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Studies on the Aqueous Solutions of Guanidinium Salts. VII. Activity Coefficients and Partial Molar Volumes for Guanidinium Salts with Hydrophobic Counter Anions in Aqueous Solutions

Koichiro MIYAJIMA,* Hiromitsu YOSHIDA, and Masayuki NAKAGAKI
Faculty of Pharmaceutical Sciences, Kyoto University,
Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto 606

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Mean activity coefficients ($\gamma_\pm$) and partial molar volumes of guanidinium salts with hydrophobic counter anions were measured by isopiestic comparison method and densitometry, respectively. The values of $\gamma_\pm$ were low and the order of $\gamma_\pm$ values found to be acetate>propionate>benzoate. The b_v values from the Redlich-Rosenfeld's equation were slightly positive at 15 and 25 °C, and decreased with increased temperature. These results were interpreted in terms of the unique structure breaking action of guanidinium ions and the interionic interactions.

In a previous paper,[1] activity coefficients and partial molar volumes for guanidinium salts with hydrophilic counter anions in aqueous solutions were reported. The low mean activity coefficients ($\gamma_\pm$) of these salts were attributed to the structure breaking actions of these salts. The order of $\gamma_\pm$ values among these salts with different anions was interpreted as the interactions between the hydrated anions and cations. Among these salts, guanidinium acetate had the largest value of $\gamma_\pm$. The acetate ion has a methyl group which is different from other counter anions used in the previous studies.

To further investigate the properties of aqueous guanidinium ions and their interactions, mean activity coefficients and partial molar volumes of some guanidinium salts with hydrophobic counter anions were obtained by the isopiestic comparison method and densitometry, respectively. The results were interpreted in terms of the structure breaking action of the guanidinium ion and the interionic interactions.

Experimental

Materials. Guanidinium acetate, propionate, and benzoate were prepared by titrating aqueous guanidine solution with the corresponding carboxylic acid below 25 °C. These salts were recrystallized twice or more from an ethanol-ether mixture, and the elementary analyses are shown in Table 1. Reagent grade potassium chloride was used without further purification for the reference solute in the isopiestic measurements. These salts were dissolved in redistilled and deionized water.

Method. Osmotic coefficients were measured at 25±0.02 °C by the isopiestic method described elsewhere.[1] From the osmotic coefficient (ϕ), $\gamma_\pm$ was calculated from

$$\ln\gamma_\pm = (\phi-1)+2\int_0^{\sqrt{m}}\frac{\phi-1}{\sqrt{m}}\mathrm{d}\sqrt{m}, \tag{1}$$

where m is the molality. Density measurements were made at 15, 25, and 35±0.01 °C using a Seiko SDM-410 densimeter. Buoyancy corrections were made for guanidinium salts on the assumption that the densities of the solid states were all unity. From the densities of aqueous solutions, the apparent molar volume (ϕ_v) and the partial molar volume ($\bar{V}_2$) were calculated by

$$\phi_v = \frac{1000(d_0-d)}{Cd_0}+\frac{M}{d_0}, \tag{2}$$

$$\bar{V}_2 = \phi_v + \frac{1000-C\phi_v}{2000+C^{3/2}(\partial\phi_v/\partial\sqrt{C})}C^{1/2}\left(\frac{\partial\phi_v}{\partial\sqrt{C}}\right), \tag{3}$$

where d and d_0 are the densities of solution and solvent, respectively, and M and C are the molecular weight and molarity of the salt, respectively. The densities and the isopiestic molalities of aqueous solutions are shown in Tables 2 and 3. The reproducibility of the density measurements was found to be 5×10^{-5} g/cm^3 and the experimental determination of isopiestic concentrations was precise to 0.2%. Viscosities of aqueous BSA solutions were measured by using a Ubbelohde type viscometer at 25±0.01 °C. The reproducibility of flow time was found to be 5×10^{-2} s.

TABLE 1. RESULTS OF ELEMENTARY ANALYSES OF GUANIDINIUM SALTS (%)

Compound		H	C	N	O
Guanidinium	(Exp)	7.49	30.23	35.34	26.94
acetate	(Calcd)	7.62	30.25	35.27	26.86
Guanidinium	(Exp)	8.19	36.24	31.86	23.71
propionate	(Calcd)	8.33	36.08	31.56	24.03
Guanidinium	(Exp)	6.23	52.78	23.03	17.96
benzoate	(Calcd)	6.12	53.03	23.09	17.56

Results and Discussion

Mean Activity Coefficients. The values of the mean activity coefficients and the osmotic coefficients are shown in Table 4. The plots of log $\gamma_\pm$ *vs.* concentration for guanidinium salts are shown in Fig. 1 together with those of the sodium salts of fatty acids.[3] Generally these curves reflect the ion-ion and ion-solvent interactions. All curves approach the Debye-Hückel limiting line (DH-L) from above at low concentrations, indicating the absense of ion pairs, as with cadmium sulfate. The relatively low values of log $\gamma_\pm$ for guanidinium salts compared with those of the sodium salts are attributed to the structure breaking action of the guanidinium ion. As seen in Fig. 1, the log $\gamma_\pm$ values of sodium acetate are lower than those of sodium propionate, while the log $\gamma_\pm$ values of guanidinium acetate are larger than those of guanidinium propionate. This may be explained as follows: The sodium ion is a hydrophilic structure maker in contrast with the guanidinium

TABLE 2. DENSITIES OF AQUEOUS GUANIDINIUM SALTS AT VARIOUS TEMPERATURES

Guanidinium acetate		Guanidinium propionate		Guanidinium benzoate	
m	*d*(g/cm^3)	*m*	*d*(g/cm^3)	*m*	*d*(g/cm^3)
		at 15 °C			
0.05053	1.00063	0.05017	1.00064	0.05023	1.01539
0.07051	1.00123	0.07079	1.00124	0.06993	1.00238
0.09996	1.00211	0.10020	1.00207	0.10017	1.00375
0.15122	1.00355	0.15380	1.00364	0.15306	1.00614
0.19923	1.00492	0.20082	1.00496	0.20140	1.00827
0.29949	1.00782	0.30072	1.00770	0.50279	1.02072
0.49330	1.01302	0.40013	1.01036	0.70189	1.02830
0.70205	1.01833	0.50772	1.01311	0.98495	1.03751
1.00361	1.02554	0.70418	1.01797		
		1.00495	1.02491		
		at 25 °C			
0.05053	0.99855	0.06513	0.99886	0.05082	0.99931
0.07051	0.99910	0.07127	0.99900	0.06896	1.00009
0.09996	0.99991	0.09772	0.99970	0.10013	1.00143
0.15123	1.00133	0.19809	1.00240	0.20351	1.00578
0.19922	1.00266	0.26313	1.00384	0.29993	1.00971
0.29949	1.00534	0.27039	1.00402	0.48480	1.01693
0.49330	1.01033	0.29795	1.00502	0.68487	1.02426
0.70205	1.01543	0.48203	1.00967	0.96872	1.03378
1.00361	1.02232	0.65695	1.01388		
		0.82827	1.01778		
		at 35 °C			
0.04709	0.99544	0.05017	0.99548	0.05023	0.99548
0.09958	0.99683	0.07079	0.99603	0.06993	0.99603
0.18908	0.99902	0.10020	0.99747	0.10017	0.99830
0.29004	1.00188	0.15380	0.99835	0.15304	1.00047
0.36268	1.00450	0.20082	0.99955	0.20140	1.00250
0.50355	1.00737	0.30072	1.00210	0.29913	1.00636
0.60763	1.00992	0.40013	1.00464	0.50279	1.01401
0.71540	1.01258	0.50772	1.00721	0.70189	1.02108
0.89557	1.01682	0.70418	1.01174	0.98495	1.03066
1.00320	1.01893	1.00495	1.01823		

TABLE 3. MOLALITIES OF ISOPIESTIC SOLUTIONS

Potassium chloride *m*	Guanidinium propionate *m*	Guanidinium benzoate *m*	Potassium chloride *m*	Guanidinium propionate *m*
0.1866	0.1968	0.2015	1.0558	1.1485
0.3299	0.3383	0.3736	1.7446	2.0086
0.3665	0.3787	0.4213	1.9271	2.2506
0.4235	0.4412	0.4960	2.5623	3.1653
0.4286	0.4437	0.4998	3.3918	4.4503
0.4828	0.5125	0.5791	3.7193	5.0231
0.5175	0.5352	0.6174	4.0070	5.5185
0.5341	0.5593	0.6556		
0.5899	0.6239	0.7392		
0.8179	0.8763	1.1204		
0.9087	0.9775	1.2569		

ion which is a structure breaker.[1,4–6] It is well known that a hydrophobic ion forms water structures around itself, which is so called "iceberg"[7] or "hydrophobic hydration."[8] The structure of the hydrated cosphere around the sodium ion and that of the hydrophobic ion are incompatible with each other and result in "structure salting out."[9] This explains why the $\gamma_\pm$ values of sodium propionate are larger than those of sodium acetate. The guanidinium ion is a large monovalent organic ion whose shape and size are quite similar to that of urea and its electric field to orient water molecules radially around itself is weaker than

TABLE 4. OSMOTIC AND ACTIVITY COEFFICIENTS OF GUANIDINIUM SALTS AT 25 °C

m	ϕ	$\gamma_\pm$	m	ϕ	$\gamma_\pm$	m	ϕ	$\gamma_\pm$
			Guanidinium propionate					
0.1	0.913	0.745	0.9	0.839	0.536	3.0	0.754	0.386
0.2	0.898	0.689	1.0	0.832	0.523	3.5	0.741	0.367
0.3	0.886	0.652	1.2	0.821	0.502	4.0	0.730	0.350
0.4	0.877	0.624	1.4	0.811	0.483	4.5	0.719	0.335
0.5	0.868	0.601	1.6	0.802	0.466	5.0	0.710	0.324
0.6	0.860	0.582	1.8	0.793	0.451	5.5	0.701	0.311
0.7	0.852	0.565	2.0	0.785	0.437			
0.8	0.845	0.550	2.5	0.768	0.409			
			Guanidinium benzoate					
0.1	0.891	0.713	0.5	0.767	0.485	0.9	0.691	0.383
0.2	0.845	0.624	0.6	0.746	0.454	1.0	0.675	0.365
0.3	0.817	0.567	0.7	0.726	0.427	1.2	0.645	0.333
0.4	0.790	0.521	0.8	0.708	0.404			

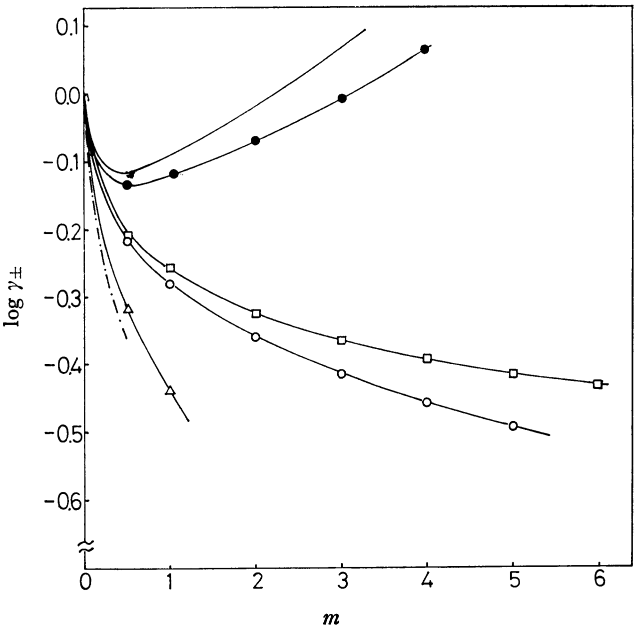

Fig. 1. Relations between log $\gamma_\pm$ and the molalities for various guanidinium salts and sodium salts at 25 °C. ——: Sodium propionate, —●—: sodium acetate, —□—: guanidinium acetate, —○—: guanidinium propionate, —△—: guanidinium benzoate, —·—: DH-L.

that of the alkali-metal ion. The guanidinium ion, however, has the ability to form hydrogen bonds with water molecules like urea, and the orientation of the hydrogen bonds with water does not fit the three dimensional network of ice-like structure. This leads to the conclusion that the structure breaking action of the guanidinium ion is different from that of the alkali-metal ions, such as Rb^+ and Cs^+. In fact, alkali halides and guanidinium halides behave in a different way when they are mixed with hydrocarbon gases[10] or symmetrical tetraalkylammonium halides[11] in aqueous solutions. Large alkali-metal halides produce salting out effects towards hydrophobic solutes,[11] while guanidinium halides produce salting in effects.[10,12]

The order of the $\gamma_\pm$ values among guanidinium salts with hydrophobic anions may be explained in two ways. As discussed above, the guanidinium ion breaks the structure of the bulk water which is in equilibrium with the structured water around the hydrophobic solute. This structure breaking action of the guanidinium ion is more pronounced in the highly structured solution containing the hydrophobic solute and results in the decrease of the $\gamma_\pm$ values of the salts. A second explanation is based on the more direct interaction between the guanidinium ion and the hydrophobic anion. Assuming that the hydrogen bond participates in the interaction between the guanidinium ion and the hydrated water molecules around it, may be possible to share the hydration cosphere with that of the hydrophobic anion. This interaction works as an attractive rather than a repulsive force and decreases the $\gamma_\pm$ values of the hydrophobic salts of guanidine. Although there is no evidence to support such an interaction, it cannot be neglected since the hydrophobic tetraalkylammonium halides form crystalline complexes with guanidinium halides and water,[13] and the $\gamma_\pm$ values of either salts in aqueous ternary solutions decrease each other as compared with those in each binary solution.[12] We cannot decide which of these mechanisms is really working at the present stage. Judging from the hydrophobicities of the counter anions, the $\gamma_\pm$ values of these salts should decrease in the order benzoate, propionate, and acetate in either mechanism.

Partial Molar Volumes of Guanidinium Salts. In general, the apparent molar volume (ϕ_v) of the salt may be expressed by Masson's equation (Eq. 4) or Redlich-Rosenfeld's equation (Eq. 5).[14]

$$\phi_v = \phi_v^0 + S_v^*\sqrt{C} \tag{4}$$

$$\phi_v = \phi_v^0 + S_v\sqrt{C} + b_vC \tag{5}$$

In these equations, ϕ_v is the apparent molar volume at infinite dilution and equal to $\bar{V}_2^0$, the partial molar volume at infinite dilution, C is the molarity and S_v^* is the experimental slope, and S_v is the constant obtained from the free energy change on pressure assuming that the $\gamma_\pm$ obeys the Debye-Hückel limiting law.[15] Therefore, b_v is the constant concerning the deviation of

TABLE 5. $\bar{V}_2^0$, S_v^*, AND b_v VALUES FOR GUANIDINIUM SALTS IN AQUEOUS SOLUTIONS AT VARIOUS TEMPERATURES

	Guanidinium acetate	Guanidinium propionate	Guanidinium benzoate	Temp (°C)
$\bar{V}_2^0$ (cm^3/mol)	88.3	102.1	113.6	15
S_v^* ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-2}}$)	2.0	2.2	2.4	
b_v ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-3/2}}$)	0.3	0.5	0.7	
$\bar{V}_2^0$ (cm^3/mol)	90.0	103.6	136.4	25
S_v^* ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-2}}$)	2.0	2.0	2.1	
b_v ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-3/2}}$)	0.1	0.2	0.2	
$\bar{V}_2^0$ (cm^3/mol)	90.7	105.0	137.8	35
S_v^* ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-2}}$)	1.7	2.0	2.1	
b_v ($\mathrm{cm^3 \cdot l^{1/2} \cdot mol^{-3/2}}$)	−0.4	−0.3	0.1	

volume from the predicted value by the Debye-Hückel theory and is regarded as a measure of the ion-solvent interactions. Negative b_v values have been reported on the aqueous solutions of various ammonium salts, such as tetraalkylammonium salts,[16] *n*-alkylamine hydrobromides,[17] and cyclic amine hydrochlorides.[18] These negative b_v values were attributed to the decrease in ice-likeness with increase of concentration by overlapping of the structural region around the hydrophobic solute,[17,18] or the increase of cage-like structure with increase of concentration.[19]

Concerning the sodium salts of fatty acids,[20] the values of b_v were slightly negative at 25 °C and these negative values increased with the carbon numbers of the chain as in the case of alkylamine hydrobromides. However, the temperature dependence of b_v values was negative in contrast with the case of the higher homolog of alkylamine salts. From the viscosity[21] and the NMR study,[5] the acetate ion was concluded to be a structure maker. Judging from the size of the ion and the delocalization of the charge,[22] the carboxylate ion may not have so much influence on the structure of water. If this is the case correct, then the structure making ability of the acetate ion originates from the methyl group of the acetate ion, that is, the acetate ion is a hydrophobic structure maker.

The formation of a cage-like structure around the hydrophobic solute makes a negative contribution to the partial molar volume of the solute.[23] If the guanidinium ion breaks the water structure around the hydrophobic ion in the unique way as discussed in the previous section, the guanidinium ion will increase the partial molar volume of the salt. Therefore the b_v values of the guanidinium salts will be positive if the structure breaking effect of the guanidinium ion dominates the structure making effect of the hydrophobic anions.

As seen in Table 5, the b_v values of three guanidinium salts were all positive at 25 °C and increased with the hydrophobicities of the counter anions. The negative b_v values of acetate and propionate at 35 °C and the negative temperature dependence of the b_v values for the each salt mean that the structure breaking ability of the guanidinium ion decreased with temperature increase, since the water structure was already broken to a considerable extent at high temperature.

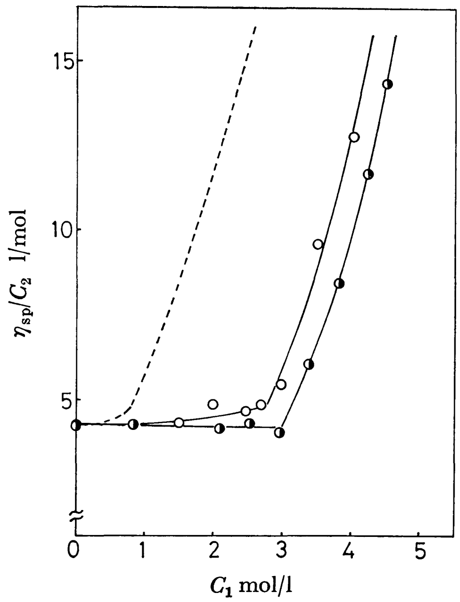

Fig. 2. Relation between the reduced viscosities of BSA and the concentrations of guanidinium salts.
----: Guanidinium chloride, —○—: guanidinium propionate, —◑—: guanidinium acetate.
C_1: Concentration of guanidinium salts (mol/l), C_2: concentration of BSA (7.6×10^{-2} g/ml aqueous salt solution).

Reduced Viscosity of BSA. The relation between the reduced viscosities of BSA (η_{sp}/C_2) and the concentration of guanidinium salts are shown in Fig. 2. The initial concentrations necessary to produce the denaturation are around 3 mol/l for these salts with hydrophobic anions and about 1 mol/l for chloride. The difference of the denaturation abilities of these two kinds of salts together cannot be compared, since the chloride ion is hydrophilic and the other two ions are hydrophobic. As discussed in the previous paper[1] and preceding sections of this paper, the modes of the interaction between the guanidinium ion and these two kinds of anions are different from each other. This suggests that the interactions of these two kinds of salts with proteins are also different. It does appear that the hydrophobic anions weaken the denaturating ability of the guanidinium ions. Assuming that the structure breaking actions of these salts play the most important role in the various interactions involved in the denaturation of BSA, the effective concentration of the propionate for the denaturation of BSA might be expected to be larger than that of acetate. In fact, the concentration of propionate was slightly smaller than that of acetate. This result is consistent with the denaturation of ribonuclease by tetraalkylammonium salts.[24] In the tetraalkylammonium salts, the homolog with larger alkyl chains promoted the denaturation more strongly than those with shorter alkyl chains.

In conclusion, it was difficult to explain the order of the denaturation abilities of the hydrophobic salts of guanidine only in terms of the $\gamma_\pm$ values and volume data of the aqueous binary solutions.

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