Solution Studies of the Nucleic Acid Bases and Related Model Compounds. Solubility in Aqueous Urea and Amide Solutions†

Theodore T. Herskovits* and Joseph J. Bowen

ABSTRACT: The solubilities of the nucleic acid bases and related nucleosides (adenine, adenosine, deoxyadenosine, thymine, guanosine, and cytosine) have been determined in water and various urea–water and amide–water mixtures. Setchenow constants and related free energies of transfer of these bases and nucleosides from water to various urea and amide solutions were evaluated and are presented. In general, the increase in chain length or hydrocarbon content of the ureas and amides is found to increase the initial solubility of these compounds, giving increasingly more negative Setschenow constants and transfer free energies. In this respect the solubilizing effects of these two series of denaturants is similar to the effects of the alcohols reported in a previous study (T. T. Herskovits and J. Harrington (1972), *Biochemistry 11,* 4800). Qualitatively, a close correlation is found between the solubility parameters, the relative hydrophobicity, and the effectiveness of the ureas and amides as DNA denaturants. The equations of Peller and Flory employed previously to account for the effects of denaturants on the denaturation transition and destabilization of proteins and polypeptides were extended to DNA denaturation, using the present Setschenow parameters corrected for self-interaction, and also the binding constants, K_B, employed in protein denaturation studies (T. T. Herskovits *et al.* (1970), *J. Biol. Chem. 245,* 2588; D. Elbaum *et al.* (1974), *Biochemistry 13,* 1278). The calculated solvent denaturation midpoints with both sets of constants are found to be in satisfactory agreement with the experimental denaturation midpoints of bacteriophage T4 DNA, obtained by Levine *et al.* (1963), *Biochemistry 2,* 168) using various ureas and amides at 73°.

Various neutral solutes of the alcohol (Geiduschek and Gray, 1956; Geiduschek and Herskovits 1961; Herskovits *et al.,* 1961; Levine *et al.,* 1963; Aubel-Sadron *et al.,* 1964; Brahms and Mommaerts, 1964; Girod *et al.,* 1973; Ivanov *et al.,* 1973), glycol (Duggan 1961; Herskovits, 1962; Eliasson *et al.,* 1963; Fasman *et al.,* 1964; Luzzati *et al.,* 1964; Nelson and Johnson, 1970; Green and Mahler, 1971; Lerman, 1971), amide (Helmkemp and Ts'o, 1961; Marmur and Ts'o, 1961; Herskovits, 1962; Levine *et al.,* 1963; Kurihara *et al.,* 1963; Massie and Zimm, 1969; McConaughy *et al.,* 1969; Elson and Record, 1974), and urea (Herskovits, 1963; Levine *et al.,* 1963) classes, as well as salts (Hamaguchi and Geiduschek, 1962; Tunis and Hearst, 1968; Von Hippel and Schleich, 1969), are known to destabilize the native helical conformation of nucleic acids.

Various studies of the association of the DNA bases themselves and other simple models such as the mono- and dinucleosides have established the importance of base to base stacking interactions in regard to the maintenance of the basic helical features of DNA and RNA in aqueous solutions (for experimental and theoretical reviews of the literature, see Ts'o, 1970; Pullman and Pullman, 1968; Cantor and Katz, 1971). It has been known for some time that the stacking interactions are enthalpy driven and entropy opposed (Sturtevant et al., 1958; Crothers and Zimm, 1964; Van Holde *et al.,* 1965; Lowe and Schellman, 1972) in comparison with the classical, largely entropy driven hydrophobic interactions (Kauzmann, 1959) encountered in the study of the side chain interactions of nonpolar amino acid residues in proteins. However, the role of the natural solvent, water, in the formation of the stacked bases, and the details concerning the base–base and base–solvent interactions have been the subject of controversy and speculation (Herskovits and Harrington, 1972; Pörschke and Eggers, 1972; Scruggs *et al,* 1972; Alvarez and Biltonen, 1973).

In a previous publication we have reported the solubility behavior of the DNA purines and pyrimidines and related nucleosides in various aqueous alcohol and glycol solutions, and have employed the derived Setschenow parameters for the assessment of the effects of these solvents on the melting temperature of DNA (Herskovits and Harrington, 1972). In this study we present similar data and analysis obtained with the water soluble urea and amide series of denaturants.

Experimental Section

Materials. The various purines, pyrimidines, and nucleosides employed were purchased from Schwarz/Mann. The reagents used were spectral grade or of the purest commercially available quality. The solid ureas and amides were once or twice recrystallized from hot ethanol and dried under vacuum. The urea used was ultrapure grade obtained from Schwarz/Mann. It was used without further crystallization. The water employed was distilled in an all glass Corning still.

Solution and Solubility Determination. Saturated solutions of the bases and nucleosides were obtained by 6–11 days stirring of the solutions in constant temperature bath maintained at 25 ± 0.03°. Solution was affected by magnetic stirring in stoppered 5- or 10-ml volumetric flasks placed in submersible microstirrers (Tri-R-Instruments

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TABLE I: Solubility of Purine and Pyrimidine Bases and Nucleosides in Aqueous Urea Solutions at 25.0.[a]

Concn of Urea (M)	Solubility (M × 10^2)					
	Adenine	Adenosine	Deoxyadenosine	Thymine	Guanosine	Cytosine
Water	0.722 ± 0.007	1.92 ± 0.03	2.69 ± 0.05	2.78 ± 0.06	0.182 ± 0.003	6.58 ± 0.05
Urea						
0.5		2.23	3.16	3.31	0.213	7.30
1.0	0.92	2.61	3.69	3.75	0.257	8.03
1.5	1.03	2.94	4.18	4.32		
2.0	1.09	3.34	4.70	4.77	0.384	9.46
3.0	1.28	4.11	5.75	3.95	0.539	10.90
4.0	1.45	4.88	6.72	3.58	0.689	12.39
5.0	1.60	5.64	7.78	3.36	0.903	13.96
6.0	1.75	6.36		3.29	1.136	15.63
7.0					1.469	17.29
8.0	1.89			3.10	1.821	18.86
Methylurea						
0.5	0.96	2.47	3.43		0.238	7.45
1.0	1.18	2.95	4.25	3.88	0.311	8.35
1.5	1.28	3.53	5.12	4.53	0.376	9.29
2.0	1.52	4.04	5.84	5.06	0.462	10.05
3.0	1.85	5.02	7.33	6.06	0.643	11.71
4.0	2.15	5.97	8.70	7.04	0.856	12.56
5.0	2.47	6.77	9.77	8.33	1.069	14.40
6.0	2.66	7.14	10.59	9.15	1.349	15.33
1,3-Dimethylurea						
0.25		2.31	3.42		0.225	
0.50	1.10	2.64	4.12	3.57	0.271	7.80
0.75		2.97	4.54		0.306	
1.00	1.48	3.33	5.18	4.26	0.341	8.87
1.50	1.82	4.07	6.14	4.71	0.421	9.85
2.00	2.10	4.67	7.62	5.26	0.495	10.72
3.00	2.68	5.64	8.88	6.29	0.603	11.72
4.00	3.10	6.37	9.86	7.05	0.747	12.54
Ethylurea						
0.25	0.83	2.24	3.31	3.20	0.202	7.09
0.50	0.96	2.63	3.76	3.45	0.247	7.67
0.75	1.07	2.97	4.25	3.76	0.279	8.18
1.00	1.24	3.36	4.93	4.11	0.313	8.68
1.50	1.51	4.10		4.55	0.411	9.67
2.00	1.78	4.80	7.24	5.12	0.504	10.48
3.00	2.46	6.38	9.63	6.64	0.725	12.43
4.00	3.11	7.92	11.85	8.08	0.893	13.26
Propylurea						
0.25	0.94[b]	2.34	3.57	3.25[b]	0.226	7.20
0.50	1.10[b]	2.83	4.49	3.66[b]	0.271	7.96
0.75	1.32[b]	3.38	5.38	4.09[b]	0.325	8.67
1.00	1.57	3.97	6.53	4.66	0.381	9.32
1.50	2.04	5.26	8.58	5.68	0.510	10.56
2.00	3.00	6.69		6.82	0.661	11.76
3.00	4.08	9.60		9.07	0.973	14.09
4.00		11.69			1.290	15.72
Butylurea						
0.2	0.97	2.41	3.55	3.28	0.232	7.30
0.4	1.22	3.02	4.60	3.78	0.281	8.16
0.6	1.54	3.75	6.04	4.23	0.342	8.99
0.8	1.90	4.52	7.28	4.71	0.411	9.74
1.0	2.56	5.44	8.98	5.37	0.487	10.60

[a] The solubilities for water represent the average of three to nine determinations while those for the urea and amide solutions represent one or two determinations (Herskovits and Harrington, 1972). [b] These values were interpolated results, based on S = 0.911, 1.023, 1.217, and 1.572 × 10^{-2} M solutions of adenine and 3.17, 3.50 3.85, and 4.17 × 10^{-2} M solutions of thymine in 0.2, 0.4, 0.6, 0.8, and 1.0 M propylurea.

TABLE II: Solubility of Purine and Pyrimidine Bases and Nucleosides in Aqueous Amide Solutions at 25.0°.

Concn of Amide (M)	Solubility (M × 10^2)					
	Adenine	Adenosine	Deoxyadenosine	Thymine	Guanosine	Cytosine
Formamide						
0.5	0.84	2.07	3.00	3.12		7.06
1.0	0.89	2.33	3.47	3.43	0.233	7.51
1.5	1.00	2.57	3.68	3.73	0.262	8.03
2.0	1.08	2.76	4.10	4.06	0.297	8.28
3.0	1.24	3.27	4.87	4.70	0.443	9.20
4.0	1.44	3.70	5.36	5.23	0.527	10.09
5.0	1.56	4.18	5.91	5.85		
6.0	1.70	4.70	6.27	6.43		
N,*N*-Dimethylformamide						
0.25	0.87			3.17	0.263	7.65
0.50	1.07			3.41	0.270	8.04
0.75	1.27			3.54	0.303	8.55
1.00	1.42			3.76	0.335	9.00
1.50	1.78			4.04	0.393	9.45
2.00	2.13			4.56	0.444	10.0
Acetamide						
0.50	0.88	2.22	3.23	3.27	0.215	7.31
0.75		2.46	3.60			
1.00	1.10	2.64	3.90	3.57	0.248	7.90
1.50	1.25	3.00	4.45	3.94	0.284	8.43
2.00	1.39	3.27	4.98	4.31	0.321	8.97
3.00	1.66	3.88	5.87	5.06	0.413	9.89
4.00	1.88	4.37	6.90	5.70	0.427	10.81
5.00	2.08			6.12	0.579	11.38
Propionamide						
0.25	0.89			3.10	0.191	7.57
0.50	0.95			3.33	0.252	7.75
0.75	1.06			3.56	0.314	8.08
1.00	1.15			3.83	0.346	8.70
1.50	1.43			4.28	0.417	9.36
2.00	1.69			4.73	0.476	9.25
3.00	2.20			5.66	0.632	10.52
4.00	2.66			6.79	0.733	11.39
Butyramide						
0.25	0.88			3.14	0.218	8.52
0.50	1.04			3.46	0.267	9.85
0.75	1.21			3.76	0.342	10.98
1.00	1.42			4.08		12.21
1.50	1.84			4.76	0.489	14.26
2.00	2.32			5.46	0.574	15.87

Inc., Rockville Center, N.Y.). Saturation was usually attained after 2 days of stirring with small 7- or 10-mm Teflon-coated stirring bars (Herskovits and Harrington, 1972; Nakano and Igarashi, 1970). The saturated solutions were usually filtered by use of analytical filter paper. However, with many of the guanosine solutions filtration through small, Millipore 500 filters was necessary. The solubilities reported were based on absorbance measurements made on a Cary 14 recording spectrophotometer after 50–1000-fold volumetric dilution of the filtrates. The following molar extinction coefficients were used to calculate solubility of the various DNA bases and nucleosides; adenine, 13.3 × 10^3 at 260.5 nm; adenosine, 14.9 × 10^3 at 259.5 nm; deoxyadenosine, 14.7 × 10^3 at 260 nm; thymine, 7.89 × 10^3 at 264.5 nm; guanosine, 13.65 × 10^3 at 252.5 nm; and cytosine, 6.13 × 10^3 at 267 nm (Beaven *et al.*, 1963). Where required, solvent blank corrections were made. With the exception of adenine, the base solubilities in water at 25° (Table I) were found to be within ±1–3% of the values reported by Robinson and Grant (1966). The solubility of the newer adenine samples employed in the present study (Schwarz/Mann Lot No. U3284 and W3396) was found to be on an average about 3% higher than the previous value of 7.04 × 10^{-3} reported from our laboratory (Herskovits and Harrington, 1972). Our present value of 7.22 ± 0.007 × 10^{-3} is about 3–10% lower than the values of 7.5–7.8 and 8.0 × 10^{-3} obtained by Nakano and Igarashi (1970) and Robinson and Grant (1966). The solubilities of the other bases in water were found to be in close agreement with our previously reported values.

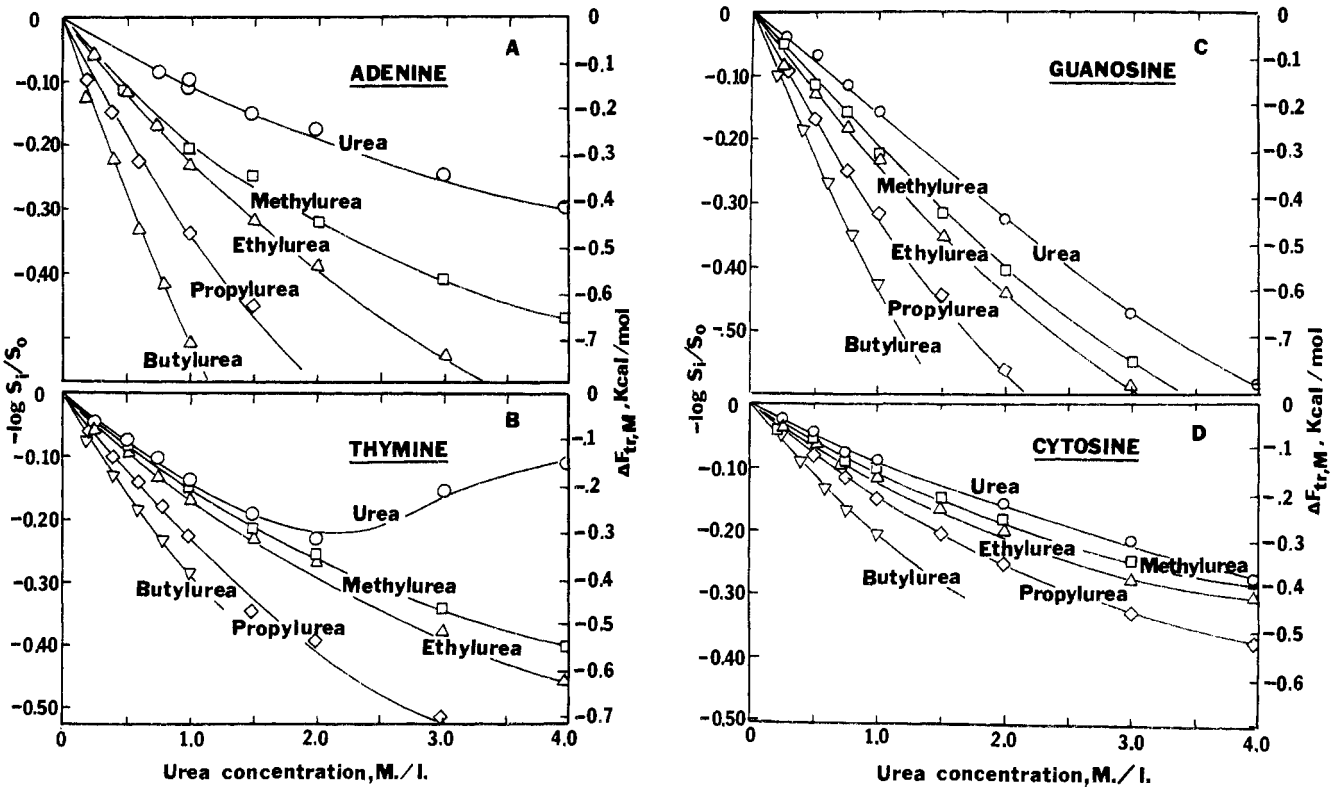


FIGURE 1: Setschenow plots and related $\Delta F_{tr.,M}$ plots of various urea solutions of (A) adenine, (B) thymine, (C) guanosine, and (D) cytosine at 25° according to eq 1 and 2 of the text.

Results

Tables I and II present the solubility data of the DNA bases together with ribose and deoxyribose derivative of adenine obtained in various aqueous urea and amide solutions at 25°. The Setschenow constants, K_s, were estimated from the initial linear portion of the log S_i/S_0 *vs.* urea or amide concentration plots based on the Setschenow equation (Long and McDevit, 1952)

$$\log (S_i/S_0) = -K_s c_s \quad (1)$$

where S_i and S_0 are the solubilities of the DNA bases or model compounds, respectively, in the presence and absence of the particular urea or amide, and c_s is the urea or amide concentration expressed in moles/liter. It should be noted that save for the constant $2.303RT$ eq 1 has the form of the more familiar free energy of transfer expression on the molar scale

$$\Delta F_{tr.,M} = -2.303RT \log (S_i/S_0) \quad (2)$$

Figures 1–3 present the solubility data obtained with some of the ureas and amides plotted according to eq 1 and 2.

Since the attachment of the ribose and deoxyribose sugars to the bases increases their solubility, the Setschenow parameters should be corrected for self-interaction. In this regard it should be noted that reliable solubility data could not be obtained with guanine alone, partly because of its low solubility, and the turbidity of the filtered solutions (Herskovits and Harrington, 1962). The solubility behavior of guanosine was investigated instead and the data obtained were corrected for self-interaction according to eq 3–6. The association of the nucleic acid bases and related compounds in aqueous solution is well documented (Ts'o, 1970; Cantor and Katz, 1971). Accordingly with self-interactions taken into account, the $K_s c_s$ term in eq 1 can be expressed as (Long and McDevit, 1952)

$$K_s c_s = k_s c_s + k_i c_i \quad (3)$$

thus

$$k_s = K_s - k_i (S_i - S_0)/c_s \quad (4)$$

and

$$\log (S_0/S_i) = k_s c_s + k_i (S_i - S_0) \quad (5)$$

where k_s is the true or corrected solvent–solute interaction constant and k_i is the solute–solute or DNA base–base self-interaction constant (Long and McDevit, 1952). The required k_i for the self-interaction correction can be estimated using eq 6 and the known activity coefficients of the bases or appropriate model compounds γ_i as a function of concentration c_i, as suggested by Robinson and Grant (1966)

$$\log \gamma_i = k_i c_i \quad (6)$$

For the calculations of the various corrected k_s data given in Table III the k_i values of −1.25 were used for adenine, adenosine, deoxyadenosine, and guanosine, −0.46, for thymine, and −0.056, for cytosine, respectively (Herskovits and Harrington, 1972). These values were based on log γ_i *vs.* c_i plots of purine, uridine, and cytidine in aqueous solutions at 25°, using the the osmotic coefficient data of Ts'o *et al.* (1963).

Table III presents both the corrected true interaction parameters, k_i, and also the K_s parameters for the various ureas and amides. It is apparent from both the data of Figures 1–3 and the data of this table that the ureas and amides increase the solubility of the bases with increasing hydrocarbon content of the cosolvent. The more negative K_s values obtained with the higher more hydrophobic members of these two series represent also more favorable ΔF_{tr} values with increasing hydrocarbon content. Attachment of the sugar moiety to the bases seems to have only a relatively

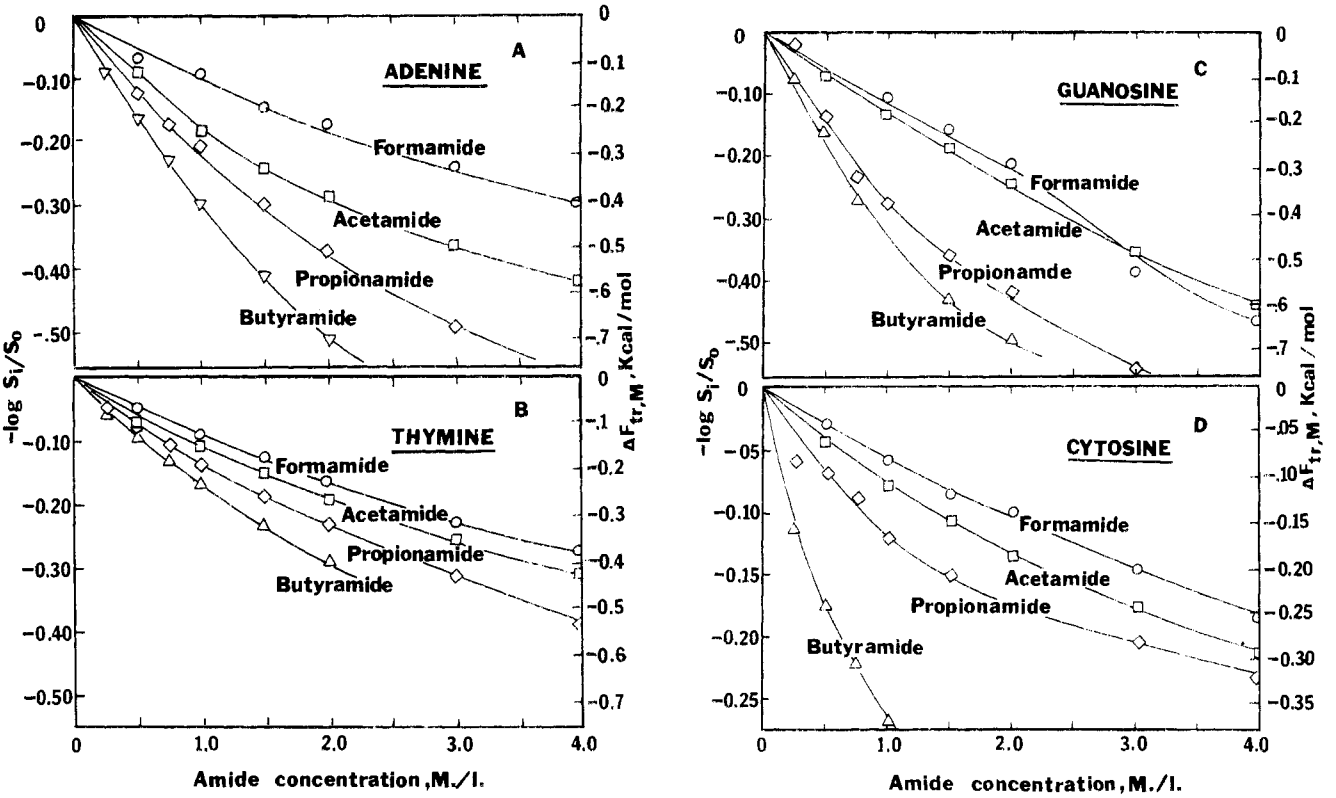


FIGURE 2: Setschenow plots and related $\Delta F_{tr,M}$ plots of various amide solutions of (A) adenine, (B) thymine, (C) guanosine, and (D) cytosine at 25° plotted according to eq 1 and 2 of the text.

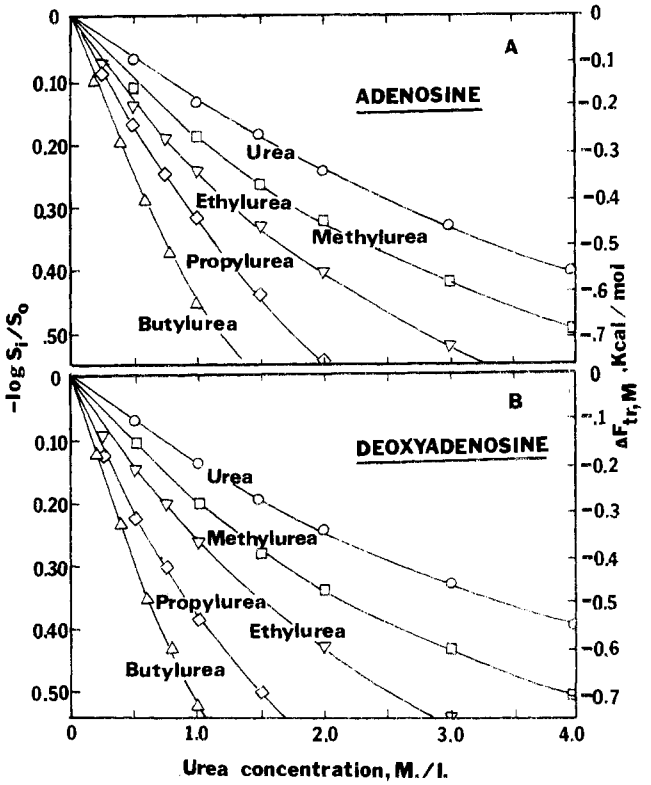


FIGURE 3: Setschenow plots and related $\Delta F_{tr,M}$ plots of various urea solutions of (A) adenosine and (B) deoxyadenosine at 25° plotted according to eq 1 and 2 of the text.

minor effect on the Setschenow parameters and the related free energies of transfer (Table III and Figures 1 and 3).

The free energies of transfer on the mole fraction scale, $\Delta F_{tr,N}$ can be expressed as (Nozaki and Tanford, 1963, 1965)

$$\Delta F_{tr,N} = -2.303RT \log N_i/N_0 + 2.303RT \log \gamma_0/\gamma_i \quad (7)$$

where N_i and N_0 and γ_i and γ_0 represent the mole fractions and activity coefficients of the solute in the aqueous urea or amide solutions and pure water, respectively. For some of our calculations (Table V and Figure 4) the purine and pyrimidine activity coefficient in water at 25° was used (Ts'o *et al.*, 1963). In the absence of any data in the urea and amide solutions actually investigated, it was assumed that the self-interactions of the base in these solutions are the same as in water at the same solute concentration (Nozaki and Tanford, 1963, 1971). Consequently, based on eq 5 and 6 one can express the correction term in eq 7 as (Herskovits and Harrington, 1972)

$$2.303RT \log (\gamma_0/\gamma_i) = -2.303RTk_i(S_i - S_0) \quad (8)$$

This correction in most cases has been found to be relatively small, *i.e.*, of the order of 2–5%.

Discussion

The enhancement of the solubility of the DNA bases and nucleosides with increasing hydrocarbon content of the urea and amide series of denaturants shown in Figures 1–3 is reminiscent of the effects of the increasing alkyl chain length of the alcohol series examined in a previous study from this laboratory (Herskovits and Harrington, 1972). Both the Setschenow and binding parameters of these series of denaturants have been used to predict their effectiveness as denaturants of globular proteins (Schrier *et al.*, 1965, Herskovits *et al.*, 1970a–c; Elbaum *et al.*, 1974) and destabilizing agents for DNA (Herskovits and Harrington, 1972). The theoretical expressions of Peller (1959) and Flory (1957) for the lowering of the melting point of a bio-

TABLE III: Solubility Parameters, K_s and k_s, of Purine and Pyrimidine Bases and of Some Ribose and Deoxyribose Nucleosides at 25.0°.[a]

Urea or Amide	Adenine		Adenosine		Deoxyadenosine	
	K_s	k_s	K_s	k_s	K_s	k_s
Urea	−0.107	−0.105	−0.133	−0.124	−0.137	0.125
Methylurea	−0.217	−0.211	−0.201	−0.188	−0.201	−0.182
Ethylurea	−0.235	−0.229	−0.262	−0.244	−0.280	−0.252
Propylurea	−0.392	−0.381	−0.337	−0.311	−0.410	−0.362
Butylurea	−0.570	−0.550	−0.480	−0.436	−0.592	−0.513
1,3-Dimethylurea	−0.320	−0.311	−0.136	−0.118	−0.174	−0.143
Formamide	−0.100	−0.098	−0.082	−0.077	−0.092	−0.082
N,N-Dimethylformamide	−0.338	−0.329				
Acetamide	−0.187	−0.182	−0.138	−0.129	−0.162	−0.147
Propionamide	−0.242	−0.237				
Butyramide	−0.338	−0.329				

	Thymine		Guanosine		Cytosine	
	K_s	k_s	K_s	k_s	K_s	k_s
Urea	−0.140	−0.136	−0.155	−0.154	−0.092	−0.084
Methylurea	−0.145	−0.140	−0.225	−0.223	−0.108	−0.098
Ethylurea	−0.172	−0.166	−0.250	−0.248	−0.128	−0.116
Propylurea	−0.230	−0.221	−0.330	−0.328	−0.163	−0.148
Butylurea	−0.310	−0.298	−0.450	−0.446	−0.228	−0.205
1,3-Dimethylurea	−0.205	−0.198	−0.330	−0.328	−0.140	−0.127
Formamide	−0.095	−0.092	−0.108	−0.107	−0.058	−0.053
N,N-Dimethylformamide	−0.188	−0.183	−0.313	−0.311	−0.182	−0.169
Acetamide	−0.115	−0.111	−0.139	−0.138	−0.084	−0.076
Propionamide	−0.168	−0.163	−0.290	−0.288	−0.122	−0.110
Butyramide	−0.192	−0.186	−0.338	−0.334	−0.360	−0.293

[a] Setschenow constants K_s estimated from initial slopes of plots of $-\log [S_i/S_0]$ *vs.* urea or amide concentration (see text and Figures 1–3). The k_s values estimated using eq 4 with the k_i values given in the text, together with the S_0 and S_i data of Tables I and II, the latter being taken mostly at 1.0 M urea or amide concentrations.

polymer, ΔT_m due to preferential binding or interaction of the denaturing agent with its denatured form can be expressed as

$$\Delta T_m = (T_m^0 - T_m) = (RT_mT_m^0/\Delta h)\bar{\nu} \ln (1 + K_B a_s) \quad (9)$$

In turn the denaturation midpoint, S_m is given by the expressions (Herskovits *et al.*, 1970a)

$$S_m = a_s = (\Delta T_m \Delta h/RT_mT_m^0\bar{\nu})/K_B \quad (10)$$

and

$$S_m = (-\Delta T_m \Delta h/2.30RT_mT_m^0\bar{\nu})/\langle k_s \rangle \quad (11)$$

where T_m and T_m^0 are the midpoints of the denaturation transition in the presence and absence of denaturant, Δh is the enthalpy change of unfolding per monomer unit of the biopolymer under study, $\bar{\nu}$ is the effective number of binding sites per monomer unit treated as an adjustable parameter (Herskovits *et al.*, 1970a–c, 1972), K_B is the association or binding constant of the denaturant with the average monomer unit, $\langle k_s \rangle$ is the Setschenow constant per average DNA base corrected for self-interaction, and a_s is the activity of the denaturant, assumed to be equal to its concentration (Schrier *et al.*, 1965; Herskovits *et al.*, 1970a–c; Elbaum *et al.*, 1974). For the calculations summarized in Table IV a Δh value of 4000 cal/mol per average DNA base was used (Bunville *et al.*, 1965), with the average Setschenow parameter, $\langle k_s \rangle$, evaluated using the k_s data of Table III and the mole fraction of the four DNA bases, f_i, according to the equation

$$\langle k_s \rangle = \sum_i f_i k_s \quad (12)$$

For T4 bacteriophage DNA the f_i values for adenine and thymine are equal to 0.32 while for guanine and cytosine these values are 0.18 (Josse *et al.*, 1961).

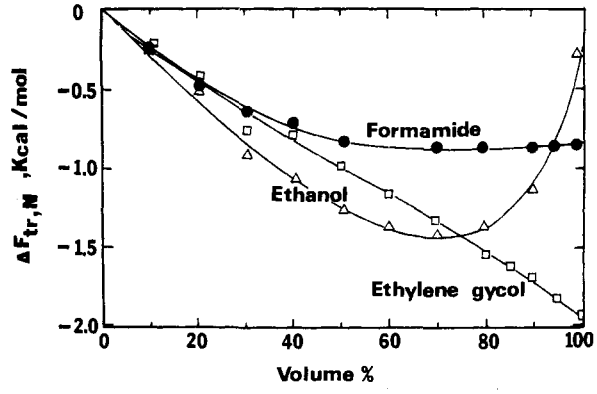


FIGURE 4: The free energies of transfer $\Delta F_{tr.,N}$ of adenine from water to formamide solution as function of formamide concentration at 25° plotted according to eq 7 of the text. For reason of comparison the alcohol and ethylene glycol data of adenine (Herskovits and Harrington, 1972) have also been included on this figure.

TABLE IV: Comparison between the Calculated and Experimental Denaturation Midpoints, S_m, of Bacteriophage T4 DNA at 73°.[a]

	R_M[b] (cm³)	$\langle k_s \rangle \times 10^2$[c]	$K_B \times 10^2$[d]	Calcd S_m (mol/l.) Eq 11 with $\langle k_s \rangle$ data	Calcd S_m (mol/l.) Eq 10 with K_B data	Exptl S_m[e] (mol/l.)
Water	3.7					
Ureas						
Urea	13.7	−1.20	3.2	1.12	1.12	1.0
1,3-Dimethylurea	21.0	−2.45	5.22	0.55	0.69	1.0
Ethylurea	22.9	−1.92	6.08	0.70	0.59	0.60
Butylurea	32.2	−3.88	23.2	0.35	0.15	(0.22)[f]
Amides						
Formamide	10.6	−0.90	2.1	1.5	1.7	1.9
N,N-Dimethylformamide	19.8	−2.50	4.12	0.54	0.87	0.60
Acetamide	19.8	−1.33	3.11	1.01	1.15	1.1
Propionamide	24.4	−2.00	4.98	0.68	0.72	0.62
Butyramide	29.0	−2.78	9.69	0.48	0.37	0.46
Alcohols[g]						
Methanol	8.2	−1.37	1.01	3.9	3.9	3.5
Ethanol	12.9	−4.60	2.88	1.26	1.35	1.2
1-Propanol	17.8	−12.4	7.59	0.47	0.51	0.54
1-Butanol	22.2	−22.7	20.0	0.26	0.20	0.33

[a] Parameters used with eq 10 and 11 were $T_m = 346.3°K$, $T_m^0 = 349.1°K$, $\Delta h = 4000$ cal/mol, $\bar{v} = 0.15$ with eq 11, and 1.3 with eq 10 for both the ureas and amides; for the alcohols the values of 0.35 and 1.2 were used, respectively (Herskovits and Harrington, 1972). [b] For urea and formamide the molar refractions, R_M, obtained by Hanlon (1966) were used; the alcohol values are from Herskovits and Harrington (1972). The higher urea and amide values were computed using the urea and formamide values plus the atomic values of 2.42 cm³ for each additional carbon and 1.1 cm³ for each additional hydrogen. [c] $\langle k_s \rangle$ values based on eq 12 and the k_s data of Table III. [d] K_B calulated assuming group additivities with 1.01, 2.88, 7.59, and 20×10^{-2} for each methyl, ethyl, propyl, or butyl group (Herskovits *et al.*, 1970a) and 0, 3.2, and 2.1×10^{-2} for each polar, -OH, -HN(CO)NH$_2$, or -CONH$_2$ group of the alcohol, urea, or amide, respectively (Herskovits *et al.*, 1970a; Elbaum *et al.*, 1974). [e] Data taken from Levine *et al.* (1963). [f] *tert*-Butylurea value of Levine *et al.* (1963). [g] Data taken from Herskovits and Harrington (1972).

In the present investigation we have obtained estimates of the denaturation midpoints for T4 bacteriophage DNA at 73° using both the average corrected Setschenow parameter, $\langle k_s \rangle$, and also the binding constants, K_B, employed in our previously published studies on the denaturation and subunit dissociation of hemoglobin and other proteins (Herskovits *et al.*, 1970a–c, Elbaum and Herskovits, 1974; Elbaum *et al.*, 1974). The same binding constants were used in our previous investigations of the effects of the alcohols and glycols on the solubility of DNA bases and their effects on the thermal stability of T4 bacteriophage DNA (Herskovits and Harrington, 1972), demonstrating the general utility of these parameters in both protein and nucleic acid studies. The additivity of the group contributions of the denaturant to K_B has been assumed in these studies (Herskovits *et al.*, 1970c, Elbaum *et al.*, 1974). Making the same assumption that the hydrophobic or nonpolar $K_{H\Phi}$ and the polar K_P contributions of the ureas or amides to K_B are additive, the binding constant to be used with eq 10 can be expressed as

$$K_B = K_{H\Phi} + K_P \qquad (13)$$

The $K_{H\Phi}$ values were estimated using the free energies of transfer of nonpolar amino acid side chains from water to alcohol (Cohn and Edsall, 1943; Tanford, 1962) and were corrected for the entropy loss of the average hydrophobic group upon binding (Schrier *et al.*, 1965; Herskovits *et al.*, 1970a).[1] Based on the protein denaturation studies, the K_P values for the alcohol, urea, and amide "polar head groups" have been taken as 0, 3.2, and 2.1×10^{-2}. The $\langle k_s \rangle$ and K_B parameters used for our calculations are given in Table IV, together with the calculated and experimental S_m data of Levine *et al.* (1963) obtained on T4 bacteriophage DNA with several of the ureas, amides, and a few of the representative alcohols. With the exception of the 1,3-dimethylurea data, the calculated and experimental S_m values are found to be in relatively satisfactory agreement, considering the various assumptions and approximations used for the evaluation of the k_s and K_B parameters (Herskovits *et al.*, 1970a, Herskovits and Harrington, 1972).

Several significant conclusions in regard to the mode of action of the ureas and amides as DNA denaturants are apparent from the data of this Table. (1) The effectiveness of the ureas and amides as denaturing, or destabilizing agents of DNA increases in a predictable way with increasing chain length or hydrocarbon content of the denaturant. (2) The hydrophobic chain length effects appear to be a general feature of the destabilization process so far encountered with all three of the series of organic denaturants—the alcohols, the ureas, and the amides—investigated in our labo-

[1] The $K_{H\Phi}$ component of the binding constant was evaluated using the standard free energy expression for binding $\Delta F^\circ_B = -RT \ln K_{H\Phi}$ where $\Delta F^\circ_B = \Delta F_{tr} - T\Delta S^\circ_B$. For these calculations ΔF_{tr} values of −610, −1180, −1750, and −2330 cal/mol were used for the CH_3, CH_2CH_3, $CH_2CH_2CH_3$, or the $CH_2CH_2CH_2CH_3$ group contributions to the free energy of binding of the various ureas or amides (Herskovits *et al.*, 1970a); an entropy loss ΔS°_B of −11 eu/mol of reagent bound was assumed (Schrier *et al.*, 1965).

TABLE V: A Comparison of the Free Energies of Transfer, $\Delta F_{tr,N}$, from Water to Alcohol, Ethylene Glycol, and Urea Solutions of Adenine, Thymine, and Representative Polar and Hydrophobic Amino Acid Groups at 25°.[a]

	Ethanol		Ethylene Glycol		
	60% (v/v)	90%	60%	90%	8 M Urea
Adenine	−1370	−1150	−1180	−1680	−730
Thymine	−750	−675	−500	−830	−230
Glycine	+1330	+3060	+605	+950	+57
Peptide Group	+65 to +425	+495 to 995	+115 to +170	+240 to +280	−145 to −310
Leucine	+520	+1325	+250	+135	−236
Leucyl side chain	−810	−1735	−355	−815	−295
Phenylalanine	+285	+1045	−60	−165	−542
Phenylalanyl side chain	−1045	−2010	−665	−1115	−600
Tryptophan	−230	+425	−705	−1395	−863
Tryptophyl side chain	−1560	−2630	−1310	−2345	−920

[a] The ethanol and glycol data for adenine and thymine are taken from Herskovits and Harrington (1972), whereas the amino acid and peptide data are taken from the papers of Nozaki and Tanford (1963,1965,1971). The latter were obtained at 25.1°.

ratory (Herskovits, 1962, 1963; Herskovits and Harrington, 1972). (3) It is possible to use the same binding constants, K_B, with the Peller–Flory theory of biopolymer destabilization (eq 10 and 11) to predict the denaturing effects of these three series of solvents of both DNA, and proteins (Herskovits *et al.*, 1970a–c, Elbaum *et al.*, 1974. (4) The $\bar{\nu}$ values of 1.2–1.3 required for the urea, amide, and alcohol calculations, that is the effective number of binding or interaction sites per average DNA base, is larger than the average number of 0.6–1.0 site found in the case of globular proteins (Schrier *et al.*, 1965; Herskovits *et al.*, 1970a–c; Elbaum *et al.*, 1974). Since the hydrophobic component of the binding interaction, as reflected by the binding term $K_{H\Phi}$ in eq 13, dominates the DNA base–solvent interaction in the case of the alcohols (*i.e.*, $K_P = 0$) and the higher members of the urea and amide series, one can reasonably argue that the hydrophobic component of the base–denaturant interactions is at least as important in nucleic acid denaturation as the average amino acid–denaturant interaction in protein denaturation.

Past studies of the denaturation of DNA by organic solvents (Herskovits *et al.*, 1961; Geiduschek and Herskovits, 1961; Helmkemp and Ts'o, 1961; Herskovits, 1962, 1963; Kurihara *et al.*, 1963; Levine *et al.*, 1963), the effects of dehydration of DNA films on its secondary structure (Falk *et al.*, 1963), and various other model investigations related to the stacking of the DNA bases in aqueous solution (see the reviews of Ts'o (1970) and Cantor and Katz (1971) have clearly shown that the supporting natural solvent, water, plays a unique role in the maintenance of the stacked conformation of the bases in the native states of the nucleic acids. All the solvents that have been examined in our laboratory are found to increase the solubility of the DNA bases and nucleosides (Tables I and II and Herskovits and Harrington, 1972) and thus lead to destabilization of stacked base conformation of the natural biopolymers. The studies of Lowe and Schellman (1972) and Johnson and Schleich (1974) have shown that organic solvents will tend to reduce the stacking enthalpy of adenine dinucleotide. Dispersion and other short range interactions that dominate the energetics of the stacking interactions in the gaseous state (DeVoe and Tinoco, 1962; Claverie *et al.*, 1966; Pullman and Pullman, 1968) should be progressively reduced in solution (Sinanoglu and Abdulnur, 1965) by similar but competing solvent–solute interactions. The stabilizing influence of the organic solvent on the unstacked conformation or the enhancement of the solubility of the DNA bases should thus correlate favorably with the polarizability or molar refraction of the solvent (R_M) as suggested by Hanlon (1966). We have included this correlation of R_M with the other denaturant binding or interaction parameters, K_B and $\langle k_s \rangle$ in Table IV, used for the quantitative examination of the DNA denaturation data.[2] With regard to water, it should be noted that no other solvent has a comparably low molar refraction. According to Hanlon (1966), it should be the least effective unstacking reagent because of its extremely low polarizability.

The Setschenow constants and the related free energies of transfer (*cf* eq 1 and 2) of the DNA bases and nucleosides examined are all negative (Figures 1–3 and Table V), indicating favorable base–organic solvent interactions. A comparison of the free energies of transfer of adenine and thymine with the transfer data of some of the hydrophobic amino acids (leucine, phenylalanine, and tryptophan) from water to alcohol, glycol, and urea solutions, presented in Table V, suggests that the relative hydrophobicities of the DNA bases should be compared to those of leucine or phenylalanine which are some of the most hydrophobic amino acid side chains involved in the stabilization of globular proteins (Kauzmann, 1959; Nemethy and Scheraga, 1962; Nozaki and Tanford, 1963, 1971). It is important to note that in case of the amino acid side chains the ΔF_{tr} presented are obtained by subtracting the ΔF_{tr} of glycine, which represents a strongly unfavorable positive contribution to the overall free energy change of the transfer reaction, from the ΔF_{tr} of the amino acid itself (Nozaki and Tanford, 1963, 1965, 1971). Such a correction for the unfavorable polar contribution of the DNA bases cannot be made since the polar and nonpolar portions of the bases are not separated physically. Despite this the free energies of transfer of the

[2] The same correlation is also applicable to protein denaturation since the same K_B parameters have been used to calculate the denaturation midpoints (Herskovits *et al.*, 1970a–c, Elbaum *et al.*, 1974).

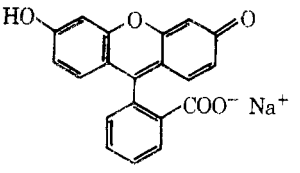
TABLE VI: Thermodynamic Parameters of Dimerization and Stacking of Selected Compounds in Aqueous Solution.[a]

Compound and Reaction	$\Delta F°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)
Fluorescein dimerization	−1.0	−7.6	−21.7
Rhodamine B dimerization	−4.4	−2.8	+5.3
Actinomycin dimerization		−15	−38
Adenylyl-(3′–5′)-adenosine stacking		−8.5	−28.5
Purine self-association	−0.44	−4.2	−13
Thymidine self-association	0.06	−2.4	−9

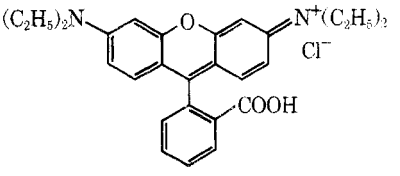
[a] The data given are from the following references: fluorescein and rhodamine B dimerization, Rohatgi and Singhal (1966); actinomycin dimerization, Crothers *et al.*, 1968; adenine dinucleotide stacking, Van Holde *et al.*, 1965; purine and thymidine self-association, Ts'o and Chan (1964) and Ts'o (1970).

DNA bases are relatively negative.

The source of the favorable free energies of transfer of nonpolar protein side chains is of entropic origin, associated with ordering of water structure around the hydrocarbon portion of the amino acids (Frank and Evans, 1945; Kauzmann, 1959; Nemethy and Scheraga, 1962). The enthalpy components of these interactions is zero or slightly positive. On the other hand, stacking interactions in the case of the nucleic acid bases and also in some of the organic dyes are characterized by favorable negative enthalpies and opposing entropies of reaction (Table VI). Perhaps it is opportune to designate the latter interactions as "solvophobic" (Lowe and Schellman, 1972) as opposed to the classical hydrophobic interactions described by Frank and Evans (1945) and Kauzmann (1959). This is not to say that positive entropy components of the stacking interactions of the DNA bases may not in fact be "hidden" by the large negative contributions produced by the polar portions of the bases, as suggested by Ts'o (1970). For example, we would expect relatively large positive contributions to the entropy of dimerization of the two ionic dyes, fluorescein (I) and rhodamine B (II), due to the water structuring of the non-



I



II

polar portions of their fused skeletal rings and the attached benzoic acid side chain. Yet only in the case of rhodamine is the entropy of dimerization actually positive. Both compounds have negative enthalpies of dimerization (Table VI). Moreover, alcohol, glycol, and urea have also been found to reduce the association of dyes (Rabinowich and Epstein, 1941; Mukerjee and Gosh, 1963; Rohatgi and Singhal, 1963).

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