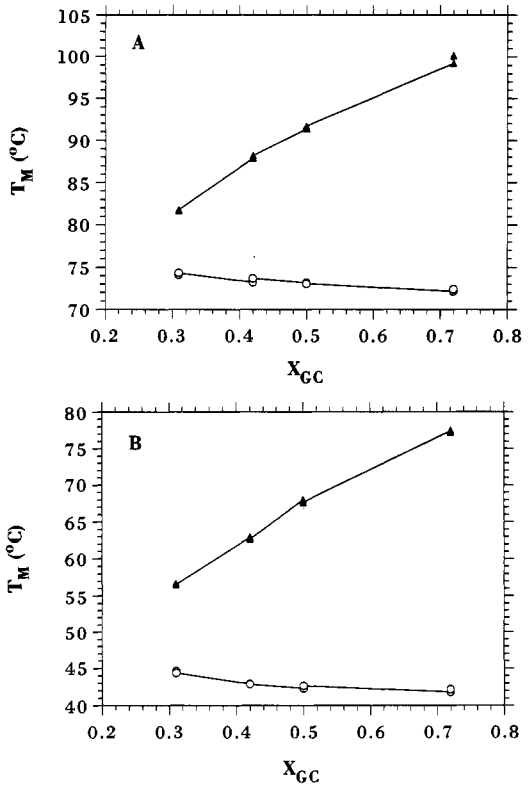

FIGURE 4: Separation of the composition dependence and the salt concentration dependence of DNA melting. (A) T_m of DNA as a function of the mole fraction of GC in buffer plus 200 mM KCl. The buffer contained 5 mM potassium phosphate/0.1 mM EDTA (pH 7.5). The upper curve (filled triangles) shows positive slope in the absence of betaine ($dT_m/dX_{GC} > 0$). The lower curve (open circles) shows approximately zero slope in the presence of 5.6 M betaine ($dT_m/dX_{GC} = 0$). (B) T_m of DNA as a function of the mole fraction of GC in buffer alone. The upper curve (filled triangles) shows positive slope in the absence of betaine ($dT_m/dX_{GC} > 0$). The lower curve (open circles) shows approximately zero slope in the presence of 5.6 M betaine ($dT_m/dX_{GC} \approx 0$).

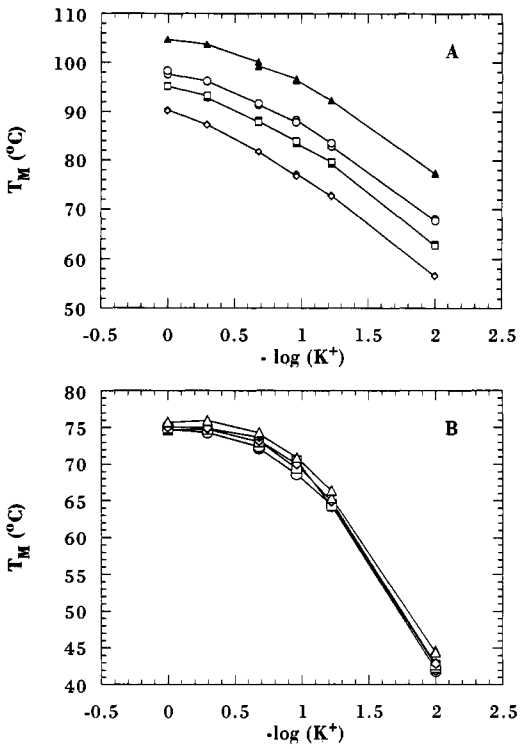

FIGURE 5: Polyelectrolyte properties of DNA in the absence and in the presence of 5.0 M betaine. (A) T_m versus –log [K^+] for natural DNAs of different base pair composition in low-salt buffer. (Filled triangles) *M. lysodeikticus* DNA (72% GC); (open circles) *E. coli* DNA (50% GC); (open squares) calf thymus DNA (42% GC); (open diamonds) *Cl. perfringens* DNA (26% GC). (B) T_m versus –log [K^+] for natural DNAs of different base pair composition in the presence of 5.6 M betaine. Symbols defined in panel A.

mM potassium phosphate, 0.1 mM EDTA (pH 7.5), and 0.2 M KCl in the presence or absence of 5.6 M betaine. Panel B shows similar data for buffer containing no added KCl, again with or without 5.6 M betaine.

Clearly, the data of both panels show the strong linear dependence on X_{GC} in the absence of betaine that is predicted by eq 1, while this dependence on the GC content of the DNA is virtually abolished in 5.6 M betaine. In addition, we see (as also shown in Figure 2) that while betaine causes a net destabilization of DNAs of all compositions at a fixed KCl concentration, the T_m for GC-rich DNA is shifted down more than that for AT-rich DNA, resulting in a net trend toward isostabilization. Finally (and most relevant to the point we wish to make here), betaine has qualitatively the same effect on DNA melting transitions at both KCl concentrations, but the isostabilized T_m is much higher (75 °C) in 0.2 M KCl than in the absence of added salt (45 °C). This positive dependence of T_m on salt concentration is, of course, consistent with eq 1 and is predicted by polyelectrolyte theory (Record et al., 1978). These findings suggest that the dependence of T_m on DNA composition/sequence-dependence effects may be altered or removed by adding betaine without significantly changing the electrostatic properties of the system.

Betaine Can Help To Separate the Composition/Sequence Effect and the Polyelectrolyte Effect in DNA Melting. To examine the preceding point more closely, we have measured the T_m values of DNAs of different GC content in betaine as a function of KCl concentration. The results of many experiments are summarized in Figure 5A,B. In Figure 5A, the well-known linear relationship between T_m and log [K^+] is demonstrated in the absence of betaine. At KCl concentrations less than ~0.2 M (corresponding to –log [K^+] > 0.7), the data fall on straight lines, and the slope of the plot of T_m vs log [K^+] is ~+19 °C (see eq 1). Deviations from linearity are observed at KCl concentrations exceeding 0.2 M (corresponding to –log [K^+] < 0.7) (Schildkraut & Lifson, 1965).

In Figure 5B, the relationship between T_m and KCl concentration is examined in 5.6 M betaine (close to the isostabilizing concentration). Here the linear relationship between T_m and log [K^+] may hold only at KCl concentrations less than ~80 mM, and the slope of the plot ($dT_m/d\{\log [K^+]\}$) is somewhat larger (~+27 °C) than that seen in dilute salt solutions. Thus, we find that composition and polyelectrolyte influences on DNA melting are indeed approximately (though not entirely) separable. The coupling that remains (manifested by the small change in dT_m/dX_{GC}, the difference in dT_m/d log [K^+], and the difference in KCl concentration at which deviations from linearity begin) may in some way arise from the fact that betaine solutions have a significantly higher bulk dielectric constant than water (Edsall & Wyman, 1935).

The Interaction between RNase A and Poly(dA-dT) Is Maintained in Concentrated Betaine Solutions. Having established conditions to isostabilize DNA using the zwitterion betaine, we have conducted preliminary experiments to investigate the potential for studying protein–DNA interactions under conditions where the electrostatic component of the binding interaction should not be greatly perturbed. To this end, the interaction between RNase A and poly(dA-dT) in betaine has been examined as a model system.

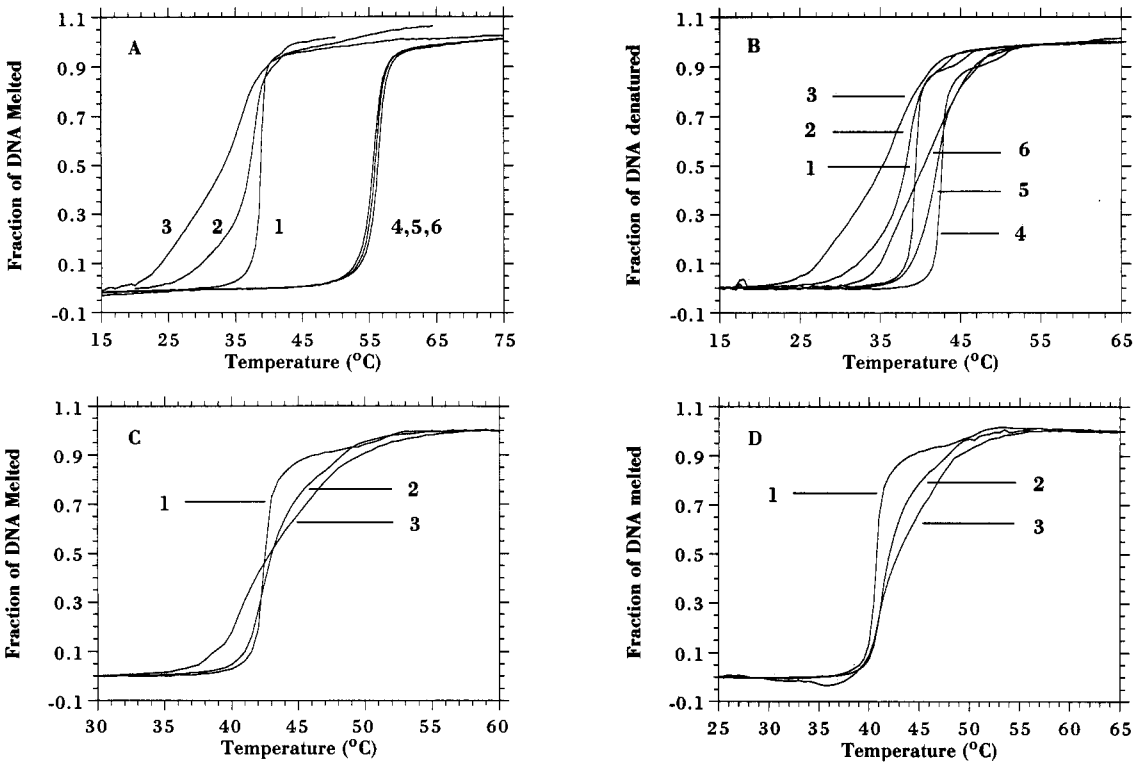

FIGURE 6: RNase A-perturbed melting profiles of poly(dA-dT) in the absence and the presence of betaine. Each reaction contained 5 mM potassium phosphate, 0.1 mM EDTA (pH 7.5), and variable concentrations of KCl, betaine, and RNase A. The poly(dA-dT) concentration was ~10 μg/mL ($OD_{260} \approx 0.2$) in all experiments. (A) Poly(dA-dT) melting profiles in buffer (curves 1–3) and buffer plus 50 mM KCl (curves 4–6) with RNase A. Curves 1 and 4, no added RNase A; curves 2 and 5, 1.0 μM RNase A; curves 3 and 6, 2.0 μM RNase A. (B) Poly(dA-dT) melting profiles in 2.0 M betaine (curves 1–3) and 3.9 M betaine (curves 4–6) with RNase A. Curves 1 and 4, no added RNase A; curves 2 and 5, 1.0 μM RNase A; curves 3 and 6, 2.0 μM RNase A. (C) Poly(dA-dT) melting profiles in 4.9 M betaine with RNase A. Curve 1, no added RNase A; curve 2, 1.0 μm RNase A; curve 3, 2.0 μM RNase A. (D) Poly(dA-dT) melting profiles in 5.6 M betaine as influenced by RNase A. RNase A concentrations: curve 1, no added RNase A; curve 2, 1.0 μM; curve 3, 2.0 μM.

RNase A binds noncooperatively to both single- and double-stranded DNA to form DNA–protein complexes (Jensen & von Hippel, 1976). The strengths of the interactions within these complexes are strongly dependent on the concentration of added salt. RNase A is a "passive"[2] DNA melting protein, binding preferentially to single-stranded DNA sequences that are transiently exposed in double-stranded DNA by thermal fluctuations. Binding can be conveniently monitored by following the reduction in the T_m of the DNA on the addition of RNase A (Jensen & von Hippel, 1976), and analyzed using the theoretical treatment of McGhee (1976).

Under the chosen reaction conditions (see Materials and Methods), the RNase A-influenced thermal denaturation of poly(dA-dT) can be studied without complications due to the thermal denaturation of the RNase A itself, since RNase A denatures at significantly higher temperatures than does poly(dA-dT). Thermal melting studies showed that RNase A begins to denature at ~55 °C in 5 mM K_2HPO_4/0.1 mM EDTA (pH 7.5) and that the T_m of this protein increases further as the concentration of betaine is increased up to 5.6 M (data not shown). These data are consistent with a recent calorimetric study by Santoro et al. (1992) of betaine as an osmolyte, which showed that RNase A is stabilized at all betaine concentrations up to 5 M (the highest concentration of betaine tested). We have shown that poly(dA-dT) melts completely at temperatures below 50 °C in betaine concentrations of 5.6 M or less (Figure 2).

In Figure 6, we show thermal melting profiles for poly(dA-dT) as a function of RNase A concentration. Increasing concentrations of RNase A shift the midpoint of the poly(dA-dT) melting curve to lower temperatures and broaden the melting transitions (Figure 6A, curves 1–3). This destabilization by RNase A is very sensitive to monovalent salt concentration, and is largely eliminated in the presence of 50 mM KCl (Figure 6A, curves 4–6). We find that the RNase A-induced decrease in the T_m of poly(dA-dT) is observed at betaine concentrations of up to 4 M (Figure 6B). Thus, it appears that RNase A is able to destabilize poly(dA-dT) as a (native) single-stranded DNA binding protein in the presence of very high concentrations of betaine.

However, Figure 6C shows that the RNase A-induced destabilization of poly(dA-dT) is no longer seen in 5 M betaine, and that in 5.6 M betaine RNase A *increases* the T_m of poly(dA-dT) (Figure 6D). A possible explanation is that RNase A, at these very high concentrations of betaine, may lose some of the aspects of its native structure that are responsible for its DNA melting properties, and thus may interact preferentially with double-stranded DNA in a partially denatured form [see Jensen and von Hippel (1976)]. However, we note that even under these conditions an electrostatic interaction between DNA and RNase A continues to be observed (see Discussion).

DISCUSSION

Betaine Can Remove the Base Pair Composition Dependence of DNA Melting without Greatly Altering Electrostatic

[2] We differentiate between "active" single-stranded DNA binding, where we mean that the protein "forces open" a specific double-stranded sequence of the DNA to permit binding, and "passive" binding, where the single-stranded binding protein "traps" and binds single-stranded DNA sequences exposed by thermal fluctuation [see Jensen and von Hippel (1976)].

Interactions. It is well established that the stability of double-helical DNA depends on base pair composition (and sequence), as well as on the ionic nature of the solvent environment (eq 1). In this paper, we have shown that the usual dependence of the melting temperature on GC content (dT_m/dX_{GC}) can be eliminated or even reversed at high concentrations of zwitterionic betaine (*N,N,N*-trimethylglycine). Thus, betaine, like TMA^+ and TEA^+, can be used to isostabilize DNAs of differing composition or sequence. However, unlike the case for TAA^+ salts, here isostabilization can be achieved without major alterations in the polyelectrolyte properties of the DNA.

The Isostabilization Mechanism. Increasing betaine concentrations sharpen the melting transitions of DNA and shift these transitions toward lower temperatures. The slope of a plot of T_m as a function of betaine concentration depends on base composition, being much larger for GC-rich than for AT-rich DNA (Figures 1 and 2). These observations are most easily interpreted in terms of the hypothesis advanced by Melchior and von Hippel (1973) to explain similar behavior evinced by the small TAA^+ ions.

These workers proposed that the observed effects of the small TAA^+ cations on DNA melting reflect a general (base composition and sequence independent) destabilization of the DNA that is partially offset by a preferential (relative to GC pairs) binding of the TAA^+ ligand to the AT base pairs of the native DNA structure. This preferential AT-stabilization effect was shown to be a linear function of both TAA^+ concentration and the AT base pair composition of the DNA. Melchior and von Hippel (1973) suggested that the preferential binding of the small TAA^+ moieties is weak and noncooperative, with respect to both AT base pair concentration and TAA^+ concentration. This hypothesis is consistent with the equilibrium dialysis measurements of Shapiro et al. (1969), who had shown directly that tetramethylammonium ions bind preferentially to AT base pairs in DNA.

The preferential AT-stabilization effect (unlike the general destabilization effect) "cuts-off" at TAA^+ sizes larger than that of the tetraethylammonium ion, thus suggesting that this preferential binding occurs in a sterically limited region of the DNA. Probing the B form of the DNA double-helix with CPK models of TMA^+ and TEA^+ showed that these moieties could bind in the major groove of the DNA and suggested that the increased affinity of these molecules for AT base pairs probably reflects a preferential hydrophobic interaction with the methyl group of thymine (Melchior & von Hippel, 1973). We note that this cutoff with TAA^+ size of the isostabilizing effect at cations larger than TEA^+ suggests that more general interpretations of these effects (e.g., involving TAA^+-dependent changes in the cooperativity of DNA melting) are not likely to be correct.

This interpretation is consistent with the observation that TMA^+ and TEA^+ are much less effective at isostabilizing T4 DNA, which contains glucosylated (hydroxymethyl)cystosine residues that largely fill the major groove of the native DNA (Melchior and von Hippel, unpublished results). As a control in these experiments, Melchior and von Hippel also demonstrated that the T4* mutant of bacteriophage T4 [which is deficient in an enzyme involved in (hydroxymethyl)cytosine glycosylation] is isostabilized like regular (cytosine-containing) DNAs by TMA^+ and TEA^+. This observation suggests that the replacement of cytosine by (hydroxymethyl)cytosine in double-stranded DNA does not perturb the proposed preferential binding of small TAA^+ ions to AT base pairs.

We propose, therefore, by the same logic as in Melchior and von Hippel (1973), that this effect is differentially offset, in direct proportion to the AT content of the DNA, by a weak and noncooperative preferential binding of betaine with AT pairs in the major groove of the B-form double-helix. One might ask why betaine behaves more like TEA^+ than its closer analogue TMA^+ in showing a general destabilizing effect. Perhaps, in terms of the previous explanation for the TAA^+ effects advanced by von Hippel and Wong (1965), one might argue that while the charge on the central nitrogen atom of TMA^+ tends to inactivate the "hydrophobic" effect of the vicinal methyl groups, this inactivation might be smaller in betaine because here the charge on the nitrogen is partially neutralized by the oppositely charged carbonyl oxygen of the zwitterion.

Stable DNA–Protein Complexes Can Be Formed and Can Function in Solutions Containing High Concentrations of Betaine. Most DNA binding proteins involve both electrostatic and nonelectrostatic components in their binding interactions with DNA (Record et al., 1976). It is possible, through the use of high concentrations of neutral salts, to eliminate the electrostatic component of the binding interaction and thus to study the nonelectrostatic component in relative isolation. We propose that a solvent additive such as betaine can achieve the complementary effect of eliminating the composition-dependent component of the binding, thus allowing the electrostatic effect to be studied in isolation under isostabilizing conditions.

A more careful consideration of this prospect, however, reveals at least three possible problems: (i) solutions containing high betaine concentrations present a somewhat different solvent environment than do the dilute salt solutions in which DNA–protein interaction studies are usually studied; (ii) betaine itself might alter the conformational stability of the protein and thus perturb its ability to interact with the DNA; and (iii) preferential binding of betaine to DNA might compete directly with protein binding.

To investigate these issues, we have conducted preliminary experiments on the binding of a model protein, RNase A, to double-stranded poly(dA-dT). RNase A binds noncooperatively to both single- and double-stranded DNA. Its binding site size is 12 nucleotide residues for single-stranded DNA and 8 base pairs for double-stranded DNA. This protein has been shown to bind preferentially to transiently single-stranded regions of DNA, and thus functions as a passive DNA melting protein (Jensen & von Hippel, 1976). The binding of RNase A to DNA has been analyzed in terms of the large-ligand/lattice binding model of McGhee and von Hippel (1974), and its effects on thermal melting profiles have been elucidated by the method of McGhee (1976).

To address concerns (i) and (ii) above, we conducted thermal melting studies of RNase A in the presence of various concentrations of betaine. We found that RNase is not significantly denatured in 5.2 M betaine (data not shown). This result is also consistent with a recent differential scanning calorimetry study that showed that the native form of RNase A is actually stabilized by up to 5 M betaine (Santoro et al., 1992). Timasheff and co-workers have also shown that various proteins are stabilized against thermal denaturation and preferentially hydrated in 0.5–2 M betaine (Arakawa & Timasheff, 1983, 1985), which is known to be an in vivo osmoprotectant.

Concern (iii) was examined by measuring the binding of RNase A to poly(dA-dT) in the absence and presence of betaine. We used thermal melting profiles to assay RNase A binding to this synthetic double-stranded DNA because RNase A binding had previously been shown to alter the

melting properties of native DNA (Jensen & von Hippel, 1976). These melting studies are summarized in Figure 6. The effect of RNase A on the melting of poly(dA-dT) is essentially the same in 2.0 and 3.9 M betaine as it is in the absence of betaine; RNase A broadens the melting profiles and shifts the T_m of poly(dA-dT) to lower temperatures in all these solvent environments.

We note that the interactions of RNase A with poly(dA-dT) that are responsible for DNA destabilization are less inhibited by 4 M betaine than they are by 50 mM KCl. This supports the view that such electrostatically-stabilized complexes can be studied at betaine concentrations approaching those needed to isostabilize DNA.

In 4.9 and 5.6 M betaine, the effects of RNase A on the poly(dA-dT) melting profile are qualitatively different from those seen at lower betaine concentrations. Thus, in 4.9 M betaine, RNase A is seen to broaden the transition without depressing T_m, and at 5.6 M betaine, RNase A actually appears to stabilize poly(dA-dT). If RNase A is partially or completely denatured in 5.6 M betaine, it would be expected to stabilize double-stranded DNA by binding preferentially, as a polycation, to this higher charge density form (Jensen & von Hippel, 1976). Alternatively, the double-stranded DNA-stabilizing activity of RNase A in 5.6 M betaine could arise from an inability of the RNase A to gain access to transiently single-stranded regions of DNA, perhaps as a consequence of the high viscosity of this medium. In either case, the fact that the melting temperature of DNA is perturbed by added RNase A even at these concentrations of betaine shows that electrostatic interactions between DNA and proteins *do* persist up to 5.6 M betaine, although the altered behavior of the RNase A–poly(dA-dT) system suggests that some aspect of the properties of the interaction must change somewhat at betaine concentrations above ~5 M.

We have also demonstrated that *E. coli* transcription elongation complexes can extend RNA transcripts at betaine concentrations up to 4 M and that *Eco*RI cleavage of its DNA target sequence proceeds at betaine concentrations up to 3.5 M (data not shown). Thus, although isostabilizing concentrations of betaine are not reached with these two interacting protein–DNA complexes without some alteration in the details of the interactions involved, these results do suggest that such biologically specific complexes *can* be studied at high betaine concentrations and that *extrapolation* to isostabilizing conditions might well be possible in such systems.

DNA Isostabilization and the Role of Betaine as an Osmoprotectant. Betaine is one of a limited number of small molecules accumulated by a variety of organisms in response to dehydrating conditions. These materials have been called "compatible solutes" or osmoprotectants, since they prevent the dehydration of cells subjected to osmotic stress without inhibiting physiological activities [for a review, see Czonka (1989)]. Betaine is a natural osmoprotectant of *E. coli* that is imported or synthesized to high concentrations in the cytoplasm of osmotically stressed cells. This accumulation occurs without decreasing cytoplasmic water content and cellular growth rates (Perroud & Lee Rudulier, 1985; Cayley et al., 1992).

Betaine is also one of many compatible solutes that stabilize proteins against thermal denaturation (Yancey et al., 1982; Arakawa & Timasheff, 1985; Santoro et al., 1992). Timasheff (1992) has proposed that one of the most significant reasons that betaine is "compatible" is because it stabilizes the native structure of biological macromolecules and is excluded from contact with their surfaces. Also, in addition to our functional studies of biological complexes described above, Pollard and Wyn Jones (1979) and Yancey et al. (1982) had earlier shown that the catalytic activity of many enzymes is not significantly inhibited in vitro by substantial betaine concentrations.

Our work represents the first published study of the effects of high concentrations of betaine on DNA structure and stability in vitro. It is not clear how this study bears on the role of betaine as a osmoprotectant, since the concentration of betaine required to isostabilize DNA is considerably higher than the 0.1–1 M concentrations found in the cytoplasm of osmotically-stressed *E. coli* cells (Perroud & Le Rudulier, 1985). To properly compare the in vivo and in vitro situations requires knowing the effective concentrations (thermodynamic activities) of betaine and water in the cytoplasm. Our CD studies, which indicate that the conformation of DNA is not altered by concentrated solutions of betaine, are clearly consistent with the view that compatible solutes do not alter the tertiary structure of macromolecules.

Practical Applications of the Isostabilization Effect of Betaine. The isostabilization phenomenon described here not only is theoretically interesting but also may have useful applications. For example, the polyelectrolyte theory of DNA melting and other conformational transitions was developed by considering DNA to be a one-dimensional lattice of point charges. Base composition and sequence effects have been ignored in these first approximation theories [see Record et al. (1978) and Zimm and Le Bret (1984)]. Critical experiments to further develop such models would benefit from the elimination of the effects of base composition and sequence. Studies in concentrated betaine solutions may therefore help advance basic knowledge of the polyelectrolyte effect in DNA.

In addition, TAA salts have been widely used to make melting temperatures independent of base composition in DNA hybridization studies [e.g., see DiLella and Woo (1987), Wood et al. (1987), Jacobs et al. (1988), Gogos et al. (1990), and Powell and Caccone (1990)]. By using betaine, it would be possible to manipulate the temperature of the hybridization reactions by varying the monovalent salt concentration while independently maintaining isostabilizing conditions. Low concentrations of TMACl have recently been used to enhance the specificity of the polymerase chain reaction which depends critically on initial priming events (Hung et al., 1990). Such experimental approaches might well benefit from the use of betaine, which, as a zwitterion, does not increase the salt concentration of the medium.

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