Thermodynamic Study of the Self-association of 6-Methylpurine in Water-1,4-Dioxane Solvent

Satoshi MORIMOTO

Research Institute for Polymers and Textiles, 1-1-4 Yatabe-Higashi, Tsukuba, Ibaraki 300-21

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Determination of the thermodynamic quantities of the self-association of 6-methylpurine in water(1)–1,4-dioxane(2) (x_2=0—0.36) mixed solvent has been made from heat of dilution measurements in various organic contents, at 25 °C. It was confirmed experimentally that the dissociation process of associating purine bases in solution is very fast by dilution. It was characteristic of this solvent system that the standard enthalpy and entropy changes, $\Delta H°$ and $\Delta S°$, of the association exhibited an abrupt behavior. These values decrease remarkably with 1,4-dioxane content up to a water-rich solvent composition below x_2=0.012. After that, they approach their relatively high values for the pure organic component, at first with a steep slope, later more gradually. This behavior is related to the structural changes of the solvent in an extremely sensitive way. In the case of water(1)–methanol(2) ($x_2<0.19$) mixed solvent system which has been investigated for comparison with the above system, minimum values of the same kind were found in the vicinity of x_2=0.12, at 25 °C.

There have been a number of studies of the self-association of purine bases in aqueous solution. So far, however, there appears to be little work[1–4] with emphasis on the solvent effects in various aqueous media. To obtain the relationships between the thermodynamic quantities of the association and the solvent characters or effects, the quantities of 6-methylpurine in water–1,4-dioxane solvent system were evaluated from directly measured integral heats of dilution and were investigated in relation to solvent composition. As the result, a characteristic behavior was found as a function of dioxane content in this system and is discussed in terms of the solvent characters.

Experimental

Materials. 6-Methylpurine from Sigma Chemical Co. (product No. M6502) was vacuum dried over P_2O_5 for about 48 h before use. The infrared spectrum of this sample agreed with the reported one.[5] The other purine derivatives, purine (No. P6880) and purine-riboside (No. P7005), were from the same source and they were subjected to the same drying and identification. Solvent water was obtained by distilling ion-exchange water from an alkaline potassium permanganate solution. 1,4-Dioxane and methanol were spectro-grade reagents of Wako Junyaku Co. Trace amounts of water remaining in them were removed by fractional distillation before use.

Heat of Dilution. The heat of dilution was measured at 25 °C by using a 8721-1 solution calorimeter of the precision calorimetry system (LKB produkter AB). At the temperature, the enthalpy of solution of tris(hydroxymethyl)-aminomethane (NBS, 724a sample) in 0.100 M HCl was found to be −29.7704 ±0.0264 kJ/mol; this agrees well with the reported values.[6] By this calibration, it was confirmed that this calorimeter was operating normally. In measuring the heat of dilution of each purine derivative solution, about 1 cm³ of sample solution was diluted in about 100 cm³ solvent with stirring at 500 r.p.m. Integral heats of dilution ΔH_∞ to zero concentration were obtained by normalizing the heats of dilution $\Delta h/n_i$ to finite dilution, where Δh is the actually observed heat and n_i is the number of moles in the initial solution. Assuming that the value $\Delta h/n_i$ at low concentrations would decrease linearly, it is regarded that the corrected heat of dilution to zero concentration added to the $\Delta h/n_i$ value is equal to the ΔH_∞ value. The calorimetric result in a typical system is shown in Table 1. Here, in the highest dilution process No. 7, the heat of dilution from m_f=0.000367 to zero concentration was obtained by extrapolating the two $\Delta h/n_i$ values at m_i=0.03977 and 0.000367 to zero concentration; all the ΔH_∞ values in this series were calculated on the basis of this corrected value, where m is molarity (mol/kg solvent) and suffixes i and f are for the initial and final situations. The heats of solution were measured at 25 °C by means of the same calorimeter and the values at infinite dilution were determined according to the same procedure.

Results

It should be noted, at first, that the dissociation process of associating purine bases in solution is very fast by dilution. It is therefore suitable to measure the dissociation enthalpy by using this calorimeter with a

TABLE 1. AN EXAMPLE OF VALUES OF THE HEAT OF DILUTION OF 6-METHYLPURINE IN WATER–1,4-DIOXANE (6.166 mol kg^{-1}) MIXED SOLVENT AT 25 °C

No.	Weight of solution / g	$m_i \cdot 10$ / mol kg^{-1}	$m_f \cdot 10^3$ / mol kg^{-1}	Δh / J	$\Delta h/n_i$ / kJ mol^{-1}	ΔH_∞ / kJ mol^{-1}
1	0.93085	6.6769	5.704	3.0449	5.2661	5.3115
2	0.48915	5.4221	2.408	1.1786	4.6012	4.6217
3	0.97957	4.2733	3.820	1.6293	4.0120	4.0482
4	0.95154	3.1753	2.794	0.9994_2	3.3073	3.3367
5	0.93990	1.6280	1.444	0.3581_9	2.1184	2.1374
6	0.84212	0.7081	0.570	0.1142_1	1.2398	1.2499
7	0.96375	0.3977	0.367	0.0704_1	0.7723	0.7795

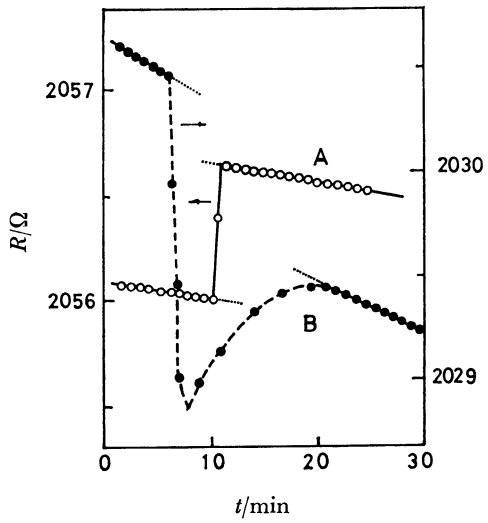

Fig. 1. Examples of thermistor resistance-time curves of dilution process, $55\Omega/K$ ($=\Delta R/\Delta T$) at 25 °C.
A: 6-Methylpurine in water–1,4-dioxane, B: 2-Pyridinol in 1,4-dioxane.

constant environment temperature. An example of the measured thermistor resistance of the dilution process for the 1,4-dioxane aqueous solution of 6-methylpurine against time is shown in Fig. 1 as curve A; the fast dissociation rate can be recognized from this curve.

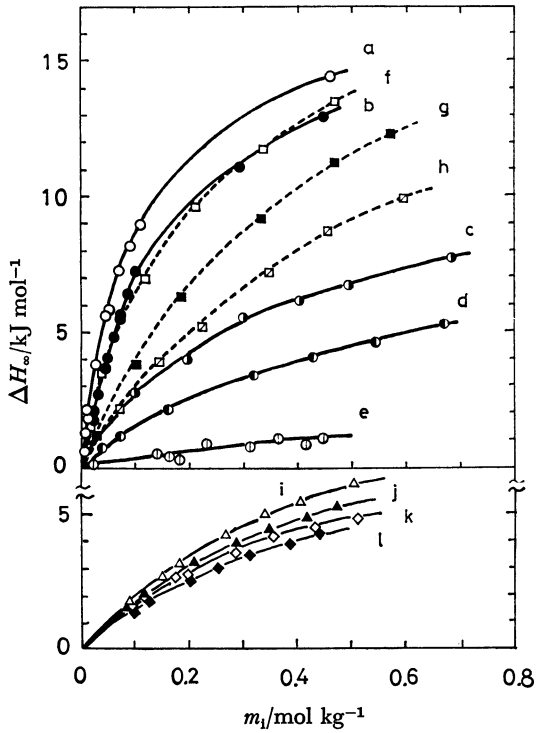

Fig. 2. Concentration dependence of the integral heats of dilution, at 25 °C.
Molarity of organic component (mol/kg water) in aqueous solvent: for 6-methylpurine solute systems; 1,4-dioxane aqueous, (a) 0, (b) 0.6400, (c) 2.761, (d) 6.166, (e) 19.58; methanol aqueous, (f) 1.423, (g) 5.359, (h) 10.67; for purine solute systems; 1,4-dioxane aqueous, (i) 0, (j) 0.6400; for purine–riboside solute systems; 1,4-dioxane aqueous, (k) 0, (l) 0.6400.

In the same figure, another example is shown: this is the dilution curve (B) of 2-pyridinol in 1,4-dioxane solvent, which can be ascribed to the association forming of the hydrogen-bonded dimers.[7] This may be compared with the above 6-methylpurine solution system. Curve B appears to reflect two thermal effects, which consist of exothermic dilution and endothermic dissociation enthalpies, and to have a time lag. The dissociation process of associating purine bases in organic aqueous solution by dilution was shown to be monotonous and very fast in this study.

In Fig. 2, the ΔH_∞ data in this work are all plotted against initial solute molarity m_i of solution. All the values are endothermic and gradually decrease with the ratio of organic component in mixed solvent, and all the curves intersect at the point zero. Curves k and l of solute purine-riboside have lower ΔH_∞ values, as compared with curves i and j of solute purine. This is probably an effect of steric hindrance from the association due to the large ribosil groups, as has been suggested by Marenchic and Sturtevant.[3]

Ts'o and Chan[8] have shown that 6-methylpurine is uncharged and that the equilibrium between various associated species in water can be characterized by a single equilibrium constant. Furthermore, the association of this monomeric solute has been supposed to proceed to an indefinite degree.[8,9] For an ideal self-association process, the ΔH_∞ values per mol of solute monomer would follow Eq. 1, as Stoesser and Gill have shown.[9] The data in Fig. 2 were all fitted

$$\Delta H_\infty = -\Delta H^\circ + (-\Delta H^\circ/K)^{1/2}(\Delta H_\infty/m_i)^{1/2} \quad (1)$$

in Fig. 3 according to this equation, where K is the equilibrium constant, and ΔH° the standard enthalpy change of association. As shown by the straight lines

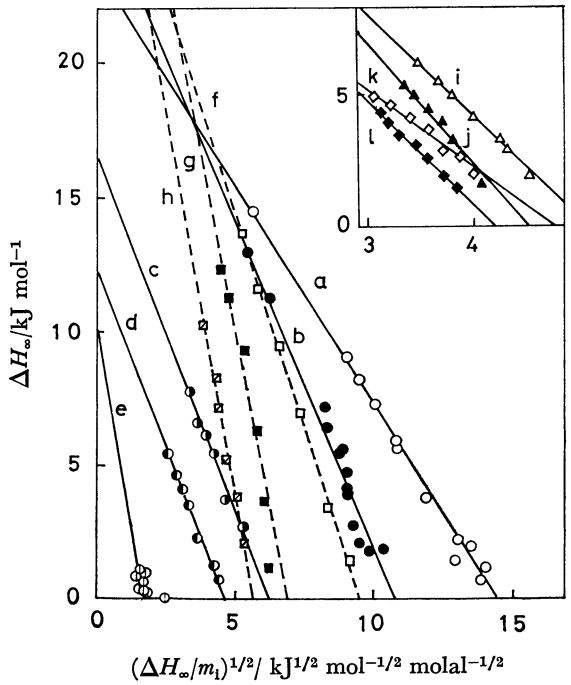

Fig. 3. Integral heat of dilution ΔH_∞ plotted against $(\Delta H_\infty/m_i)^{1/2}$, according to Eq. 1. All symbols are referred to Fig. 1.

Table 2. Evaluated thermodynamic quantities of the self-association of 6-methylpurine in water–1,4-dioxane and –methanol solvents, at 25 °C

Organic component in solvent		K	$-\Delta G^\circ$	$-\Delta H^\circ$	$-\Delta S^\circ$
mol kg^{-1}	x_2[a]	mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	J deg^{-1} mol^{-1}
0	0	8.55±0.81	5.31±0.21	23.6±0.6	61.1±1.3
0	0	(8.6 ±1.1)	(5.36±0.42)	(23.4±0.8)	(60.7±2.9)[9]
0	0	(7.75±1.6)	(5.06±0.29)	(23.3±0.2)	(61.1±0.4)[3]
[1,4-Dioxane]					
0.6400	0.0115	4.42±1.00	3.68±0.50	26.1±1.7	75.3±2.9
2.761	0.0497	2.27±0.27	1.72±0.04	16.8±1.1	50.6±3.8
6.166	0.1111	1.74±0.14	1.38±0.20	12.3±0.6	36.8±1.7
19.58	0.3527	0.42±0.24	2.30±1.26	10.4±2.9	27.2±13.8
[Methanol]					
1.423	0.0256	3.12±0.12	2.76±0.04	30.3±0.3	92.5±1.3
5.359	0.0965	1.09±0.15	0.21±0.33	41.5±1.7	139 ±7
10.67	0.1922	1.05±0.13	0.08±0.42	40.8±1.4	136 ±6

a) Mole fraction of organic component.

Table 3. Evaluated thermodynamic quantities of the self-association of purine derivatives in water–1,4-dioxane solvents, at 25 °C

Organic component in solvent		K	$-\Delta G^\circ$	$-\Delta H^\circ$	$-\Delta S^\circ$
mol kg^{-1}	x_2[a]	mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	J deg^{-1} mol^{-1}
[Purine]					
0	0	1.28±0.11	0.63±0.21	19.5±0.42	47.7±16.7
0.6400	0.0115	1.04±0.13	0.08±0.29	20.4±0.62	65.3± 1.1
[Purine-Riboside]					
0	0	1.60±0.31	1.17±0.42	13.9±0.6	42.7± 0.6
0.6400	0.0115	1.05±0.12	0.13±0.25	16.6±0.5	55.2± 0.8
[6-Dimethylaminopurine][3]					
0	0	61.7±1.0	10.2±0.04	38.1±0.42	94.1± 1.3
0.64	0.012	26.3±0.03	8.08±0.04	39.3±0.42	105 ± 0.8

a) Mole fraction of organic component.

in Fig. 3, the ΔH_∞ values determined for all systems in this study satisfy the equation under the assumption of the ideal process. Values of K and ΔH° were obtained from Eq. 1, and values of standard free energy change ΔG° and of entropy change ΔS° of association which were derived from these are shown in Tables 2 and 3 with the values for a few reported systems. These values in 6-methylpurine–water system in Table 2 agree well with the reported values determined by flow calorimetry[3,9] and the results in purine and purine-riboside–water systems in Table 3 agree approximately with reported values[10–13] determined by other methods, although the concentration ranges were slightly different.

Discussion

All the integral heats of dilution were in good agreement with ideal solution behavior, as is evident from Fig. 3. However, this result contains two problems, at least. One of them is the concentration range. It is better to measure the heat of dilution at very low concentrations[9] where more ideal solution behavior always occurs and dimerization becomes predominant. However, this could not be done here because it was difficult to obtain the heat of dilution at very low concentrations in this mixed solvent system. The other problem is the heat effect, which is the heat of dilution of solute species in each solvent. However ,as shown earlier,[14,15] the heat effect in this study can be taken to be insignificant compared with the experimental ΔH_∞ values. From Eq. 9 in Ref. 16, this effect is sufficiently less than 1% of each ΔH_∞ value even if the enthalpy parameter χ_H, for example, equals 2, which is quite large for a solute-solvent system which does not have particular interactions. This discussion will be done, therefore, on the basis of the thermodynamic quantities in Tables 2 and 3; we wish to focus attention on their solvent composition dependence.

Thermodynamic quantities evaluated in this study are shown in Fig. 4, including the values reported in references, as a function of solvent composition. The higher organic component systems were impossible to measure due to their solubilities. Our result, which covers a more extensive organic composition range in aqueous solvent, therefore helps to clarify the solvent effects of the association. In Fig. 4, thermodynamic values of the association in the same solvent system vary with the kinds of purine derivatives. The absolute values of 6-methylpurine are smaller than those of the other purine derivatives in the figure, *e.g.* 6-dimethylaminopurine, and they

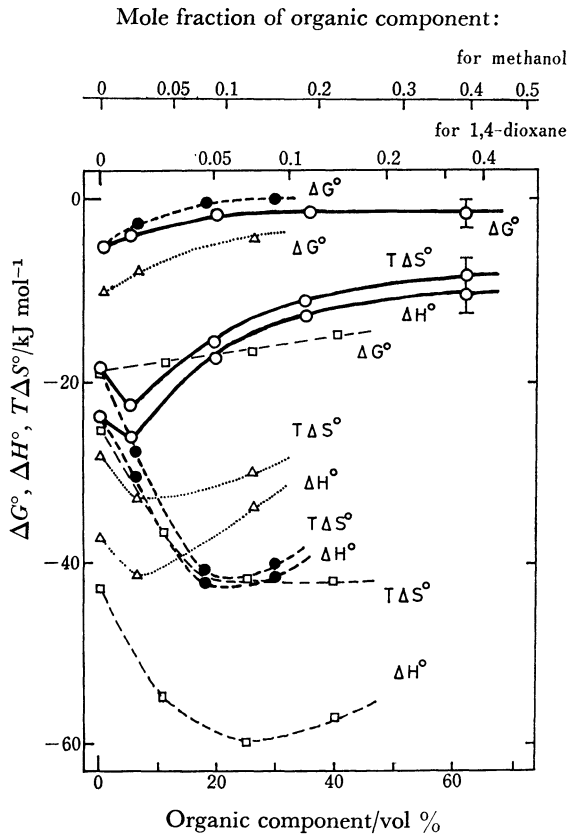

Fig. 4. Evaluated thermodynamic quantities of association plotted against organic component in aqueous solvent: (-○-) 6-methylpurine in 1,4-dioxane–, (-●-) 6-methylpurine in methanol–, (-□-) actinomycin-deoxyguanosine in methanol–[2], and (-△-) 6-dimethylaminopurine in acetonitrile–water[3] systems.

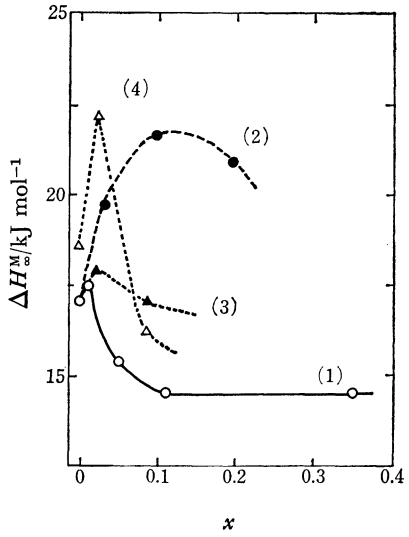

Fig. 5. Heats of solution ΔH^{M}_{∞} in purine derivatives–aqueous solvent systems, at 25 °C, plotted against mole fraction x of organic component in aqueous solvent: (1) 6-methylpurine–1,4-dioxane–, (2)–methanol–, (3)–acetonitrile– and (4) 6-dimethylaminopurine–acetonitrile–aqueous solvent systems.

are larger than those of purine in Table 3. These decreased and increased associations, as described by Marenchic and Sturtevant,[3] can be attributed mainly to the decreased and increased polarizabilities of the π-electron system[16] of these purine derivatives. Some part of the increasing and decreasing associations with variety of solvent system is presumed to be attributed to hydrophobic interactions,[17] which supposedly promote the stacking reactions of purine bases in aqueous solutions. Quantitative details of these phenomena must be left for a future study, which will compare the theoretical aspects[18–20] and the needed experimental quantities.

Recently, Leifer *et al.*[4] have studied aqueous purine solutions in the absence and presence of NaCl by vapor-pressure osmometry and high-resolution proton-magnetic-resonance spectroscopy. They have found that there is a sharp decrease in purine stacking at temperatures above 42 °C in the presence of NaCl; this salt-induced destacking of purine is consistent with the salt-induced breaking of the structure of solvent bulk water at the higher temperatures. This phenomenon is suggestive of biological systems and it must be emphasized that the solvent structure plays an important role in the association behavior. From our result, such a characteristic behavior is disclosed in the water–1,4-dioxane system. The values of $\Delta H°$ and $\Delta S°$ decrease remarkably with 1,4-dioxane content in the aqueous solvent until a water-rich binary solvent composition is reached. After the decrease, the values increase steeply, then approach gradually to their relatively high values, *i.e.*, low negative values, for pure dioxane. This tendency resembles the tendency of heat effects which occur from the solute-solvent interactions due to adding a third component to aqueous binary solvents,[21–24] although the decreasing and increasing tendencies of this study are of course opposite to the latter.

The heats of solution, *i.e.*, heats of mixing ΔH^{M}_{∞} at infinite dilution, of 6-methylpurine and 6-dimethylaminopurine in water or in some water-organic component solvents have thus been measured at the same temperature to determine the solvation effects on the $\Delta H°$ behavior. The results are shown in Fig. 5 as a function of mole fraction x of organic component in aqueous solvent. One can recognize the abrupt behavior similar to that of $\Delta H°$. Values of ΔH^{M}_{∞} do not agree, of course, with those of $\Delta H°$ even if their absolute values are compared. Fig. 6 shows a comparison of $\Delta H°$ with $-\Delta H^{M}_{\infty}$ in 6-methylpurine–1,4-dioxane aqueous solvent system. The heats of solution ΔH^{M}_{∞} would be more subjected to effects of solvation than the $\Delta H°$ because of the surface dimensions of the solute molecule. In the case of ΔH^{M}_{∞}, the solvation would be done all over the molecular surface including the hydrophilic part of solute, which is outside of the hydrophobic part which participates in the self-association. The difference between $\Delta H°$ and $-\Delta H^{M}_{\infty}$, consequently, indicates the contribution to the interaction energy of the hydrophilic part of solute. Some problems, for example, with the cavity term, the entropy term, and the relaxation term of association in the theoretical treatments[18,20] can be eliminated by this comparison between the two values, $\Delta H°$ and ΔH^{M}_{∞}. In Fig. 6, the difference between two curves appears to change the contribution from exother-

mic in water or water-rich binary solvent to endothermic in the higher organic contents. Therefore, the dependence of ΔH° on solvent composition is more remarkable than that of ΔH^{M}_{∞}. For the dissociation process of a third component, the maxima of enthalpy and entropy changes in water-rich binary solvents occur in the position of maximum structuredness of the solvent and, at this position, the interactions with solvent or the solvations of solvent molecules are minimized as a result of the solvent structure. The data of heat of mixing show that addition of 1,4-dioxane[25] or methanol[26] to pure water causes at first an increase in the solvent structuredness. The ΔH° and $T\Delta S^\circ$ curves in 1,4-dioxane, or methanol aqueous solution systems in Fig. 4, may be closely related to such variation of solvent structures.

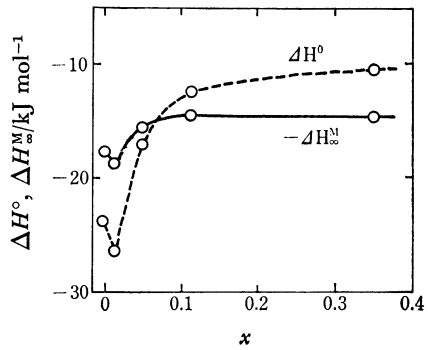

Fig. 6. Comparison of ΔH° with $-\Delta H^{M}_{\infty}$ in 6-methylpurine–1,4-dioxane aqueous solvent system, at 25 °C.

The heats of vaporization of purine derivatives have been reported to be about 80—130 kJ/mol.[27] That of 6-methylpurine has not yet been made clear, but the association heat of 6-methylpurine in gas phase will probably exhibit a similarly large negative value. From the trends shown in Figs. 5 and 6, it is assumed in this 6-methylpurine aqueous solution that the contributions of solvation or de-solvation due to hydrophobic interactions are strongly connected with the ΔH° values. The ΔH° behavior with organic component seems to be more influenced by the behavior of the other contributions, which reflect the variation of solvent structure. It could be considered that the characteristic behavior of ΔH° and ΔS° with the organic content generally come from the nature or the structure of the bulk solvent itself. The effects of solvent atmosphere, and especially the effects of the variation of solvent structures on the association, would considerably affect both ΔH° and ΔS° behaviors.

From Tables 2 and 3, a characteristic feature is recognized for purine and three purine derivatives: 6-methylpurine, 6-dimethylaminopurine, and purine-riboside, in water and 0.64 mol kg^{-1} 1,4-dioxane–water systems. Although the hydrophobic strength of the purine rings is considered to be different for each purine derivative, the thermodynamic values of association ΔH° and ΔS° in both solvent systems were almost identical, irrespective of the sort of purine derivatives. In all four systems, the entropy changes in aqueous solution of 0.64 mol kg^{-1} of 1,4-dioxane showed a decrease of about 12 to 17 J/deg mol, and the enthalpy changes showed a decrease of about 2.5 kJ/mol, in comparison with the ones of the system in pure water. These significant differences may be ascribed to the solvation changes of purine rings, and to the variation of bulk solvent structures around the dissolved purine derivative solutes. As a result, a characteristic abrupt change in thermodynamic quantities was found to be expressible as a function of 1,4-dioxane content in this solution system.

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