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Solvent Effects on the Stability of A_7U_7p†

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ABSTRACT: The thermodynamics of double-helix formation were measured spectrophotometrically for A_7U_7 in water at 1 M NaCl and for A_7U_7p in a variety of solvent mixtures and salt. Comparison of the A_7U_7 results with calorimetric measurements indicates duplex formation involves intermediate states. For A_7U_7p between 0.06 and 0.55 M Na^+, $dT_m/d(\log [Na^+]) = 17.4$ °C, similar to the value of 19.6 °C for poly(A)·poly(U) [Krakauer, H., & Sturtevant, J. M. (1968) *Biopolymers 6*, 491-512]. At 1 M NaCl, the A_7U_7p duplex is most stable in 100% water. For 10 mol % solutions, the order for A_7U_7p duplex stability is ethylene glycol > glycerol > ethanol > 2-propanol > dimethyl sulfoxide > 1-propanol > formamide > *N*,*N*-dimethylformamide > urea > dioxane. Comparison of changes in stability and thermodynamic parameters with literature results for proteins suggests proteins and A_7U_7p interact differently with solvent. The results suggest hydrophobic bonding is not a major contributor to the stability of the A_7U_7p duplex. Comparisons with bulk solvent surface tension suggest the energy of cavity formation is also not a major contributor to duplex stability.

Solvent is thought to make important contributions to the stabilities of nucleic acids (Cantor & Schimmel, 1980; Bloomfield et al., 1974). It has been suggested that either classical hydrophobic bonding (Kauzmann, 1959; Tanford, 1973) or the energies of solvent cavities (Sinanoglu & Abdulnur, 1964, 1965; Sinanoglu, 1968, 1980, 1982) drive formation of double helices. The effects of solvent and the environment on stabilities of nucleic acids have implications for predicting the structures and properties of nucleic acids in both aqueous and partially aqueous environments. The latter are of increasing importance. For example, many RNA–protein complexes are being discovered (Kole et al., 1980; Stark et al., 1978; Lerner & Steitz, 1981; Walter & Blobel, 1982, 1983); a powerful new method for detecting sequence changes in DNA depends on denaturation by cosolvents (Lerman et al., 1984; Fischer & Lerman, 1983); many hybridization experiments are conducted on solid-phase supports. Despite the importance of understanding environmental effects on nucleic acids, there is relatively little experimental data available (Levine et al., 1963; Lowe & Schellman, 1972; Herskovits & Harrington, 1972; Herskovits & Bowen, 1974; Breslauer et al., 1978; Dewey & Turner, 1980; Freier et al., 1981; Albergo & Turner, 1981). This paper reports the thermodynamics of duplex formation by A_7U_7p in water and aqueous cosolvent mixtures. The results in water have implications for deriving thermodynamic parameters useful in predicting RNA structure (Tinoco et al., 1971, 1973; Nussinov et al., 1982; Nussinov & Tinoco, 1981; Pipas & McMahan, 1975; Salser, 1977; Zuker & Stiegler, 1981; Auron et al., 1982; Borer et al., 1974; Gralla & Crothers, 1973). The results from solvent perturbations suggest hydrophobic and solvent cavity effects are relatively unimportant in determining nucleic acid stability.

Materials and Methods

Synthesis of A_7U_7p. A_7U_7p was synthesized in three steps. All reactions were monitored by high-performance liquid chromatography (HPLC)[1] as outlined by Petersheim & Turner (1983).

Poly(U) (Sigma) at 15 mg/mL was dialyzed 4 times against 10 mM NaCl and 10 mM Tris, pH 7.5, for 12 h each. The first dialysis solution also contained 10 mM EDTA. The poly(U) was hydrolyzed with 0.9 M KOH (Borer, 1972; Martin et al., 1971) at 0 °C for 4 h and neutralized with concentrated $HClO_4$, producing $KClO_4$ precipitate. The supernatant and washings were combined, the pH was lowered to 3 with 1 M HCl, and the solution was incubated for 3 h at 37 °C to break cyclic phosphates. The solution was brought to pH 7, and the products were isolated. $p(Up)_7$ was prepared by incubating $(Up)_7$ with 15 units/mL T4 polynucleotide kinase for 6 h at 37 °C (Uhlenbeck & Cameron, 1977).

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; CD, circular dichroism; DEAE, diethylaminoethyl; DMF, *N*,*N*-dimethylformamide; Me_2SO, dimethyl sulfoxide; DSC, differential scanning calorimetry; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; Form, formamide; HPLC, high-performance liquid chromatography; poly(U), poly(uridylic acid); 1-PrOH, 1-propanol; 2-PrOH, 2-propanol; TEAB, triethylammonium bicarbonate; TEACl, tetraethylammonium chloride; TMACl, tetramethylammonium chloride; Tris, tris(hydroxymethyl)aminomethane; UV, ultraviolet.

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A_7 (Boehringer Mannheim) was checked for purity by HPLC and for identity by ^1H NMR in D_2O. A_7 was coupled to $p(Up)_7$ by using T_4 RNA ligase (Uhlenbeck & Cameron, 1977). Reaction conditions were 1.6 mM A_7, 0.8 mM $p(Up)_7$, 225 units/mL T4 RNA ligase (P-L Biochemicals), 4 mM ATP, 16 mM $MgCl_2$, 10 μg/mL BSA, 8 mM dithiothreitol, and 160 mM Tris, pH 8.6. The mixture was incubated at 4 °C for 16 h, at room temperature for 0.5 h, and at 37 °C for 5–24 h.

Purification. $(Up)_7$, $p(Up)_7$, and A_7U_7p were purified by anion-exchange chromatography with DEAE-Sephadex (Petersheim & Turner, 1983; Petersheim, 1982). Products of the poly(U) alkaline hydrolysis were isolated with a TEAB gradient. Kinase and ligase reaction products were isolated with NaCl/7 M urea gradients with 10 mM Tris, pH 8.2. Pooled fractions were desalted by elution from a second DEAE-Sephadex column with 1.5 M TEAB. Water and TEAB were removed from all products by rotary evaporation under reduced pressure with methanol as a final wash.

Solutions. All solvents were the highest purity commercially available. The absorbance of DMF was 0.278/cm at 275 nm. Formamide was recrystallized 3 times (Casey & Davidson, 1977; Robberson et al., 1971) and had an absorbance of 0.073/cm at 270 nm. Oligomer solutions were 50 mM sodium cacodylate and 1 mM EDTA, pH 7.0 ± 0.3, as measured by a Sigma glass calomel electrode. Solutions also contained 1 M NaCl unless otherwise stated.

Extinction Coefficients. An extinction coefficient (ε) of 1.48×10^5 L (mol of strand)$^{-1}$ cm^{-1} at 70 °C was used for A_7U_7 and A_7U_7p. This was derived by assuming $\epsilon = 10.3 \times 10^3$ L (mol of base pair)$^{-1}$ cm^{-1} at 50 °C for A_nU_n oligomers (Borer, 1972) and extrapolating to 70 °C by using experimental data for A_7U_7p at low concentrations. Measurements on solutions containing 8.9×10^{-6} M A_7U_7p in 10 mol % ethanol, formamide, DMF, urea, and glycerol indicate solvent effects on ε are less than 10%, so no corrections were made for solvent effects on extinction coefficients.

Spectra. UV spectra were measured on a Gilford 250 spectrophotometer and CD spectra on a Jasco J-40 spectropolarimeter. The bandwidth was 2 mm for UV and CD spectra.

Melting Curves. Absorbance at 260 nm vs. temperature curves were measured on a Gilford 250 spectrophotometer. Temperature was controlled with a Gilford 2527 thermoprogrammer. It was raised 30 °C/h except for glycerol and sucrose solutions which were heated 15 °C/h. The spectrophotometer and thermal programmer were interfaced to a PDP 11/34 computer for data collection and analysis.

Cosolvent solutions were degassed by standing at room temperature for several days before being mixed with A_7U_7p. This minimized the loss of volatile cosolvents. Absorbances of all cells were measured at 0 °C before and after melting experiments to check for degradation. Experiments with absorbance changes greater than 4% were not analyzed. No corrections were made for volume expansion (Albergo & Turner, 1981).

Analysis of Melting Curves. Melting curves were analyzed as previously described (Petersheim & Turner, 1983). In brief, the extinction coefficient as a function of temperature, $\epsilon(T)$, is fit to the equation for a two-state transition:

$$\epsilon(T) = \frac{A(T)}{lc_T} = \alpha_{ds}\epsilon_{ds} + (1 - \alpha_{ds})\epsilon_{ss} \quad (1)$$

Here, $A(T)$ is the absorbance of the solution at temperature T, l is the path length, c_T is the total strand concentration, and

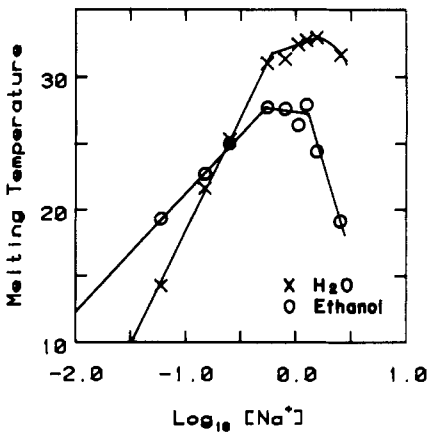

FIGURE 1: T_m vs. log $[Na^+]$ for 3.9 μM A_7U_7p in H_2O (×) and for 4.9 μM A_7U_7p in 10 mol % ethanol (○). Buffer is 1 M NaCl, 50 mM sodium cacodylate, and 1 mM EDTA, pH 7.

ϵ_{ds} and ϵ_{ss} are extinction coefficients for the double- and single-stranded species, respectively, and are assumed to be linear functions of temperature:

$$\epsilon_{ds} = m_{ds}T + b_{ds} \quad (2)$$

$$\epsilon_{ss} = m_{ss}T + b_{ss} \quad (3)$$

α_{ds} is the fraction of strands in the double-stranded state and is related to the enthalpy, $\Delta H°$, and entropy, $\Delta S°$, changes for the reaction by

$$K = \frac{\alpha_{ds}}{2(1 - \alpha_{ds})^2 c_T} = \exp\left(\frac{-\Delta H°}{RT} + \frac{\Delta S°}{R}\right) \quad (4)$$

The parameters $\Delta H°$, $\Delta S°$, m_{ds}, b_{ds}, m_{ss}, and b_{ss} are fit by using a nonlinear least-squares algorithm. In practice, eq 1 did not give good fits to the melting curves. However, if curves were truncated about 30 °C above the temperature, T_m, corresponding to $\alpha = 0.5$, then good fits were obtained (supplementary material; see paragraph at end of paper regarding supplementary material). This indicates eq 3 and the two-state model are reasonable approximations over a limited temperature range.

When T_m is less than 20 °C, m_{ds} is not well determined. In such cases, m_{ds} was set equal to the average found for melting curves in the same solvent with $T_m \geq 20$ °C. For cosolvents where $T_m < 20$ °C, and only one experiment was performed, m_{ds} was set equal to the average measured in water.

The $\Delta H°$ and $\Delta S°$ values determined from fitting melting curves are used to calculate the T_m of the sample. A second measure of the thermodynamic parameters is then obtained by plotting T_m^{-1} vs. log c_T since (Borer et al., 1974; Martin et al., 1971)

$$\frac{1}{T_m} = \frac{2.3R}{\Delta H°} \log c_T + \frac{\Delta S°}{\Delta H°} \quad (5)$$

Results

Figure 1 contains plots of T_m vs. log $[Na^+]$ for A_7U_7p in H_2O and 10 mol % ethanol. In H_2O and 10% ethanol, respectively, $dT_m/d(\log [Na^+])$ is 17.4 and 8.8 °C between 0.06 and 0.55 M Na^+ and 2.9 and −1 °C between 0.55 and 1.3 M Na^+, suggesting electrostatic effects are largely saturated by 1 M Na^+. Thus, solvent studies were conducted at 1 M NaCl to minimize complications due to electrostatics. This also permits comparisons with previous studies of A_7U_7 in H_2O at 1 M NaCl (Martin et al., 1971; Breslauer et al., 1975).

Representative melting curves at 1 M NaCl of 1.9×10^{-5} M A_7U_7p in water and various cosolvents at 10 mol % and in 1 M sucrose are shown in Figure 2 and the supplementary

Table I: Fitted Thermodynamic Parameters for A_7U_7p Duplex Formation in Water and 10 mol % Cosolvent Mixtures[a]

salt	cosolvent	cosolvent concn (M)	dielectric constant, ϵ^b	surface tension, σ^b (dyn/cm)	$-\Delta H°$ (kcal/mol of duplex)	$-\Delta S°$ [cal K^{-1} (mol of duplex)$^{-1}$]	T_m (°C)
1 M NaCl	H_2O		78.5	72.0	65.5	189.9	36.8
	sucrose	1.0	70.2	76.1[e]	63.1	185.4	31.7
	ethylene glycol	4.6	70.5	60.0[f]	66.4	197.8	29.1
	glycerol	4.3	69.8	68.1	63.3	187.8	28.9
	ethanol	4.6	65.5	36.6	77.0	233.7	28.5
	2-propanol	4.3	59.1	27.6[g]	72.0	217.2	28.4
	dimethyl sulfoxide	4.4	77.0[j]	60.9[l]	73.4	222.1	27.9
	1-propanol	4.3	59.7	26.4	71.7	216.5	27.7
	formamide	5.0	87.9[c]		56.1	168.3	22.0
	N,N-dimethylformamide	4.3	71.7[d]	53.1[h]	72.0	224.3	19.9
	urea	4.8	90.7	71.3[k]	51.0	153.1	19.0
	dioxane	4.1	48.7	45.3[i]	60.8	187.0	18.1
1 M TMACl	H_2O				61.6	181.4	30.1
	ethanol	4.6			71.2	220.0	21.5
1 M TEACl	H_2O				55.4	170.2	15.6
	ethanol	4.6			60.8	195.7	6.7

[a] Parameters derived from fitting single melts of A_7U_7p at 18.7 μM. Buffer was 50 mM sodium cacodylate and 1 mM disodium ethylenediaminetetraacetic acid, pH 7. Errors in $\Delta H°$ and $\Delta S°$ are roughly ±5% as judged by comparison with more extensive data for H_2O, ethanol, glycerol, urea, formamide, and *N,N*-dimethylformamide. [b] Values for the dielectric constant and surface tension at 25 °C are from Timmermans (1960), except where noted. [c] Rohdewald & Möldner (1973). [d] Average of values from Douheret & Morenas (1967) and Reynaud (1968). [e] At 21 °C from Landt (1931). [f] Monick (1968). [g] At 23 °C from Hirata et al. (1958). [h] Mellan (1977). [i] At 26 °C from Weast (1983). [j] Lindberg & Kenttamaa (1960). [k] Jäger et al. (1965). [l] At 30 °C from Murakami & Yamada (1962).

Table II: Thermodynamic Parameters for A_7U_7p Duplex Formation in Water and 10 mol % Solvent Mixtures[a]

cosolvent parameter	units	H_2O	urea	formamide	DMF	glycerol	ethanol	ethanol/ TMACl	H_2O/ TMACl
$-\Delta H°$, log c_T	kcal (mol of duplex)$^{-1}$	77.9	61.8	72.8	83.8	84.3	88.4	86.1	80.0
$-\Delta H°$, fits	kcal (mol of duplex)$^{-1}$	62.8	54.4	58.0	71.7	64.4	76.7	70.2	60.1
($\Delta H°$, fits)/($\Delta H°$, log c_T)		0.81	0.88	0.80	0.86	0.76	0.87	0.82	0.75
$-\Delta S°$, log c_T	cal K^{-1} (mol of duplex)$^{-1}$	229.7	190.7	225.1	264.2	257.7	271.1	269.8	241.8
$-\Delta S°$, fits	cal K^{-1} (mol of duplex)$^{-1}$	180.9	165.1	174.7	223.0	191.4	232.1	215.5	176.0
($\Delta S°$, fits)/($\Delta S°$, log c_T)		0.79	0.87	0.78	0.84	0.74	0.86	0.80	0.73
$-\Delta C_p°$[b]	cal K^{-1} (mol of duplex)$^{-1}$	662	446	315	311	523	342	1523	1577
lower base-line slope	L mol^{-1} cm^{-1} K^{-1}	131	221	184	231	157	181	200	164
upper base-line slope	L mol^{-1} cm^{-1} K^{-1}	163	118	129	60	78	113	160	204

[a] Buffer is 50 mM sodium cacodylate, 1 mM EDTA, and 1 M NaCl, pH 7, except for TMACl in which 1 M tetramethylammonium chloride is substituted for 1 M NaCl. [b] Determined from the average slopes of $\Delta H°$ vs. T_m and $\Delta S°$ vs. ln T_m plots.

material. Melting curves at 1 M tetramethylammonium chloride or tetraethylammonium chloride were also measured in water and 10 mol % ethanol. At 1 M cation, all cosolvents destabilize the helix relative to water. Thermodynamic parameters derived from fits of these melts are listed in Table I. Melting curves of 1.9×10^{-5} M A_7U_7p were also measured as a function of cosolvent concentration for ethanol, formamide, urea, and DMF. The dependence of T_m and fitted $\Delta H°$ and $\Delta S°$ on the mole fraction of cosolvent is shown in Figure 3. While T_m varies almost linearly with cosolvent concentration, $\Delta H°$ and $\Delta S°$ change little above 10 mol %. The dependence on urea concentration up to 10 mol % is similar to that measured for DNA using calorimetry (Klump & Burkart, 1977).

Melting curves at 1 M NaCl as a function of oligomer concentration were measured for A_7U_7 in water and for A_7U_7p in water and 10 mol % ethanol, urea, formamide, DMF, and glycerol. Measurements were also made at 1 M tetramethylammonium chloride in water and 10 mol % ethanol. Plots of T_m^{-1} vs. log c_T are shown in Figure 4. Thermodynamic parameters derived from these plots and from fitted parameters are listed in Tables II and III. The fitted parameters were a function of T_m, as shown in the supplementary material. Heat capacities derived from this temperature dependence are listed in Table II. The error in these heat capacities is roughly ±30% due to scatter in the data.

To determine if the conformation of A_7U_7p at 1 M NaCl is the same in all the solvents listed in Table II and in 10 mol % 1-propanol, CD and absorption spectra were measured at 0 and 70 °C for the highest concentration used in each set of melting curves. Typical CD spectra are shown in Figure 5. Absorption spectra and spectra of the Kuhn dissymetry factor, $\Delta\epsilon/\epsilon$, are contained in the supplementary material. Corresponding spectra in different solvents are similar, indicating the conformation of A_7U_7p is the same. The CD spectrum of 1.1×10^{-4} M A_7U_7p in water was also measured at 10 °C intervals between 0 and 70 °C. A CD melting curve derived

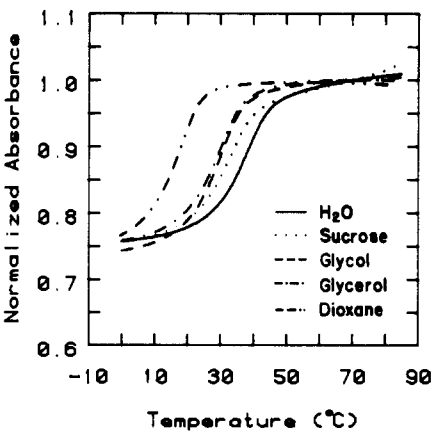

FIGURE 2: Absorbance normalized at 70 °C vs. temperature for 19 μM A_7U_7p in 1 M NaCl, 50 mM sodium cacodylate, and 1 mM EDTA, pH 7 in H_2O (—), 1 M sucrose (···), 10 mol % ethylene glycol (– –), 10 mol % glycerol (–·–), and 10 mol % dioxane (–··–).

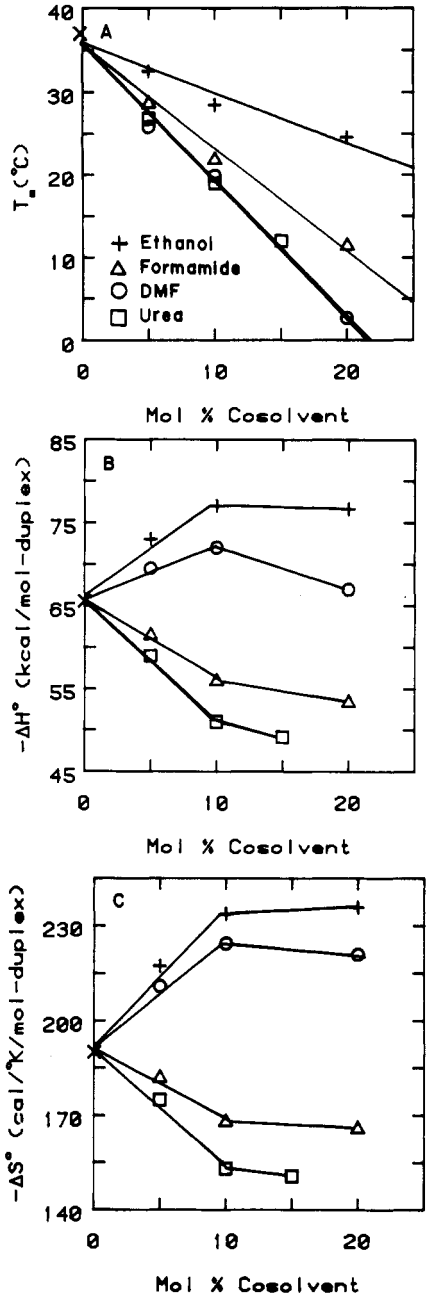

FIGURE 3: Thermodynamic parameters for 19 μM A_7U_7p vs. mole percent cosolvent for ethanol (+), formamide (△), DMF (○), and urea (□). Buffer is 1 M NaCl, 50 mM sodium cacodylate, and 1 mM EDTA, pH 7.

from these data is consistent with the thermodynamics reported in Table II and shows no signs of additional equilibria such as aggregation (Freier et al., 1983).

Discussion

The thermodynamic parameters reported here for the melting of A_7U_7 and A_7U_7p in water can be compared with literature values for poly(A)·poly(U) and A_7U_7. The recent explosion in the use of thermodynamic parameters for prediction of RNA structure (Tinoco et al., 1971, 1973; Gralla & Crothers, 1973; Borer et al., 1974; Pipas & McMahan, 1975; Salser, 1977; Zuker & Stiegler, 1981; Nussinov & Tinoco, 1981; Nussinov et al., 1982; Auron et al., 1982) makes a critical evaluation of such parameters particularly important (Freier et al., 1984; Albergo et al., 1981).

Table IV lists thermodynamic parameters measured calorimetrically for melting of poly(A)·poly(U) (Rawitscher et al., 1963; Ross & Scruggs, 1965; Krakauer & Sturtevant, 1968; Neumann & Ackermann, 1969; Suurkuusk et al., 1977; Filimonov & Privalov, 1978). For comparison with A_7U_7 and A_7U_7p, the parameters have been extrapolated to 36 °C, the average T_m of the experiments reported in Table III. The average calorimetric $\Delta H°$ at 36 °C is −6.6 kcal/mol of A·U. For A_7U_7 and A_7U_7p, the average $\Delta H°$ is −4.8 kcal/mol of stack for fitted parameters and −5.9 kcal/mol of stack for T_m^{-1} vs. log c_T parameters. The calorimetric $\Delta H°$ is greater than either van't Hoff $\Delta H°$, suggesting A_7U_7 melting is not a two-state transition (Tsong et al., 1970). The difference be-

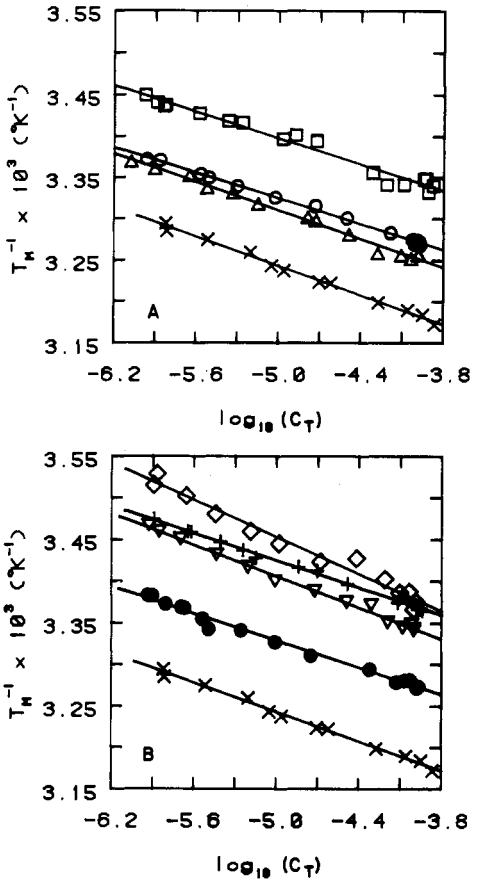

FIGURE 4: T_m^{-1} vs. log c_T for A_7U_7p in 1 M NaCl (or TMACl where indicated), 50 mM sodium cacodylate, and 1 mM EDTA, pH 7, in the following solvents: (A) H_2O (×), H_2O/1 M TMACl (△), 10 mol % ethanol (○), and 10 mol % ethanol/1 M TMACl (□); (B) H_2O (×), 10 mol % glycerol (●), formamide (▽), DMF (+), and urea (◇).

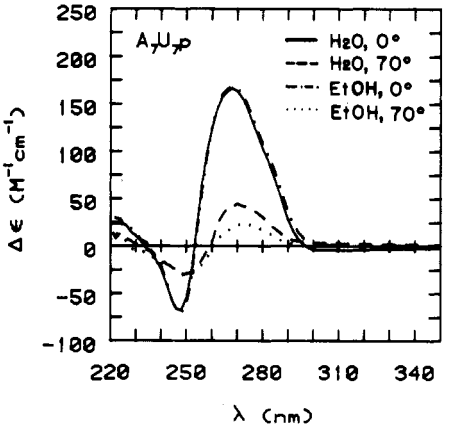

FIGURE 5: Circular dichroism spectra for 0.11 mM A_7U_7p in H_2O at 0 (—) and 70 °C (– –) and in 10 mol % ethanol at 0 (–·–) and 70 °C (···). Buffer is 1 M NaCl, 50 mM sodium cacodylate, and 1 mM EDTA, pH 7.

Table III: Thermodynamic Parameters for A_7U_7 and A_7U_7p in Water with 1 M NaCl

		this work		lit. values for A_7U_7		
					Breslauer et al. (1975)	
parameter	units	A_7U_7p	A_7U_7	Martin et al. (1971)	optical	calorimetric
$-\Delta H°$, log c_T	kcal (mol of duplex)$^{-1}$	77.9	75.7	107	78.3[a]	99.3[c]
$-\Delta S°$, log c_T	cal K^{-1} (mol of duplex)$^{-1}$	229.7	222.5	323.8[b]	232.4[a,b]	296.3[c,b]
$-\Delta G°$ at 25 °C, log c_T	kcal (mol of duplex)$^{-1}$	9.2	9.3	10.5	9.0[a]	12.4[c]
T_m, 4.38×10^{-4} M	°C		44.9[g]	42.3[g]	42.9	45.5
T_m, 1×10^{-5} M	°C	35.2	35.4[g]	35.5[g]	33.6	38.0[c]
$-\Delta H°$, fits	kcal (mol of duplex)$^{-1}$	62.8	61.6	72[d]	57.4,[e] 70.3[e]	73.2[f]
$-\Delta S°$, fits	cal K^{-1} (mol of duplex)$^{-1}$	180.9	176.8		204.2[e,b]	214.4[f,b]
$-\Delta G°$ at 25 °C, fits	kcal (mol of duplex)$^{-1}$		8.8		9.4[e]	9.3[f]

[a] From temperature-jump amplitudes. [b] When $\Delta S°$ was not reported, it was calculated from $\Delta H°$, T_m, and c_T. [c] From calorimetry using integrated heat capacity for $\Delta H°$. [d] From slope of melting curve at T_m. [e] From slope of melting curve at T_m of 46.1 °C using flat (57.4) and sloping (70.3) lower base lines. From plots of $\Delta H°$ vs. T_m, the fitted $\Delta H°$ predicted for A_7U_7 from this work at 46.1 °C is −69.4 kcal (mol of duplex)$^{-1}$ when sloping base lines are used. $\Delta C_p°$ for A_7U_7 for this work is −811 cal K^{-1} (mol of duplex)$^{-1}$. [f] From slope of enthalpy vs. temperature curve measured calorimetrically. [g] From $\Delta H°$ and $\Delta S°$ derived from log c_T plots.

Table IV: Calorimetric Values of Thermodynamic Parameters for Poly(A)·Poly(U) Duplex Formation

source	T[a] (°C)	salt	$-\Delta H°$ at T [kcal (mol of bp)$^{-1}$]	$-\Delta C_p°$ [cal K^{-1} (mol of bp)$^{-1}$]	$-\Delta H°$ at 36 °C [kcal (mol of bp)$^{-1}$]	$-\Delta S°$ at T [cal K^{-1} (mol of bp)$^{-1}$]	$-\Delta S°$ at 36 °C [cal K^{-1} (mol of bp)$^{-1}$]
			Mixing Experiments				
Rawitscher et al. (1965)	40	0.1 M KCl	6.74	0.206 (T + 273) −11.09	6.6	23.0[b]	19
Ross & Scruggs (1965)	37	0.5 M NaCl	6.69	110.4[c]	6.6		
			DSC Experiments				
Krakauer & Sturtevant (1968)	44.5	0.018 M NaCl	7.38	60[d]	6.9	23.2	22
Neumann & Ackermann (1969)	54.1	0.5 M NaCl	8.1	71[d]	6.8	24.8	21
Suurkuusk et al. (1977)	42.2	0.016 M NaCl	6.8	80[e]	6.3	21.6	20
Filimonov & Privalov (1978)	57.9	0.1 M NaCl	8.56	86[d]	6.7	25.9	20

[a] For mixing experiments, T is the temperature of the experiment. For DSC experiments, T is the T_m for the experiment. [b] At 58 °C. [c] Calculated from ΔH vs. T by using data at 0.5 M NaCl. [d] Calculated from plot of $\Delta H°$ vs. T_m shown in Filimonov & Privalov (1978). [e] Taken from Table II of Suurkuusk et al. (1977).

tween the two van't Hoff $\Delta H°$ values also suggests non-two-state behavior since it has previously been shown fits and log c_T plots give identical $\Delta H°$ values for oligomers with two-state transitions (Albergo et al., 1981; Petersheim & Turner, 1983; Freier et al., 1983). Alternatively, the differences between calorimetric and van't Hoff $\Delta H°$ values could be due to experimental error, the presence of one AU stack in A_7U_7, or the possibility that only base pairs not exposed to water contribute to $\Delta H°$ (Levitt, 1972). While these possibilities appear less probable, they cannot presently be ruled out.

Thermodynamic parameters for melting of A_7U_7 in water at 1 M NaCl have been studied extensively (Martin et al., 1971; Borer et al., 1974; Breslauer et al., 1975) and are listed in Table III. The calorimetric $\Delta H°$ is −99.3 kcal/mol of duplex, which is larger than the log c_T $\Delta H°$ of −75.7 kcal/mol of duplex (see Table III), providing additional evidence for non-two-state behavior. Previous spectroscopic measurements on A_7U_7 involving melting curves (Martin et al., 1971) and temperature-jump relaxation amplitudes (Breslauer et al., 1975) have provided values of −107 and −78.3 kcal/mol of duplex, respectively. One test of thermodynamic parameters is to compare predicted and measured melting temperatures. For 4.38×10^{-4} M A_7U_7, Breslauer et al. (1975) report a calorimetric T_m of 45.5 °C. The predicted T_m's from log c_T plots are 44.9 °C (this work) and 42.3 °C (Martin et al., 1971). The comparison suggests recent advances in data acquisition and analysis (Petersheim & Turner, 1983) make possible more reliable determinations of thermodynamic parameters.

The comparisons of van't Hoff and calorimetric enthalpy changes indicate there are more than two states in A_7U_7 melting. For a reaction with all steps equal, the total number

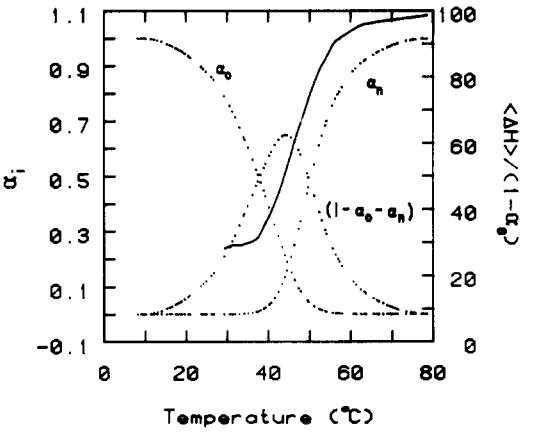

FIGURE 6: Fraction of A_7U_7 strands in state i, α_i (···), and average enthalpy, $\langle\Delta H\rangle$, divided by fraction of molecules not in duplex, $1 - \alpha_0$, (—), vs. temperature. α_0 and α_n are fractions of strands in the initial and final states, respectively. Data for this analysis were taken from Breslauer et al. (1975).

of species in the reaction, n, can be derived from (Tsong et al., 1970; Tanford, 1968)

$$\frac{\Delta H_{vH}}{\Delta H_{cal}} = \frac{n+1}{3(n-1)} \quad (6)$$

Combinations of the various $\Delta H°$ values from Table III all give an n between 2 and 3, suggesting a single intermediate state. Freire & Biltonen (1978a) have developed a procedure for deconvoluting differential scanning calorimetric data to obtain information on intermediates. By use of the data of Breslauer et al. (1975), this procedure was used to generate plots of the fraction of strands in state i, α_i, and the average

enthalpy, $\langle \Delta H \rangle$, divided by the fraction of molecules not in duplex, $1 - \alpha_0$, as a function of temperature (see Figure 6). The results indicate there is one intermediate, and the enthalpy difference between this intermediate and the double strand is about 30 kcal/mol at 45.5 °C. This suggests the intermediate contains roughly 10 base pairs and is therefore not a hairpin conformation of A_7U_7. For comparison, Freire & Biltonen (1978b) concluded that for poly(A)·poly(U) at 0.16 M Na^+, the average helical lengths when the fraction of base pairs is 0.5 and at the midpoint of strand separation are 12 and 7 base pairs, respectively.

The values for $dT_m/d(\log [Na^+])$ for A_7U_7p and poly(A)·poly(U) in water are 17.4 and 19.6 °C, respectively. The slope for A_7U_7p is somewhat higher than the 14 °C predicted from the theory of Record & Lohman (1978) by using parameters derived from melting of $d(AT)_n$ oligomers (Elson et al., 1970). This slope is related to the number of ions released, Δi, by (Anderson & Record, 1982; Record et al., 1978; Manning, 1978)

$$\frac{dT_m}{d \log Na^+} = \frac{2.3}{2\Delta\xi}\frac{RT_m^2}{\Delta H^\circ} = \frac{2.3RT_m^2}{\Delta H^\circ}\Delta i \qquad (7a)$$

$$\Delta\xi = \frac{q^2}{\epsilon kT}\left(\frac{1}{d_{dh}} - \frac{1}{d_{ss}}\right) \qquad (7b)$$

Here q is the protonic charge, ϵ is the solvent dielectric constant, and d_{dh} and d_{ss} are the axial charge separations in the double helix and the single strand, respectively. The calorimetric results for A_7U_7p and poly(A)·poly(U) suggest ΔH° is the same for both and is independent of salt (Breslauer et al., 1975). Since $\Delta i = 0.16$ ion released per phosphate for poly(A)·poly(U) (Record et al., 1978; Record & Lohman, 1978), this suggests $\Delta i \approx 0.14$ ion per phosphate for A_7U_7p. This differs from the 0.06 ion reported in a preliminary communication (Freier et al., 1984) because of the difference in ΔH° determined calorimetrically and spectroscopically. When ethanol is added to the solvent, ϵ and T_m decrease, ΔH° increases, and d_{ss} probably increases, reflecting a more unstacked single strand. On the basis of eq 7, these effects should decrease $dT_m/d(\log [Na^+])$. The measured slope of 8.8 °C in 10 mol % ethanol is consistent with this prediction. Below 0.15 M Na^+, the A_7U_7p double helix is more stable in 10 mol % ethanol than in water, presumably reflecting increased neutralization of phosphate charges by Na^+.

The comparisons presented above have implications for the determination and use of thermodynamic parameters for prediction of RNA structure (Borer et al., 1974; Gralla & Crothers, 1973; Tinoco et al., 1971). First, the ΔH° values measured calorimetrically for poly(A)·poly(U) are only in good agreement when extrapolated to a common temperature, indicating the coil to helix transition has a substantial heat capacity change. Thermodynamic parameters derived from fitting optical melting curves also show temperature dependence (Table II; Appleby & Kallenbach, 1973; Pörschke et al., 1973; Petersheim & Turner, 1983; Freier et al., 1983). Thus, ΔH° and ΔS° values for different oligomers should be extrapolated to a common temperature before thermodynamic parameters are derived for nearest-neighbor interactions. After extrapolation, the calorimetric ΔH° values are still larger than those determined optically. This probably reflects non-two-state behavior. Thus, oligomers with two-state transitions should be used to provide thermodynamics for helix propagation when measurements are made optically. A comparison of free energy changes derived for the A_7U_7 coil to helix transition has been presented previously (Freier et al., 1984). It shows that ΔG° near the T_m of an experiment is relatively insensitive to the method of measurement and analysis but that extrapolation to other temperatures does depend on method. Finally, it is necessary to consider ionic strength when predicting absolute stabilities of RNA helices. Ionic strength may also be important for predicting relative stabilities since such effects are sequence dependent for DNA (Frank-Kamenetskii, 1971; Blake & Haydock, 1979).

In comparing thermodynamic parameters for the coil to helix transition of A_7U_7p in different solvents, we assume the only difference is the solvent surrounding coil and helix. CD spectra indicate the conformations of coil and helix are not sensitive to solvent. Comparison of the ratios of thermodynamic parameters available from fits and log c_T plots indicates the cooperativity of the transition is also relatively independent of solvent (see Table II).

The melting temperatures listed in Table I provide qualitative insight into the action of 11 denaturants on A_7U_7p stability. At 1 M cation, the T_m of A_7U_7p is highest when the solvent is pure water. Similar observations have been made with DNA (Levine et al., 1963; Wetmur & Davidson, 1968), $(dG\text{-}dC)_3$ (Albergo et al., 1981; Freier et al., 1983), poly(A) (Dewey & Turner, 1979), and poly(C) (Freier et al., 1981). This contrasts with the solvent dependence of protein stability where glycerol and sucrose stabilize native confromations, whereas other cosolvents destabilize (Gerlsma & Stuur, 1974; Bull & Breese, 1978; Shifrin & Parrott, 1975; Gekko & Timasheff, 1981a,b; Lee & Timasheff, 1981; Brandts & Hunt, 1967; Prakash et al., 1981; Schreier et al., 1965; Parodi et al., 1973; Velicelebi & Sturtevant, 1979; Back et al., 1979). Gekko & Timasheff (1981a,b) attribute protein stabilization by glycerol to an unfavorable interaction between glycerol and protein. This is consistent with studies on the interactions of small alcohols and amides in aqueous solution by Wood and co-workers (Okamoto et al., 1978). They find an unfavorable free energy for $CHOH/CH_2$ interactions and larger favorable free energies for CH_2/CH_2 and CONH/CONH interactions. The contrasting effects of polyhydric alcohols on proteins and nucleic acids suggest the buried portions of proteins have substantial CH_2 character whereas nucleic acids do not.

Most measurements of ΔH° and ΔS° were made at 10 mol % cosolvent, where solvent effects appear to saturate (see Figure 3). Results in Tables I and II indicate urea and formamide make the ΔH° for duplex formation less favorable than water; ethanol probably makes ΔH° more favorable and ΔS° less favorable (see Tables I and II). To determine if the effect of ethanol was due to an ethanol/Na^+ interaction, measurements were made with tetramethylammonium cation. As shown in Table II, the results are similar, indicating the ethanol/cation interaction is not dominant.

The effects of cosolvents on the thermodynamic parameters for A_7U_7p helix formation contrast with effects seen on protein denaturation. Monohydric alcohols can change the ΔH° for protein folding by 40% or more (Velicelebi & Sturtevant, 1979; Brandts & Hunt, 1967; Parodi et al., 1973). The largest change observed with A_7U_7p is 13%. Moreover, changes with proteins typically show a pronounced maximum as a function of alcohol concentration (Brandts & Hunt, 1967; Velicelebi & Sturtevant, 1979). This is not observed with A_7U_7p (see Figure 3). Thus, thermodynamic parameters also indicate denaturants affect stabilities of proteins and nucleic acids differently.

Classical hydrophobic bonding (Kauzmann, 1959; Tanford, 1973) is considered a major contributor to protein stability. The above comparison of cosolvent effects on proteins relative

to A_7U_7p suggest hydrophobic bonding is not a major contributor to A_7U_7p stability. Moreover, the $\Delta S°$ values for duplex formation by A_7U_7p and poly(A)·poly(U) at 36 °C are −16 and −20 cal K^{-1} (mol of base pair)$^{-1}$, respectively. The constraint of backbone bonds in the double helix gives rise to a negative configuratioinal entropy estimated to be roughly −16 to −30 cal K^{-1} mol of base pair^{-1} (Longuet-Higgins & Zimm, 1960; Dewey & Turner, 1979; Freier et al., 1984), thus largely accounting for the measured $\Delta S°$. Hydrophobic bonding provides a substantial positive entropy contribution. For example, for dimerization of benzene in water, $\Delta S° = 20$ cal K^{-1} mol^{-1} (Tucker et al., 1981). Since the measured and predicted entropy changes for base pair formation are similar, there is no evidence for any contribution from such a large favorable $\Delta S°$.

The energy of cavity formation has also been suggested as a major contributor to nucleic acid stability (Sinanoglu, 1968, 1980, 1982; Sinanoglu & Abdulnur, 1965) and to the stabilization of proteins by sucrose (Lee & Timasheff, 1981). The results presented here suggest cavity formation is not important for the stability of A_7U_7p duplexes. For example, the free energy of cavity formation depends on the surface tension of the solvent. Monohydric alcohols decrease, sucrose increases, and urea affects little the surface tension of water (Timmermans, 1960; Landt, 1931; Jäger et al., 1965). However, all these cosolvents decrease the stability of A_7U_7p. From the data of Teitelbaum et al. (1951), the surface enthalpies of water and 10 mol % ethanol at 35 °C are roughly 122 and 67 dyn/cm^2, respectively. Thus, if cavity terms were dominant, the $\Delta H°$ of duplex formation would be less favorable in 10% ethanol than in water. The oposite is observed, and the change is relatively small. The results suggest theoretical treatments of solvent effects on nucleic acid stability must include more than cavity terms (Sinanoglu, 1982). Recent theoretical calculations on the contribution of water to stacking of purine also indicate small cavity effects (Langlet et al., 1980).

We have previously used kinetic data for stacking of poly(A) and poly(C) to argue that solvent cavity terms are relatively unimportant for nucleic acid stability (Dewey & Turner, 1980; Freier et al., 1981). The experimental observation is that rates of unstacking change little when cosolvents are added. One criticism of the interpretation is that rates of stacking depend on inverse solvent viscosity and decrease on addition of alcohols. Therefore, for the single-step mechanism, unstacking rates should also decrease if cosolvents have no effect on stacking. However, if a correction for solvent viscosity is applied, the unstacking rates change by less than a factor of 3 and do not correlate with solvent surface tension. Thus, rates of single-strand unstacking are also relatively insensitive to solvent composition. Moreover, poly(C) has a higher melting temperature than poly(A) (Freier et al., 1981), even though the surface area per base is less. This is opposite the trend expected if cavity terms dominate the thermodynamics.

There is no good correlation of the thermodynamic parameters reported in Tables I and II with any single solvent or cosolvent property, including surface tension, viscosity, dipole moment, solubility of adenine (Levine et al., 1963; Herskovits & Harrington, 1972; Herskovits & Bowen, 1974), or dielectric constant. However, the $\Delta H°$ and $\Delta S°$ of A_7U_7p duplex formation correlate well with bulk solvent dielectric constants when data are plotted for ethanol, DMF, formamide, and urea at concentrations of 0, 5, and 10 mol % (see supplementary material). The thermodynamic parameters for $(G\text{-}dC)_3$ (Albergo & Turner, 1981; Freier et al., 1983) show a similar correlation, except 10 mol % propanol fits the correlation but 10 mol % DMF does not. It is unlikely this correlation results from polyelectrolyte effects since the enthalpy change associated with such effects arises from the temperature dependence of the product of dielectric constant and temperature (Manning, 1978), and this dependence is small for the solvent mixtures. Solute/solvent interactions are another potential origin for the correlation. When the solvent dielectric constant is large, it is roughly proportional to the square of the effective electric dipole of the solvent (Onsager, 1936; Wyman, 1936; Kosower, 1968). Thus, the correlation may indicate solute/solvent interactions are enhanced by an increase in the effective solvent dipole moment. Of course, the correlation is not completely general, so it may only be fortuitous.

The above results indicate denaturants affect stabilities of nucleic acids and proteins differently. Classical hydrophobic bonding and solvent cavity terms appear to be relatively unimportant in stabilizing nucleic acids. The complexity of a solvent mixture interacting with a macromolecule makes it difficult to provide more quantitative interpretations. Fortunately, progress is being made in theories of solutions (Pratt & Chandler, 1980 a,b; Adelman & Chen, 1979; Berkowitz & Adelman, 1980; Pangali et al., 1979; Chandler et al., 1982). Studies of pair-wise interactions between small molecules in solution (Okamoto et al., 1978; Tucker et al., 1981) provide data that can be correlated with denaturation studies and treated theoretically (Pratt & Chandler, 1980). The results on A_7U_7p stability can be used to test resulting theories of solvent interactions with nucleic acids.

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Supplementary Material Available

Figure 1 showing absorbance vs. temperature for A_7U_7p in H_2O with least-squares fits, Figures 2 and 3 showing absorbance normalized at 70 °C vs. temperature for A_7U_7p in H_2O and 10 mol % solutions of ethanol, 1-propanol, 2-propanol, Me_2SO, formamide, urea, and DMF with 1 M NaCl, Figures 4 and 5 showing thermodynamic parameters vs. dielectric constant for A_7U_7p and $(dG\text{-}dC)_3$ in various cosolvents, Figures 6–9 showing $-\Delta H°$ vs. T_m and $-\Delta S°$ vs. ln T_m for A_7U_7p in H_2O with 1 M NaCl and 1 M TMACl, in 10 mol % ethanol with 1 M NaCl and 1 M TMACl, and in 10 mol % solutions of glycerol, DMF, formamide, and urea with 1 M NaCl, Figures 10 and 11 showing the CD spectra of A_7U_7p at 0 and 70 °C in 10 mol % solutions of urea, DMF, formamide, glycerol, and 1-propanol, Figures 12–15 showing $\Delta\epsilon/\epsilon$ for A_7U_7p at 0 and 70 °C in H_2O and 10 mol % ethanol, glycerol, 1-propanol, urea, formamide, and DMF, and Figures 16–22 showing UV spectra at 0 and 70 °C in H_2O and the various 10 mol % cosolvents (22 pages). Ordering information is given on any current masthead page.

Registry No. Form, 75-12-7; Me_2SO, 67-68-5; EtOH, 64-17-5; 1-PrOH, 71-23-8; 2-PrOH, 67-63-0; A_7U_7, 54651-08-0; A_7U_7p, 90599-30-7; Na, 7440-23-5; sucrose, 57-50-1; ethylene glycol, 107-21-1; glycerol, 56-81-5; *N*,*N*-dimethylformamide, 68-12-2; urea, 57-13-6; dioxane, 123-91-1.

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