Laser Temperature Jump Study of Solvent Effects on Poly(adenylic acid) Stacking†

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ABSTRACT: The dynamics of the single-stranded, helix–coil transition of poly(adenylic acid) have been investigated by using the Raman laser temperature jump technique. The driving forces of this conformational transition have been probed by varying the cosolvent in mixed aqueous solutions. The rate of helix formation correlates well with the inverse of the solution viscosity. This correlation and the low activation barrier of ~4 kcal/mol for this process indicate a rotational diffusion controlled reaction. The rate of coil formation has a much higher activation barrier, presumably due to the strength of base stacking in the helix. This unstacking rate is virtually unaffected by alcohol cosolvents. A significant increase in this rate occurs when polar cosolvents such as urea, formamide, or acetonitrile are added. Absorbance changes with temperature suggest that urea specifically solvates the adenine base. The polar cosolvents appear to break up the stacked, helical conformation by interacting with the bases.

The driving forces for conformational stability in macromolecules are strongly influenced by solvent. The effect of denaturants on the thermodynamics of conformational transitions has been used to probe these forces. Unfortunately, it is often difficult to interpret these results on a molecular level. Kinetic data on the folding of proteins (Tanford, 1970) and synthetic polymers (Hammes & Schimmel, 1967) allow a more detailed interpretation. These results have stressed the importance of both specific interactions and solvent structure.

Although considerable work exists on solvent effects in proteins, surprisingly little has been done with nucleic acids. The driving force for the formation of helical structures in nucleic acids is commonly attributed to base stacking interactions. These interactions are still poorly understood. Debate exists over whether they are dominated by electronic interactions or "solvophobic" forces (Pullman, 1968). The denaturing of DNA by alcohols suggested that hydrophobic interactions may be important (Herskovits, 1963; Levine et al., 1963). However, polar cosolvents like urea or formamide also are potent denaturants. These denaturation effects have been correlated best with solubility (Herskovits & Harrington, 1972; Herskovits & Bowen, 1974; Levine et al., 1963).

Single-stranded nucleic acids also form ordered helical structures (Holcomb & Tinoco, 1965) and provide a simpler system for studying effects on stacking (Lowe & Schellman, 1972). This work reports the effects of mixed aqueous solvents on the thermodynamics and kinetics of the helix–coil transition in poly(adenylic acid) [poly(A)].[1] Previous studies have characterized this transition in aqueous solution (Dewey & Turner, 1979; Pörschke, 1973, 1978).

Experimental Section

Spectra. Circular dichroism spectra were measured on a Jasco J-40 spectropolarimeter, and absorption spectra were measured on a Cary 219. For measurements of $\Delta\epsilon/\epsilon$ the CD and UV absorbance spectra were measured on the same sample and both instruments were adjusted to have a 2-nm bandwidth. This allowed the ratio $\Delta\epsilon/\epsilon$ to be calculated directly from ΔA and A without any correction due to concentration.

Thermodynamics. The temperature dependence of the absorbance was analyzed to determine the thermodynamics of the helix–coil transition. Melting curves were obtained from a Gilford 250 spectrophotometer interfaced to a PDP 11/34 computer, as described previously (Dewey & Turner, 1979). For most mixed solvents the temperature was varied from −5 to 85 °C with a heating rate of 30 °C/h. For 10 mol % glycerol this heating rate resulted in erratic absorbance readings due to convective currents in the viscous media. A 1-mm path length cell was used instead of a 1-cm cell and the heating rate was reduced to 18 °C/h to eliminate these problems. All results are an average of at least three runs. Absorbances were monitored at 260 or 270 nm.

Kinetics. The observed relaxation times were less than 1 μs and were measured with a laser temperature jump apparatus (Dewey & Turner, 1978; Turner et al., 1972). The procedure was identical with that described previously (Dewey & Turner, 1979).

Chemicals. Poly(A) was obtained from Sigma and dialyzed as previously described (Dewey & Turner, 1979). Formamide was purified by recrystallization (Casey & Davidson, 1977). The absorbance at 270 nm was 0.07/cm. Water was doubly distilled, and absolute ethanol was used. Methanol was spectral grade from Mallinckrodt, 1-propanol "distilled in glass" was from Burdick and Jackson, urea "ultrapure" was from Schwarz/Mann, and acetonitrile was spectral grade from Eastman.

Mixed solvents were made by diluting the dialyzed stock with cosolvent. Cosolvents were buffered appropriately to maintain a 0.05 M sodium cacodylate concentration, pH 7 to 8, after mixing. The polymer was checked for degradation after a melting or a temperature jump experiment. Electrophoresis in 10% polyacrylamide (Dewey & Turner, 1979) showed that the poly(A) length was maintained.

Results

Equilibrium. The change in absorbance with temperature for poly(A) in various mixed solvents is shown in Figures 1 and 2. These melting curves were analyzed with a two-state

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[1] Abbreviations used: poly(A), poly(riboadenylic acid); AMP, adenosine monophosphate; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; PrOH, 1-propanol; CD, circular dichroism.

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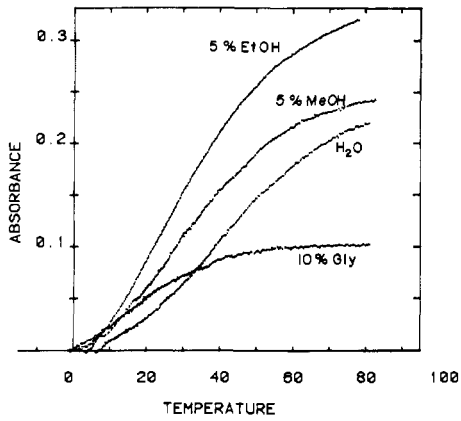

FIGURE 1: Change in poly(A) absorbance at 270 nm vs. temperature. Initial absorbance in a 1-cm path length cell is 0.574 for water, 0.511 for 5 mol % methanol, and 0.757 for 5 mol % ethanol. Initial absorbance for 10 mol % glycerol in a 0.1-cm path length cell is 0.394. All solutions contained 0.05 M sodium cacodylate.

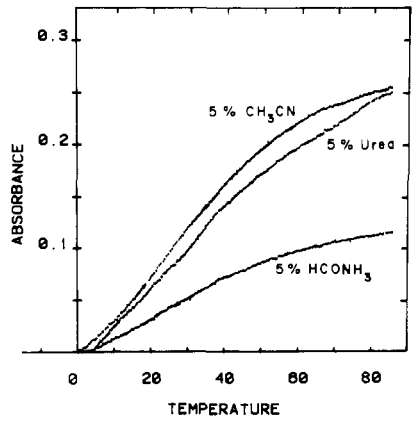

FIGURE 2: Change in poly(A) absorbance at 270 nm vs. temperature. Initial absorbance in a 1-cm path length cell is 0.683 for 5 mol % acetonitrile, 0.273 for 5 mol % formamide, and 0.726 for 5 mol % urea. All solutions contained 0.05 M sodium cacodylate.

model as described before (Dewey & Turner, 1979). The calculated thermodynamic parameters are listed in Table I. From testing the fitting procedure with computer-generated data, it was shown that temperature-dependent extinction coefficients cause large errors in ΔH and ΔS. Such effects are clearly apparent in urea solutions. The extinction of 2.6 $\times 10^{-5}$ M AMP in 5 mol % urea changes 8% at 270 nm between 5 and 70 °C.

Despite the large errors in the thermodynamic parameters for some of the mixed solvents, the errors in the equilibrium constants are not unreasonable. This in part is due to the strong correlation between ΔH and ΔS in the fitting of the data. The variance of the free energy, $\sigma_{\Delta G}^2$, is given by

$$\sigma_{\Delta G}^2 = \sigma_{\Delta H}^2 + T^2\sigma_{\Delta S}^2 - 2\rho T\sigma_{\Delta H}\sigma_{\Delta S}$$

where ρ is the correlation factor for ΔH and ΔS (Snedecor & Cochran, 1971). The estimate of this factor from the algorithm gives a value of 1. This strong correlation has the effect of reducing the error in ΔG and consequently in K. The error in K was found to be larger between runs than within a run. The standard deviation is at most 15% of the equilibrium constant. This value is used in all subsequent error analysis. It is interesting to note that in addition to showing strong correlations within a fit, ΔH and ΔS are well correlated over the range of solvents studied. Thus, this system apparently exhibits the commonly observed enthalpy–entropy compensation (Leffler & Grunwald, 1963; Lumry & Rajender, 1970).

Table I: Thermodynamics of the Poly(A) Coil-Helix Transition in 0.05 M Sodium Cacodylate

solvent	λ (nm)	$-\Delta H$ (kcal/mol)	$-\Delta S$ (eu)	K (25 °C)
H_2O	260	11.3 ± 1.1	36.3 ± 3	2.2
	270	11.8 ± 1.5	38.0 ± 5	2.2
	285	10.9 ± 1.6	35.7 ± 5	1.5
	av	11.3 ± 1.4	36.7 ± 4	2.0
5% MeOH	270	12.5 ± 1.1	41.1 ± 4	1.5
5% EtOH	260	9.6 ± 1.1	31.7 ± 3.6	1.3
	270	9.7 ± 0.9	32.4 ± 2.6	1.1
	av	9.7 ± 1.0	32.1 ± 3.0	1.2
10% EtOH	260	9.3 ± 1.7	31.7 ± 3.9	0.8
	270	9.1 ± 1.5	30.5 ± 4.7	1.0
	av	9.2 ± 1.6	31.1 ± 4.3	0.9
5% PrOH	260	9.7 ± 1.7	32.0 ± 5.6	1.3
	270	10.9 ± 1.1	35.7 ± 3.7	2.0
	av	10.3 ± 1.5	33.9 ± 4.7	1.7
5% CH_3CN	260	10.2 ± 1.0	33.9 ± 3.3	1.2
	270	10.3 ± 2.0	34.1 ± 6.7	1.3
	av	10.3 ± 1.5	34.0 ± 5.0	1.2
5% urea	260	7.6 ± 1.8	25.4 ± 5.7	1.1
	270	7.4 ± 1.7	24.2 ± 5.6	1.4
	av	7.5 ± 1.8	24.8 ± 5.7	1.2
5% formamide	270	9.9 ± 0.8	33.0 ± 2.7	1.1
10% glycerol	270	7.5 ± 1.3	26.9 ± 4.0	0.4

CD and UV Spectra. In comparing the effects of mixed solvents on the helix–coil transition of poly(A), it would be useful to know if these solvents affect the conformations of helix and coil. Unfortunately, there is no unambiguous way to determine this. The absorption and CD spectra were measured at three temperatures for poly(A) in water, 5 mol % urea, 10 mol % ethanol, and 10 mol % glycerol to provide an indication of the conformations in the various solvents (see paragraph at end of paper regarding supplementary material). The thermodynamics derived from melting curves were then used with these data to extrapolate to the spectra of the fully stacked and unstacked species. These spectra were then used to calculate the Kuhn dissymmetry factor, $\Delta\epsilon/\epsilon$, as a function of wavelength (Kuhn, 1958). These plots are shown in Figure 3. The Kuhn dissymmetry factor was chosen in order to normalize for the change in extinction coefficient that occurs in mixed solvents even if there is no conformational change (e.g., for monomers). This does not correct for all expected solvent effects on polymer optical activity but rather represents a first approximation. The spectra for the unstacked states indicate little structure in all four solvents. The spectra for the stacked states differ significantly only for 10 mol % EtOH. The shapes of the spectra are very similar, but the amplitudes of the bands vary. Some of this difference may be due to the errors in the thermodynamics. Unfortunately, it is not possible to separate out the effects due to conformational changes from those due to solvent perturbation of the interactions producing optical activity. The results suggest, however, that the states in different solvents are similar but probably not identical.

Kinetics. The relaxation times measured at 25 °C for various mixed aqueous solvents are shown in Table II. The relaxation was measured at 270 and 285 nm. These wavelengths are in the hypochromic and hyperchromic regions, respectively. No measurement was made in 15 mol % glycerol at 285 nm because there was not a large enough absorbance change to observe. In previous work, the appearance of a wavelength-dependent relaxation in aqueous solutions of high ionic strength suggested that more than two states are present (Pörschke, 1973, 1978). At low ionic strength this wavelength dependence disappears and a two-state model is applicable (Dewey & Turner, 1979). The lack of wavelength dependence

Table II: Relaxation Times and Rate Constants of Poly(A) at 25 °C in 0.05 M Sodium Cacodylate: Two-State Model and Kinetic Ising Model[a]

solvent	τ_{270} (ns)	τ_{285} (ns)	τ_{av} (ns)	$k_{-1} \times 10^{-6}$ (s^{-1})	$k_1 \times 10^{-6}$ (s^{-1})
H_2O	95 ± 15	105 ± 13	100 ± 14	3.2 ± 0.6 (5.5)	7.0 ± 1.5 (10.6)
5% MeOH	126 ± 16	116 ± 25	120 ± 21	3.3 ± 0.6 (6.4)	5.0 ± 1.2 (7.7)
5% EtOH	120 ± 27	128 ± 22	124 ± 25	3.7 ± 0.8 (6.2)	4.4 ± 1.2 (7.5)
10% EtOH	123 ± 30	130 ± 17	127 ± 24	4.1 ± 0.8 (7.1)	3.7 ± 0.9 (6.4)
5% PrOH	129 ± 25	127 ± 20	128 ± 23	3.4 ± 0.7 (5.7)	4.4 ± 1.1 (7.4)
5% CH_3CN	64 ± 7	68 ± 12	66 ± 10	6.9 ± 1.2 (11.1)	8.3 ± 1.9 (14.4)
5% urea	68 ± 12		68 ± 12	6.7 ± 1.3 (11.9)	8.0 ± 2.0 (13.1)
5% formamide	99 ± 15	99 ± 8	99 ± 12	4.8 ± 0.7 (8.2)	5.3 ± 1.1 (9.0)
10% glycerol	211 ± 39		211 ± 39	3.4 ± 0.6 (4.8)	1.4 ± 0.6 (2.9)
15% glycerol	392 ± 91		392 ± 91		

[a] Values for the kinetic Ising model are in parentheses.

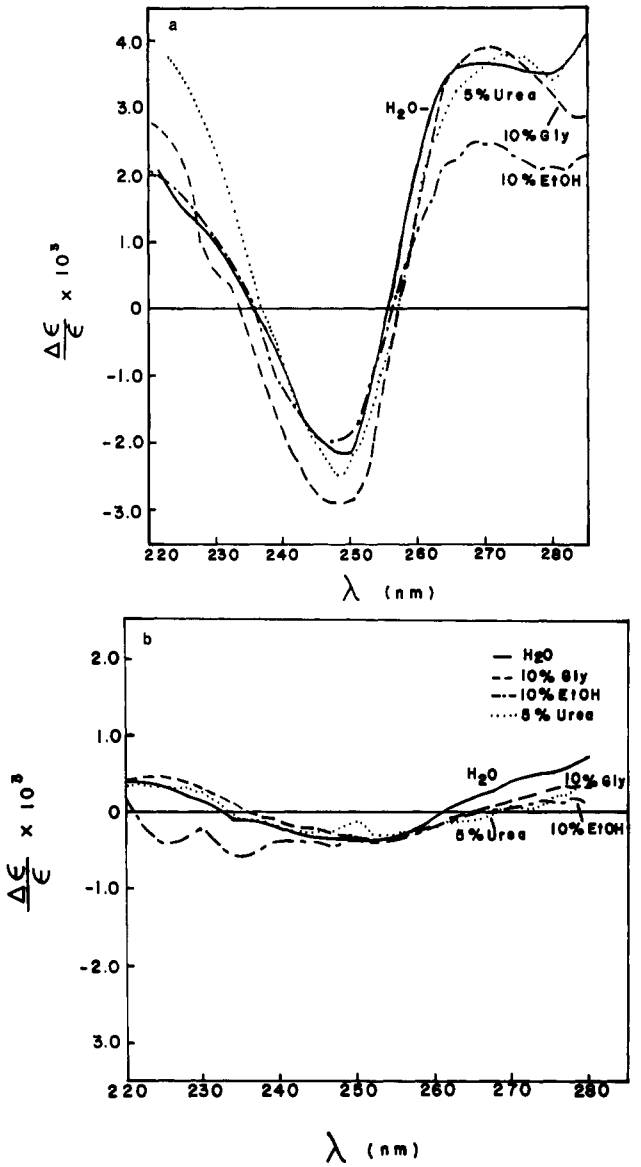

FIGURE 3: Extrapolated $\Delta\epsilon/\epsilon$ vs. wavelength for poly(A) in (a) the stacked conformation and (b) the unstacked conformation: (—) water; (---) 10 mol % glycerol; (-·-) 10 mol % ethanol; (···) 5 mol % urea. All solutions contained 0.05 M sodium cacodylate.

in mixed aqueous solvents indicates that no additional states are stable, and the equilibrium is described by

$$\text{poly(A) (unstacked)} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{poly(A) (stacked)}$$

The relaxation time, τ, is then given by

$$1/\tau = k_1 + k_{-1}$$

Table III: Relaxation Times, Rate Constants, and Activation Parameters for Poly(A) in 0.05 M Sodium Cacodylate: Two-State Model and Kinetic Ising Model[a]

T (°C)	τ_{270} (ns)	τ_{285} (ns)	$k_{-1} \times 10^{-6}$ (s^{-1})	$k_1 \times 10^{-6}$ (s^{-1})
		H_2O		
10	174 ± 19		0.8 (1.6)	4.9 (6.2)
15	126 ± 15	126 ± 19	1.5 (2.7)	6.4 (8.5)
25	95 ± 16	105 ± 13	3.2 (5.5)	7.0 (10.6)
35	64 ± 12	57 ± 10	7.4 (12.3)	9.2 (15.8)
E_a (kcal/mol)			15.2 (14.1)	4.0 (6.2)
$\Delta S^{\ddagger}$ (eu)			20.3 (17.6)	−15.8 (−7.5)
		5% Urea		
5	141 ± 29	139 ± 23	1.7 (3.4)	5.5 (7.6)
10	108 ± 22	107 ± 28	3.0 (5.8)	6.3 (9.3)
25	68 ± 12		6.7 (11.9)	8.0 (13.1)
35	40 ± 14		13.2 (22.4)	11.8 (20.1)
E_a (kcal/mol)			11.8 (10.8)	4.2 (5.6)
$\Delta S^{\ddagger}$ (eu)			10.4 (8.1)	−14.6 (−9.2)
		5% EtOH		
5		274 ± 49	0.8 (1.4)	2.9 (3.9)
10		227 ± 38	1.2 (2.0)	3.2 (4.7)
25	120 ± 27	128 ± 22	3.7 (6.2)	4.4 (7.5)
35		72 ± 17	8.2 (12.5)	5.7 (11.2)
E_a (kcal/mol)			13.1 (12.5)	3.8 (5.8)
$\Delta S^{\ddagger}$ (eu)			13.6 (12.5)	−17.2 (−9.4)
		10% EtOH		
10	274 ± 33	285 ± 25	1.2 (2.3)	2.4 (3.7)
15	154 ± 16		2.6 (4.8)	3.9 (6.2)
25	123 ± 30	130 ± 17	4.1 (7.1)	3.7 (6.4)
35	55 ± 13		11.8 (18.5)	5.9 (11.1)
E_a (kcal/mol)			14.6 (13.3)	5.1 (6.6)
$\Delta S^{\ddagger}$ (eu)			19.2 (15.8)	−12.9 (−7.1)

[a] Values for the kinetic Ising model are in parentheses.

By use of the equilibrium constant determined from the melting curve, the relaxation time can be partitioned into the forward and reverse rate constants. The activation energy E_a was determined from the temperature dependence of the rate constants according to the Eyring equation

$$k = \frac{eRT}{Nh} \exp(-E_a/RT) \exp(\Delta S^{\ddagger}/R)$$

The activation entropies, $\Delta S^{\ddagger}$, were determined from the rates at 25 °C. The results for H_2O, 5 mol % EtOH, 10 mol % EtOH, and 5 mol % urea are shown in Table III. The Eyring plots for these solvents are shown in the supplementary material. The error in the activation energy is estimated at ±1 kcal/mol and at ±3 eu for $\Delta S^{\ddagger}$. Given the accuracy of these parameters, the results in ethanol–water mixtures are the same as in pure water. However, the reverse rate parameters for 5% urea are significantly different from H_2O. The data were also analyzed with the cooperative model of Schwarz as described previously (Schwarz, 1965, 1972; Dewey & Turner,

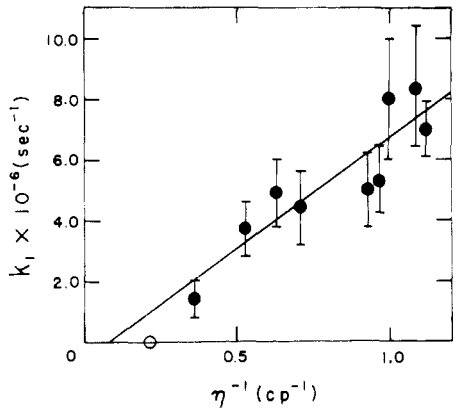

FIGURE 4: Plot of forward rate (k_1) for the two-state model vs. reciprocal solvent viscosity. Open circle is the value for 15 mol % glycerol as estimated in the text.

1979). These results are also shown in Tables II and III.

The signal in 5% urea shows a fast process with an amplitude of opposite sign to that for the unstacking of poly(A). This signal was beyond the resolution of the instrument. A similar signal was observed with AMP in 5 and 10 mol % urea solutions. This evidence along with the temperature-dependent extinction observed in poly(A) and AMP suggests that urea is specifically solvating the adenine chromophore. Similar fast initial jumps in the relaxation signal were seen in 10 mol % EtOH. In these instances the first 20 ns of the trace was ignored in evaluating the relaxation times.

Most of the changes in equilibrium constant with different solvent systems are due to changes in the forward rate. These rates do not correlate well with surface tension or dielectric constant. However, they have an excellent correlation with the reciprocal of the viscosity. This is shown in Figure 4 for the two-state model. A similar plot is obtained by using the cooperative model. In 15 mol % glycerol the reciprocal relaxation time is 2.6×10^6 s^{-1}. For 10 mol % glycerol the reverse rate of 3.4×10^6 s^{-1} was essentially unchanged from that of water while the forward rate decreased by about a factor of 5. On the assumption that the reverse rate changes little in going from 10 to 15 mol % glycerol, the relaxation time predominantly reflects the reverse rate. This is indicated in Figure 4 by the open circle indicating a forward rate of zero for 15 mol % glycerol.

The reverse rates show very little change with cosolvent with the exception of the polar cosolvents. Five mole percent urea and acetonitrile approximately double the reverse rate while 10 mol % EtOH has very little effect.

Discussion

The largest effect observed in this study is the decrease in forward rate constants in glycerol–water mixtures. There is an almost fivefold decrease in the forward rate going from water to 10 mol % glycerol. Since the reverse rate is essentially unchanged at 10 mol %, the large increase in relaxation time at 15 mol % glycerol is probably due to a further decrease of the forward rate. The large effect of glycerol implicates viscosity as a controlling factor. Measurements of rotational correlation times for dyes have indicated that macroscopic viscosity is a good measure of microscopic viscosity (Eisenthal, 1975; Chuang & Eisenthal, 1971).

The good correlation of reciprocal viscosity with the forward rate shown in Figure 4 suggests that this rate is rotational diffusion controlled. The activation energy of ~4 kcal/mol for the four solvents listed in Table III is consistent with this interpretation. Phosphorus NMR relaxation studies on poly(A) in aqueous solution indicate that the backbone phosphates have relaxation times from 10^{-8} to 10^{-10} s (Akasaka, 1974; Pan & Bobst, 1973). Thus, forward rates of $\sim 10^6$ s^{-1} may appear too slow for a process controlled by rotational diffusion. However, previous work has demonstrated that the forward rate in poly(A) is limited by a considerable conformational entropy barrier (Dewey & Turner, 1979). Formation of a stacked state requires the restriction of several backbone rotations plus a base–sugar rotation. The linear correlation of k_1 with inverse viscosity shown in Figure 4 suggests that the stacking process requires strong coupling to a single internal rotational motion while the backbone is in a specific, fixed conformation. The internal rotation is a diffusion-controlled process that is viscosity dependent while the restriction to a fixed conformational state contributes a large unfavorable entropy of activation and thereby slows the process.

The only significant changes on the reverse rate occur with polar cosolvents. The reverse rate is increased in 5 mol % formamide and is approximately doubled in 5 mol % urea and 5 mol % acetonitrile. The polar cosolvents might be expected to stabilize the stacked states since they can more effectively shield the charged phosphate backbone. However, this is not the case. The destabilization may be due to the interaction of the cosolvent with the bases. The "instantaneous" signal observed for relaxations in urea solutions suggests specific solvation of AMP and poly(A) by the cosolvent. The polar cosolvents may increase the reverse rate by attacking the stacked state and solvating the bases. This is consistent with the 10-eu less favorable activation entropy and 3.4 kcal/mol lower activation energy measured in 5 mol % urea. Evidently, the bases are polar and can have electrostatic interactions with other polar species whether they are other bases or cosolvents (Devoe & Tinoco, 1962). The ineffectiveness of the nonpolar solvents in destabilizing the stacked state is expected in such a view. This suggests that in a noninteracting solvent the reverse rate would be dominated by electronic interactions.

The driving force for helical stacking is not well understood. One clever but largely untested theory is that the energy difference between forming a solvent cavity around a random coil and a helical polymer provides the stabilization energy (Sinanoglu, 1968; Sinanoglu & Abdulnur, 1964, 1965). This energy is related to the surface tension of the solvent. An attractive feature of the model is that it qualitatively predicts the correct thermodynamics for base stacking. However, if cavity forces were maintaining the stacked state, a large decrease in surface tension would be expected to significantly decrease the lifetime of this state. Such an effect is not seen in the data of Table II. For example, the surface tensions of water, 5 mol % EtOH, 10 mol % EtOH, and 5 mol % PrOH at 25 °C are 72.0, 46.4, 36.6, and 34.7 dyn/cm while the measured stacked state lifetimes ($1/k_{-1}$) are 303, 270, 243, and 294 ns, respectively. Recent studies of dye stacking kinetics also do not provide evidence for surface tension effects (Dewey et al., 1978, 1979).

These results demonstrate the advantage of the kinetic approach to the investigation of conformational stability of nucleic acids. The thermodynamics show that the helix–coil transition of poly(A) is destabilizated by both polar and nonpolar solvents. The kinetics reveal that the destabilization in nonpolar solvents is due to viscosity effects on the forward rate. The forward rate is susceptible to such media effects because it is a rotational diffusion controlled process. On the other hand, polar solvents predominantly increase the reverse rate, presumably by a cosolvent attack on the stacked conformation. To achieve this detailed a description from ther-

modynamics alone would have been extremely difficult.

Supplementary Material Available

One figure showing melting curves of poly(A) in 5 mol % propanol and 10 mol % ethanol; three figures showing Eyring plots of poly(A) in 5 mol % ethanol, 5 mol % urea, and 10 mol % ethanol for both the two-state and kinetic Ising models; and eight figures of absorption and circular dichroism spectra at three temperatures for poly(A) in water, 5 mol % urea, 10 mol % ethanol, and 10 mol % glycerol (12 pages). Ordering information is given on any current masthead page.

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Binding of T4 Endonuclease V to Deoxyribonucleic Acid Irradiated with Ultraviolet Light†

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ABSTRACT: Endonuclease V of bacteriophage T4 binds to UV-irradiated deoxyribonucleic acid (DNA) but not to unirradiated DNA. We have developed an assay to detect this binding, based on the retention of enzyme–DNA complexes on nitrocellulose filters. The amount of complex retained, ascertained by using radioactive DNA, is a measure of T4 endonuclease V activity. The assay is simple, rapid, and specific, which makes it useful for detecting T4 endonuclease V activity both in crude lysates and in purified preparations. We have used it to monitor enzyme activity during purification and to study binding of the enzyme to DNA under conditions that minimize the ability of the enzyme to nick DNA. From our data we conclude that (1) T4 endonuclease V binds to UV-irradiated DNA but not to DNA that has been previously incised by the endonuclease, (2) equilibrium between the free and complexed form of the enzyme is attained under our reaction conditions, (3) dissociation of enzyme–DNA complexes is retarded by sodium cyanide, and (4) retention of enzyme–DNA complexes on nitrocellulose filters is enhanced by high concentrations of saline–citrate.

Endonuclease V of bacteriophage T4 is an important biochemical probe for identifying and quantitating pyrimidine dimers in DNA. It produces nicks in DNA strands containing dimers, making approximately one incision for each dimer (Friedberg & King, 1969, 1971; Yasuda & Sekiguchi, 1970; Simon et al., 1975). To complement studies of the incising properties of T4 endonuclease V (Minton et al., 1975; Yasuda & Sekiguchi, 1976), we have developed an assay to measure the binding of the enzyme to DNA. The assay, a modification of previously described procedures (Jones & Berg, 1966; Riggs et al., 1970; Hinkle & Chamberlin, 1972; Madden et al., 1973; Braun & Grossman, 1974), involves a binding reaction in which enzyme and radioactive, UV-irradiated DNA are mixed under conditions that inhibit incision but permit the endonuclease to bind to UV-irradiated DNA. After dilution to prevent formation of new enzyme–DNA complexes the mixture is filtered through nitrocellulose filters that retain enzyme–DNA complexes but not free DNA. The amount of

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