The Enthalpy of Self-Association of Pyrimidine Derivatives in Water*

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ABSTRACT: The relative molal enthalpies of a series of pyrimidine compounds have been measured over a range of concentrations approaching the solubility limit in water at 25°. These measurements, when used in conjunction with the osmotic coefficient data of other workers, enable the evaluation of the enthalpy of self-association for these materials. The values of ΔH° range from -2.2 to -3.0 kcal/mole. The relatively small values found for these materials differ significantly from estimations made on oligomers. This apparently reflects a difference in the nature of the geometry or solvation of the complex.

In a previous study (Gill *et al.*, 1967) we reported enthalpy results for the self-association of selected purine compounds. We have extended these measurements to a set of pyrimidine compounds where osmotic coefficient data are available (Ts'o *et al.*, 1963; Ts'o and Chan, 1964). The theory of calculating the enthalpy of self-association from a combination of relative apparent molal enthalpy (ϕ_L) and the osmotic coefficient (ϕ) is discussed in the first paper. The basic assumption is that the heat effect of diluting a solution containing a mixture of complex molecular species is due to the dissociation of the complexes. Further the enthalpy of dissociating one molecule from a particular complex is assumed independent of the size of the complex. Under these conditions the enthalpy (ΔH°) for the general reaction

$$\mathrm{P} + \mathrm{P}_{n-1} = \mathrm{P}_n \qquad (1)$$

is given by

$$\Delta H^\circ \pm \phi_L/(1 - \phi) \qquad (2)$$

Consistency for these assumptions is tested by measurements at various concentrations.

Materials and Methods

All compounds were obtained from Sigma Chemical Co. and were dried under vacuum for 16 hr prior to use. The purity of the compounds in solution was checked by comparison (within 2%) of the molar absorbancy indices with published values (Chargoff and Davidson, 1955). The special mixing calorimeter described previously (Stoesser and Gill, 1967) was used for all dilution measurements. Solutions prepared from doubly distilled water were diluted with various amounts of water within the calorimeter. All measurements were reproducible to within 1% and were corrected for the mechanical heat of mixing of solvent with solution.

Results

In Table I are summarized the results of the calorimetric measurements, (ϕ_L), as well as the osmotic coefficient data (ϕ), and the calculated heats of self-association from eq 2.

Discussion

The enthalpy of self-association for these pyrimidine riboside compounds in water is about -2.5 kcal. This is about half that found for purine and 6-methylpurine (Gill *et al.*, 1967), but quite comparable to the purine riboside and deoxyadensosine cases. The most appropriate comparison should be with purine riboside for which recent osmotic coefficient data (Broom *et al.*, 1967), in conjunction with previous calorimetric measurements, give a consistent value of -2.5 ± 0.1 kcal for the ΔH° of self-association.

The ΔH° values obtained for cytidine, uridine, and thymidine do not show any concentration dependence over the range tested. However for 5-bromouridine the calculated ΔH° values increase as the concentration decreases. These effects agree with the observation of Ts'o who found for the first materials (cytidine and uridine) that a single constant describes the equilibria, whereas more than one constant is needed in the case of solutions of 5-bromouridine. The consequence of this situation is that the calculated values of ΔH° from the combination of calorimetric and osmotic coefficient data give a value for 5-bromouridine which depends upon the specific amount of the various species present at the given concentration. In Table II we have listed the thermodynamic information now available for purine riboside, cytidine, and uridine in water.

These particular cases are well characterized by a

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TABLE I: Relative Molal Enthalpy of Pyrimidine Ribosides in Water at 25°.

m	$-\phi_L$ (cal/mole)	ϕ (osmotic coef)	$-\Delta H$ (kcal)
	Cytidine		
0.790	931 ± 30	0.670[a]	2.82 ± 0.09
0.400	603 ± 33	0.786	2.82 ± 0.15
0.300	491 ± 35	0.826	2.82 ± 0.25
0.200	334 ± 38	0.876	2.70 ± 0.32
0.100	116 ± 45	0.935	1.79 ± 1.10
	Uridine		
0.806	721 ± 17	0.730[a]	2.67 ± 0.07
0.500	532 ± 26	0.801	2.67 ± 0.14
0.400	470 ± 30	0.833	2.81 ± 0.18
0.300	388 ± 50	0.866	2.89 ± 0.39
0.200	293 ± 70	0.901	2.96 ± 0.72
0.100	132 ± 85	0.943	2.32 ± 0.92
	Thymidine		
0.202	307 ± 7	0.862 ± 0.008[b]	2.22 ± 0.10
0.149	254 ± 7	0.887 ± 0.010	2.24 ± 0.20
0.111	223 ± 7	0.914 ± 0.014	2.59 ± 0.30
	5-Bromouridine		
0.310	705 ± 24	0.742[c]	2.73 ± 0.10
0.200	530 ± 24	0.801	2.66 ± 0.12
0.150	452 ± 24	0.844	2.90 ± 0.16
0.100	348 ± 29	0.894	3.28 ± 0.23
0.050	204 ± 32	0.948	3.92 ± 0.62

[a] Values of ϕ interpolated from results given by Ts'o *et al.* (1963). [b] Values of ϕ interpolated from results given by Solie (1965). [c] Values of ϕ interpolated from results given by Ts'o and Chan (1964).

TABLE II: Self-Association Quantities at 25°.

Material	$\Delta H°$ (kcal)	$\Delta G°$ (kcal)	$\Delta S°$ (eu)	K (m^{-1})
Purine riboside	−2.5 ± 0.1[a]	−0.380[b]	− 7	1.9[b]
Cytidine	−2.8 ± 0.1	0.080[c]	−10	0.87[c]
Uridine	−2.7 ± 0.1	0.290[c]	−10	0.61[c]

[a] Calculated from calorimetric dilution data of Gill *et al.* (1967) in conjunction with osmotic coefficient data of Broom *et al.* (1967). [b] Broom *et al.* (1967). [c] Ts'o *et al.* (1963).

multiple-step equilibria with equal equilibrium constants for each step. The determined $\Delta H°$ values are also insensitive to concentration. The enthalpies of interaction here are nearly the same within experimental error and so the differences in values of K or $\Delta G°$ are attributable to the entropy changes. The entropy effects involve a complex combination of solvent and base interactions and are difficult to interpret without more information than is currently available.

Insufficient detail of reaction processes occurring here prevents making a precise assignment relative to the measured heats with a given geometry of interaction or degree of solvation. The interaction heats measured could be due to the formation of a laterally formed hydrogen-bonded complex or to the formation of a vertically stacked complex. It should be noted that the heats of interaction reported here differ with those determined indirectly in pyrimidine ribonucleotide dimers (Brahms *et al.*, 1967). These are known to stack. However the difference in magnitude of the measured heats could be due to effects of geometry or hydration.

Theoretical calculations of interaction energies (Claverie *et al.*, 1966) suggest the importance of solvent effects and are in qualitative agreement with the enthalpy determinations. In these calculations a difference is anticipated between the cases of free monomer units and oligomers. Thus interactions occurring within polynucleotides or between other monomer species of pyrimidines may be expected to display quite different $\Delta H°$ values.

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