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Solvent Effects on Thermodynamics of Double-Helix Formation in $(dG\text{-}dC)_3$†

Diane DePrisco Albergo and Douglas H. Turner*

ABSTRACT: The thermodynamics of double-helix formation by $(dG\text{-}dC)_3$ have been measured in aqueous solvent mixtures containing 10 mol % methanol, ethanol, 1-propanol, formamide, *N*,*N*-dimethylformamide, or urea and 20 mol % ethanol. Optical activity measurements indicate the conformation of the double helix at 3 °C is the same in all the solvent mixtures except 20 mol % ethanol. All the cosolvents destabilize the helix relative to water. With 10 mol % alcohol cosolvents, this destabilization is associated with a more unfavorable entropy change averaging ~8% and a more favorable enthalpy change averaging ~5%. This is consistent with a small contribution of hydrophobic bonding to stability. In contrast, the destabilization by formamide, *N*,*N*-dimethylformamide, and urea is associated with a more unfavorable enthalpy change averaging ~23% and a more favorable entropy change averaging ~21%. Since all three of these cosolvents have dipole moments larger than water, this is consistent with increased competition for dipolar interactions between the nucleic acid bases. None of the results correlate with any one bulk solvent parameter such as surface tension, viscosity, or dielectric constant. With 20 mol % ethanol, optical activity measurements are consistent with a partial B to C form transition. This is associated with a 27% less favorable enthalpy and 25% more favorable entropy for helix formation relative to water. Since the B to C transition is associated with helix dehydration, this may imply a significant contribution of bound water to stability.

It has been suggested that solvent plays an important role in the conformational stability of nucleic acids (T'so et al., 1969; Levine et al., 1963; Lowe & Schellman, 1972; Breslauer et al., 1978; Girod et al., 1973; Cantor & Schimmel, 1980). However, there is debate over the mechanism involved. The denaturation of deoxyribonucleic acid (DNA) by alcohols initially implicated hydrophobic interactions (Herskovits, 1963). However, the helix was subsequently shown to be stabilized by a favorable enthalpy, whereas hydrophobic bonding is associated with a favorable entropy (Kauzmann, 1959; Tanford, 1973). The only theory of solvent participation that qualitatively predicts the correct thermodynamics suggests the energy of cavity formation plays a dominant role (Sinanoglu & Abdulnur, 1964, 1965; Abdulnur, 1966; Sinanoglu, 1968). This has been called "solvophobic" bonding. Although the theory is in qualitative agreement with trends in denaturation by mixed solvents, it has never been thoroughly tested. The only parameter that has been quantitatively correlated with the degree of destabilization of DNA is solubility (Herskovits & Harrington, 1972; Herskovits & Bowen, 1974; Levine et al., 1963).

The availability of oligonucleotides of defined length and sequence provides the opportunity for a more detailed investigation of the role of solvent. This paper reports the thermodynamics associated with the coil to helix transition of the deoxyhexanucleotide $(dG\text{-}dC)_3$ in mixed aqueous solvent systems. Previous studies have characterized this transition in H_2O and D_2O (Pohl, 1974; Albergo et al., 1981).

Experimental Procedures

Oligonucleotide. d-GpCpGpCpGpC was purchased from Collaborative Research. Tests for purity and the extinction coefficients used are described in the preceding paper (Albergo et al., 1981). The effect of solvent on the extinction coefficient at 280 nm at 3 °C was determined by measuring the absorbance of a concentrated sample in the solvent mixture (e.g., 10 mol % EtOH),[1] diluting it by 10 with water buffer, and remeasuring the absorbance. No change in extinction at 280 nm was observed for the solvents used here.

Solvents. Formamide was purified by recrystallization (Casey & Davidson, 1977). The absorbance at 270 nm was 0.07/cm. Spectral grade *N*,*N*-dimethylformamide was from Mallinckrodt. The absorbance was 0.28/cm at 275 nm. Water was double-distilled, and absolute ethanol was used. Methanol was spectral grade from Mallinckrodt, 1-propanol "distilled in glass" was from Burdick and Jackson, and urea was "ultra pure" from Schwarz/Mann.

All solvents were checked for purity by index of refraction and NMR. Water and alcohol solvents were checked for cation content by Eriochrome Black T–EDTA titration. The cation concentration was below the detection limit of 10^{-6} M in all cases. Solutions were made by diluting a 4.5 M caco-

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[1] Abbreviations used: MeOH, methanol; EtOH, ethanol; PrOH, 1-propanol; DMF, *N*,*N*-dimethylformamide; CD, circular dichroism; CMP, cytidine monophosphate; EDTA, ethylenediaminetetraacetic acid.

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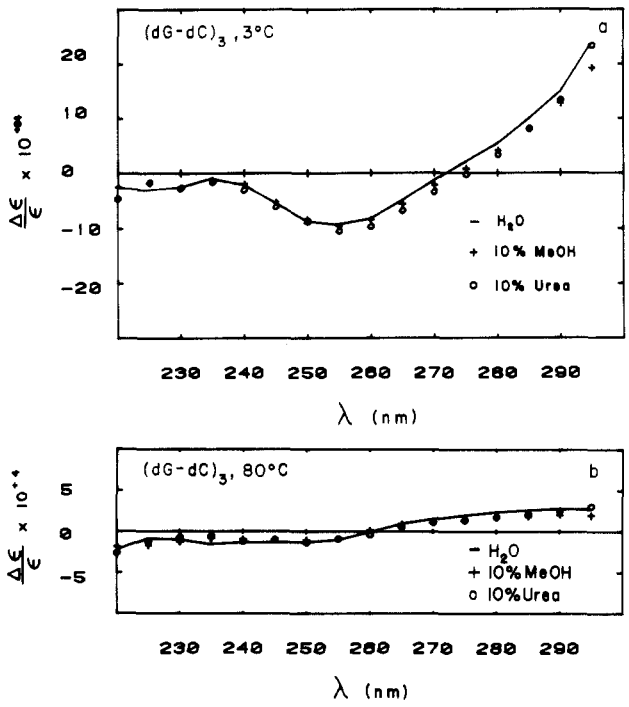

FIGURE 1: Plots of $\Delta\epsilon/\epsilon$ vs. wavelength for $(dG\text{-}dC)_3$ in H_2O (—), 10 mol % MeOH (+), and urea (O) (a) at 3 °C and (b) at 80 °C. Buffer is 1.0 M NaCl and 45 mM cacodylate, pH 7.

dylate buffer in H_2O at pH 7.7 by 100 with 1.0 M NaCl in the appropriate solvent mixture. The final pH was 7.0 ± 0.3 as measured with a Sigma glass calomel electrode.

Spectra. Absorption spectra were measured on a Gilford 250 spectrophotometer. Ultraviolet spectra of $(dG\text{-}dC)_3$ below 250 nm vary significantly with solvent. Normalized UV spectra in H_2O and aqueous mixtures containing 10 mol % methanol, ethanol, propanol, or urea are available in the microfilm edition (see paragraph at end of paper regarding supplementary material).

Circular dichroism (CD) spectra were measured with a Jasco J-40 spectropolarimeter. The Kuhn dissymmetry factor, $\Delta\epsilon/\epsilon$, vs. wavelength is reported. This partially corrects for solvent effects on polymer optical activity by normalizing for changes in extinction due to the solvent. These curves can only be plotted from 220 to 295 nm since the absorbance approaches 0 rapidly above 295 nm which results in large errors in $\Delta\epsilon/\epsilon$. Spectra in 10 mol % formamide and 10 mol % *N*,*N*-dimethylformamide could not be recorded below 275 nm due to significant solvent absorption. The experimental CD curves are available in the microfilm edition.

Thermodynamics. Absorbance vs. temperature curves were measured as described in the preceding paper (Albergo et al., 1981). Sample cells were weighed before and after melting curves to check for loss of solvent. The loss was ~1% and therefore negligible.

No corrections were made for volume expansion of the solvents. Approximate measurements of these effects were made by determining the weight of solvent required to fill a 10-mL volumetric flask at 0, 18, 42, 55, and 72 °C. Corrections for the expansion of the pyrex glass were determined by using the known volume expansion for H_2O (Felsenfeld, 1971). The largest volume change between 0 and 72 °C was 5% for 10 mol % PrOH containing 1 M NaCl. The lowest was 2% observed for H_2O. It is not correct to simply apply these correction terms since the mononucleotides have temperature-dependent extinction coefficients. Thus, for example, the absorbance of a CMP solution at 280 nm is independent of temperature because the temperature-dependent extinction

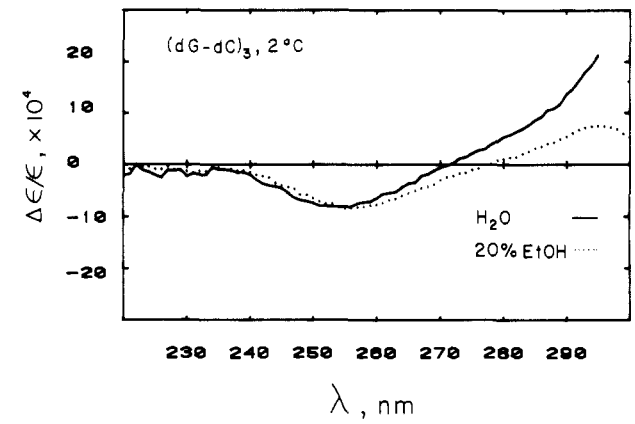

FIGURE 2: Plot of $\Delta\epsilon/\epsilon$ vs. wavelength for $(dG\text{-}dC)_3$ in H_2O (—) and 20 mol % EtOH (···) at 2 °C. Buffer is 1.0 M NaCl and 45 mM cacodylate, pH 7.

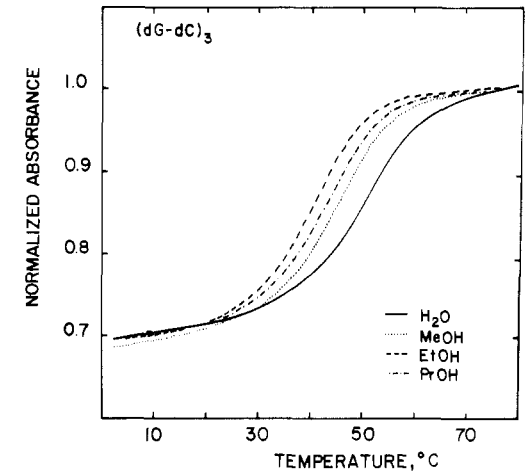

FIGURE 3: Normalized absorbance vs. temperature for $(dG\text{-}dC)_3$. Solvents and strand concentrations are as follows: (—) H_2O, 29.1 μM; (···) 10 mol % MeOH, 49.5 μM; (---) 10 mol % EtOH, 44.6 μM; (-·-) 10 mol % PrOH, 43.4 μM. The measured absorbances are 1.240, 2.080, 1.845, and 1.778/cm, respectively, at 80 °C. All solutions contain 1.0 M NaCl and 45 mM cacodylate, pH 7.

change compensates the volume change (Pörschke, 1976). We have not made any volume corrections to the data since (a) the effect is small and not highly solvent dependent, (b) it is partially compensated at 280 nm by temperature-dependent extinction coefficients, and (c) the helix to coil transition occurs over a narrow temperature region.

Results

It is important to determine if the conformation of $(dG\text{-}dC)_3$ is the same for all the solvent mixtures employed. This is especially true since poly(dG-dC) is known to adopt an unusual conformation under conditions of high salt or ethanol (Pohl & Jovin, 1972; Pohl, 1976; Patel et al., 1979). This conformation is reflected by marked changes in optical activity. Spectra of the Kuhn dissymmetry factor, $\Delta\epsilon/\epsilon$, for $(dG\text{-}dC)_3$ at 3 and 80 °C in the solvents studied are shown in Figures 1 and 2 and in the microfilm edition. Only the low-temperature spectrum observed in 20 mol % ethanol differs significantly from that in water. The spectrum in high ethanol concentration exhibits a diminution of the long-wavelength positive band similar to that observed for DNA in high alcohol concentrations (Girod et al., 1973). Thus 20 mol % ethanol is the only solvent used in this study that induces a conformational change detectable by optical activity.

Typical melting curves for $(dG\text{-}dC)_3$ in mixtures containing 10 mol % of various cosolvents are shown in Figures 3 and 4.

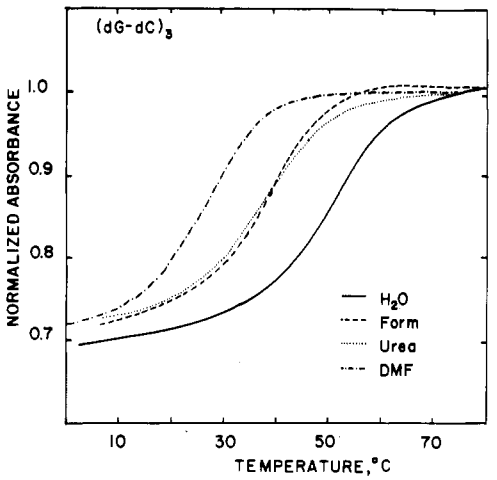

FIGURE 4: Normalized absorbance vs. temperature for (dG-dC)$_3$. Solvents and strand concentrations are as follows: (—) H_2O, 29.1 μM; (···) 10 mol % urea, 47.2 μM; (---) 10 mol % formamide, 48.2 μM; (-·-) 10 mol % DMF, 40.6 μM. The measured absorbances are 1.240, 1.930, 1.615, and 1.871/cm, respectively, at 80 °C. All solutions contain 1.0 M NaCl and 45 mM cacodylate, pH 7.

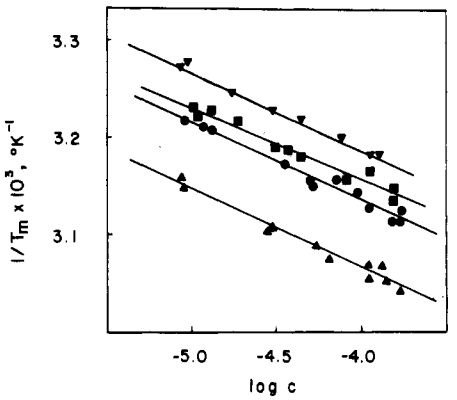

FIGURE 5: Plots of inverse melting temperature vs. log of concentration for (dG-dC)$_3$ in (▲) H_2O, (●) 10 mol % MeOH, (▼) 10 mol % EtOH, and (■) 10 mol % PrOH. All solutions contain 1.0 M NaCl and 45 mM cacodylate, pH 7.

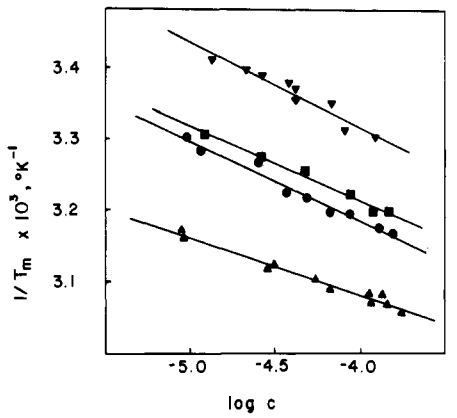

FIGURE 6: Plots of inverse melting temperature vs. log of concentration for (dG-dC)$_3$ in (▲) H_2O, (●) 10 mol % formamide, (■) 10 mol % urea, and (▼) 10 mol % DMF. All solutions contain 1.0 M NaCl and 45 mM cacodylate, pH 7.

All the cosolvents destabilize the helix relative to water. They also alter the shape of the curve at high temperatures but not at low temperatures. This is reflected in the slopes of the low- and high-temperature base lines which are listed in Table I. The thermodynamics derived from melting curve data by three methods described in the preceding paper are listed in Tables II and III (Albergo et al., 1981). Plots used for the analysis

Table I: Base Line Slopes for Melting Curves [L/(mol cm deg)][a]

solvent	mol %	lower base line	upper base line
H_2O	100	53.4	44.9
MeOH	10	46.6	22.8
EtOH	10	40.7	15.0
PrOH	10	32.0	26.6
formamide	10	47.1	25.2
DMF	10	42.3	23.8
urea	10	46.4	20.8
D_2O	100	46.5	58.5
EtOH	20	47.7	20.7

[a] Calculated by using a linear least-squares fit to the base line from the equation $\epsilon_{280}(T \text{ in K}) = \text{slope} \times \text{temperature (K)} + \epsilon_{280}(0 \text{ K})$.

Table II: Enthalpy Changes for Double-Helix Formation by (dG-dC)$_3$ (kcal/mol)

solvent	mol %	derivative of $1/T_{max}$ vs. log c	sloping base line subtraction, $1/T_m$ vs. log c	nonlinear two-state fit	av
H_2O	100	−56.6	−57.4	−56.6	−56.9
D_2O	100	−66.6	−61.8	−59.7	−62.7
MeOH	10	−58.0	−58.5	−58.6	−58.4
EtOH	10	−56.7	−61.1	−57.7	−58.5
PrOH	10	−65.7	−62.6	−57.6	−62.0
formamide	10	−44.4	−46.8	−50.8	−47.3
DMF	10	−38.6	*a*	*a*	−38.6
urea	10	−46.2	−50.3	−52.1	−49.5
EtOH	20	−40.1	−41.9	−48.2	−43.4

[a] Melting temperature too low to define lower base line.

Table III: Entropy Change for Double-Helix Formation by (dG-dC)$_3$ [cal/(mol deg)]

solvent	mol %	derivative of $1/T_{max}$ vs. log c	sloping base line subtraction, $1/T_m$ vs. loc c	nonlinear two-state fit	av
H_2O	100	−154.2	−157.5	−154.9	−155.5
D_2O	100	−184.1	−171.1	−164.1	−173.1
MeOH	10	−161.7	−164.8	−162.1	−162.9
EtOH	10	−161.7	−175.9	−162.0	−166.5
PrOH	10	−187.2	−179.0	−161.4	−175.9
formamide	10	−121.0	−130.1	−140.5	−130.5
DMF	10	−107.5	*a*	*a*	−107.5
urea	10	−127.6	−142.0	−147.0	−138.9
EtOH	20	−109.8	−117.4	−136.2	−121.1

[a] Melting temperature too low to define lower base line.

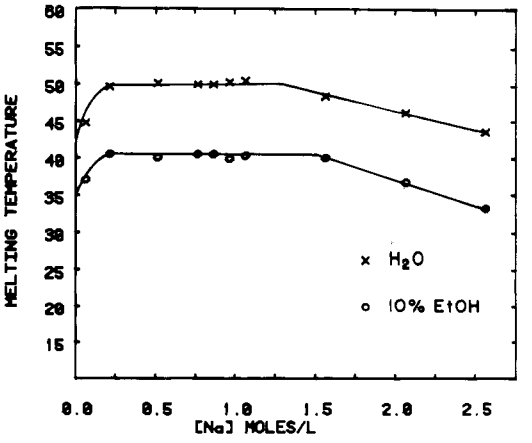

FIGURE 7: Plots of melting temperature vs. NaCl concentration for (dG-dC)$_3$ in H_2O (×) and 10 mol % EtOH (○). All solutions contain 45 mM cacodylate, pH 7.

Table IV: Solvent Parameters at 25 °C

cosolvent	mol %	internal pressure (atm)	cohesive energy density[a] (atm)	surface tension[d] (dyn/cm)	viscosity[d] (cP)	dielectric constant[d]	dipole moment[e,f] (D)
H_2O	100	1694[a]	22684	72.0	0.89	78.5	1.87
MeOH	10	2579[a]	20456	50.0	1.24	71.5	1.70
EtOH	10	3766[b]		36.6	1.90	65.5	1.7
EtOH	20			29.6	2.35	52.3	1.7
PrOH	10			26.4	2.15	59.7	1.68
formamide	10	3625[c]			1.04	87.9	3.37
urea	10				1.15	90.7	4.56
DMF	10					72.0	3.86

[a] MacDonald et al. (1971). [b] Calculated by using $P_i = T(\alpha/\beta)$, $\alpha = 52.0 \times 10^{-5}$ and $\beta = 41.2 \times 10^{-6}$ at 25 °C from Drecker (1883). [c] Extrapolated from Dack et al. (1976). [d] Timmerman (1960). [e] Values are for pure solvents. [f] The calculated dipole moments for G and C are 6.9 and 6.4, respectively (Clementi et al., 1969).

are shown in Figures 5 and 6. Melting curves of 2.5×10^{-5} M oligomer in water and 10 mol % ethanol were also measured as a function of salt concentration. The results shown in Figure 7 indicate melting temperature is essentially constant from 0.20 to beyond 1 M NaCl.

Discussion

This paper presents the first measurements of the enthalpy and entropy of double-helix formation as a function of solvent. For the $(dG\text{-}dC)_3$ model system studied, all the cosolvents destabilize the double helix relative to water. The thermodynamics associated with this effect are considerably different for alcoholic and polar cosolvents, suggesting a difference in mechanism of denaturation. An exception to this is found for 20 mol % ethanol. However, this is also the only solvent that induces a conformational change in the helix, so that direct comparisons are not valid. Thus the following discussion will concentrate on the results for 10 mol % cosolvent.

The destabilization of the oligomer double helix induced by 10 mol % alcohols at 1.0 M NaCl concentration results from a more unfavorable ΔS for helix formation. The data in Figure 7 show the melting temperature in 10 mol % ethanol is essentially independent of NaCl concentration from 0.20 to 1.5 M, suggesting salt effects are not responsible. The entropy change is consistent with the perturbation expected for classical hydrophobic bonding (Kauzmann, 1959; Tanford, 1973). However, the effect is not large. The entropy term for 1-propanol is the only parameter to vary by >10% from that measured in water. For comparison, in the denaturation of lysozyme at pH 2, the ΔS term changes >50% in going from water to 10 mol % ethanol at 40 °C (Velicelebi & Sturtevant, 1979). Thus, there is no evidence for substantial hydrophobic bonding in the $(dG\text{-}dC)_3$ double helix.

The enthalpy change measured for helix formation in 10 mol % alcohol cosolvents is slightly more favorable than in water. The difference is small, and potential systematic errors discussed below may change the sign but will not greatly alter the magnitude of this difference. One immediate conclusion is that the destabilization in these solvents cannot be explained by a decrease in "solvophobic bonding". This effect is expected to provide a substantially less favorable enthalpy term as surface tension decreases (Sinanoglu & Abdulnur, 1964, 1965; Abdulnur, 1966; Sinanoglu, 1968). This is because the decrease in aqueous surface tension due to perturbation by alcohols is roughly paralleled by a decrease in surface enthalpy. While exact surface enthalpies are not available for the solvent mixtures used in this study, surface tensions have been measured. As shown in Table IV, the surface tension of the solvents employed decreases by over a factor of 2, whereas the measured enthalpy of helix formation becomes slightly more favorable. It is reasonable to compare macroscopic surface tensions in calculating the energy required to form solvent cavities for nucleic acids because the curvature dependence of surface tension is negligible (Buff, 1971, 1955; Kirkwood & Buff, 1949; Tolman, 1949). However, other parameters such as internal pressure, cohesive energy density, or isothermal compressibility may be better measures of cavity energy (Dack, 1975; Oakenfull & Fenwick, 1974; Hildebrand et al., 1970). Unfortunately, these are only available for a limited number of mixed solvents. The most extensive data provide internal pressures for aqueous methanol and 1-propanol solutions (Macdonald et al., 1970; Macdonald & Hyne, 1971). These are approximately 2000, 3000, and 4000 atm at 30 °C for water, 10 mol % methanol, and 10 mol % 1-propanol, respectively. The large magnitudes of these changes seem to preclude correlation with the small effects observed in $(dG\text{-}dC)_3$ melting. Thus, these results suggest solvophobic bonding is not an important contributor to helix stability. Recent results on stacking dynamics in mixed solvents and observations that DNA is double stranded in solvent systems containing only 5 vol % water support this interpretation (Dewey et al., 1978, 1979; Dewey & Turner, 1980; Kay, 1976; Girod et al., 1973).

A more quantitative interpretation of the effects observed in alcohol solutions is not possible at this time due to the lack of effective theories describing mixed solvents (Dack, 1975; Franks & Ives, 1966). Qualitatively, however, it is clear that alcohols in the concentrations used here induce substantial solvent structural changes. Table IV lists some parameters reflecting this. In contrast, the thermodynamics of helix formation are not very sensitive to addition of alcohol. Future theories of solvent effects on nucleic acids must take this into account.

Formamide, urea, and *N*,*N*-dimethylformamide destabilize the helix by affecting the enthalpy change for the transition (see Tables II and III). The entropy change becomes more favorable with these cosolvents that are more polar than water. Thus these cosolvents compete with the interaction providing the enthalpy that drives double-helix formation. Quantum mechanical calculations indicate these are dipolar interactions between the bases (Pullman, 1965; Pullman & Pullman, 1968, 1969). The enthalpy and entropy changes induced by addition of small molecules with large dipole moments are consistent with this idea (see Table IV for dipole moments). These polar cosolvents may also compete with hydrogen bonding between the bases. However, *N*,*N*-dimethylformamide has a more pronounced destabilizing effect than formamide, even though it possesses no hydrogen bond donor sites. Polar cosolvents are also known to disrupt single-strand stacking in poly(adenylic acid) where hydrogen bonding presumably is not important (Dewey & Turner, 1980). Thus, the effects of polar

cosolvents seem to be more dependent on dipole moment than on hydrogen bonding ability.

In considering the thermodynamics reported here, it is important to be aware of potential systematic errors. The largest error probably arises from the assumption used in the analysis that the single-strand to double-helix transition is two state. As discussed in the preceding paper, the (dG-dC)$_3$ double helix may not be a single conformation, and the single strand can exist in conformations ranging from totally stacked to random coil (Albergo et al., 1981). Thus the initial and final states of the transition may vary with temperature and solvent. The potential effects of these changes need to be examined.

The stacking equilibrium in single-strand poly(adenylic acid) and poly(cytidylic acid) is known to be solvent dependent (Dewey & Turner, 1980; Freier et al., 1981). The transition temperature is lowered by addition of cosolvents, and a similar effect is expected for single-strand (dG-dC)$_3$. A qualitative indication of this is the change in the high-temperature slopes of the absorbance profiles in Figures 3 and 4, since residual single-strand stacking is reflected in continued absorbance changes after the cooperative transition. The average slopes of the linear high-temperature portions of the curves are listed in Table I as a function of solvent. All the cosolvents decrease this slope, indicating the final state contains less single-strand stacking than in water. To correct for this before comparing water with a given solvent would require decreasing the ΔH of the water value by the enthalpy associated with the extra single-strand stacking. An upper limit for this correction can be obtained by comparing the calorimetric and spectrophotometric values for ΔH in water reported in the preceding paper. The calorimetric value of −59.6 kcal/mol helix refers to the essentially unstacked final state at 95 °C while the spectroscopic final state includes residual stacking. Thus the upper limit for the correction is −59.6 − (−56.9) = −2.7 kcal/mol of helix. The slopes in Table I suggest the actual correction will be less than this. The small value of the correction is not surprising in view of the broad, uncooperative nature of single-strand melting. The effect of a typical correction term on the data in Table II is to increase the changes induced by polar solvents and to decrease that induced by alcohols (perhaps changing the sign of the difference in ΔH). The overall picture and interpretation are not altered.

The nature of the various conformations available to the double strand is not as well understood as single-strand stacking. Possible intramolecular rearrangements include "fraying" of terminal bases and chain sliding to a species with less than six base pairs. The good agreement between calorimetric and spectrophotometric values of ΔH in H_2O and D_2O indicates these effects do not greatly affect values obtained for this parameter. The low-temperature slopes of the absorbance profiles listed in Table I may reflect these reactions. The slopes are very similar suggesting solvent composition has little effect on the nature of the helix. The CD spectra at 3 °C are consistent with this. Thus, it does not seem that intramolecular reactions of the helix present a major source of error.

The thermodynamic parameters measured in 20 mol % ethanol cannot be directly compared with the above results because the CD spectrum indicates a different helix conformation in this solvent. The change in CD is similar to that observed for DNA going from B to C form (Tunis-Schneider & Maestre, 1970). This has been attributed to dehydration of the helix (Girod et al., 1973; Wolf & Hanlon, 1975). The thermodynamics in this case indicate a less unfavorable entropy term for helix formation. This is consistent with the formation of a structure that binds fewer water molecules. The less favorable enthalpy term would then arise from the loss of these oligomer–water interactions, coupled with the revised base stacking reflected in the CD spectrum. Interestingly, the CD spectra of the hexamer never approach that observed for poly(dG-dC) in high salt (see microfilm edition for $\Delta\epsilon/\epsilon$ spectra as function of NaCl concentration in H_2O and 10 mol % EtOH) (Pohl & Jovin, 1972; Patel et al., 1979). This suggests it is too short to form this unusual structure, which may be a left-handed helix (Wang et al., 1979). The salt concentration required to induce the conformation in solution is known to be chain length dependent (Pohl & Jovin, 1972).

The purpose of this research is to increase our understanding of the forces controlling nucleic acid stability. It may also help rationalize protein–nucleic acid interactions and the chemistry of nucleic acids in environments that are not truly aqueous such as found in a ribosome or membrane. The major experimental results are that alcohol cosolvents produce rather minor, mostly entropic effects on the thermodynamics of helix formation whereas polar cosolvents substantially decrease the absolute magnitude of both ΔH and ΔS. These data can be used to test present and future theories of helix stabilization. A working hypothesis consistent with the results is that most of the solvent contribution to helical stability comes from tightly bound water rather than from bulk solvent effects. There are many studies demonstrating the existence of such water (Bloomfield et al., 1974; Tunis & Hearst, 1968; Texter, 1978).

In this view the destabilization observed with 10 mol % alcohols may be due to hydrophobic bonding. However, a much larger effect is observed at 20 mol % EtOH where dehydration may be occurring. Thus, it appears changes in the bulk solvent have minor effects on the helix until it begins to be dehydrated. This is also consistent with the effects of D_2O on stability and with recent calculations of solvent-accessible surfaces of nucleic acids (Albergo et al., 1981; Alden & Kim, 1979). The large effects observed with polar cosolvents can then be attributed to a competition for base–base dipolar interactions. Work in progress will test these ideas further.

Acknowledgments

We thank Dr. F. P. Buff for several stimulating discussions.

Supplementary Material Available

Two figures showing the normalized UV spectra at 3 and 80 °C for (dG-dC)$_3$ in H_2O and 10 mol % solutions of MeOH, EtOH, PrOH, and urea, one figure showing the CD spectra at 3 and 80 °C for (dG-dC)$_3$ in H_2O and 10 mol % solutions of MeOH, EtOH, PrOH, and urea, and five figures showing $\Delta\epsilon/\epsilon$ plots for (dG-dC)$_3$ in various solvents (8 pages). Ordering information is given on any current masthead page.

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