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Studies on the Aqueous Solutions of Guanidinium Salts. X. Activity Coefficients of Biguanide Salts in Aqueous Solutions at 25 °C

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Synopsis. Activity coefficients and molar conductivities of biguanide salts (Bg·HX) in aqueous solutions were determined at 25 °C. The values of mean activity coefficients of these salts were relatively low and lay in the order Bg·HOAc>Bg·HCl>Bg·HBr>ButylBg·HCl. These results were discussed in terms of the ion-ion interactions and the concomitant structural change of water.

Biguanide is a condensed compound of two molecules of guanidine and its salts are known to induce a significant change of conformation of protein in aqueous solutions.[1] Some of its alkyl and arylalkyl derivatives have been used clinically as hypoglycemic agents.

In a previous paper,[2] we reported that there was a close relation between $\gamma_\pm$ values and the denaturation abilities of various guanidinium salts with different counter anions, that is, the lower the $\gamma_\pm$ values are, the stronger the denaturation abilities are. The minimum concentration of a biguanide salt to produce the denaturation of BSA is about a half of that of the guanidinium salt.[3] On the basis of the similarities of functional group and the molecular structure between these two kinds of salts, the denaturation mechanism of biguanide salts seems to be similar to that of the guanidinium salts. As for the protein denaturation, the structure breaking action of the guanidinium salt is considered to be the most important factor. From these points of view, we determined the osmotic and activity coefficients of some biguanide salts and discussed their effects on the structure of water by using the cosphere overlap model of Desnoyers.[4]

Experimental

Materials. Biguanide hydrochloride, hydrobromide, and acetate were prepared from biguanide sulfate monohydrate obtained from Aldrich Chemical Co., Ltd. The crude compounds were recrystallized twice or more from ethanol. Butylbiguanide hydrochloride was obtained from Sankyo Co., Ltd. and used without further purification. The purities of these salts were described elsewhere.[3] These salts were dissolved in redistilled and deionized water.

Methods. Osmotic and activity coefficients were determined at 25±0.02 °C by the isopiestic comparison method described elsewhere.[2] From the osmotic coefficient (ϕ), mean activity coefficient ($\gamma_\pm$) was calculated from Eq. 1.

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

where m is the molality of salt. Buoyancy corrections were made for biguanide salts using the densities of solid salts reported previously.[3] For the determination of isopiestic molality, buoyancy correction was also made to the aqueous solution on the assumption that the densities of aqueous solutions were all unity. The isopiestic molalities of aqueous solutions of potassium chloride (reference solute) and the biguanide salts are shown in Table 1. The experimental determination of isopiestic concentrations is precise to ±0.2%. Conductance was measured by the universal bridge manufactured by Yokogawa-Hewlet-Packard Co., Ltd. at 25± 0.05 °C. The conductance of the solution was obtained by subtracting the experimental value of conductance of water from that of the solution to avoid the effect of CO_2 dissolved in water. The molar conductivity at infinite dilution was obtained graphically by using the Fuoss-Onsager equation.[5]

Results and Discussion

The values of ϕ and $\gamma_\pm$ are shown in Table 2. The values of log $\gamma_\pm$ *vs.* $\sqrt{m}$(mol kg^{-1})$^{1/2}$ curves for various biguanide salts approach the Debye-Hückel limiting line (DHL-L) from above at low concentration, indicating the absence of such ion pairs as seen in the case of cadmium sulfate. The relatively low values of log $\gamma_\pm$ for Bg·HOAc, Bg·HCl, and Bg·HBr as compared with the alkali metal salts with the same counter anions, may be attributed to the nature of structure breaking of the biguanide ion. The values of log $\gamma_\pm$ for three biguanide salts lie in the same order ($OAc^- > Cl^- > Br^-$) as the guanidinium salts with the corresponding counter anions.[2] The values of log $\gamma_\pm$ for Bg·HCl and Bg·HBr are smaller than those of guanidinium chloride and bromide respectively, while the value of log $\gamma_\pm$ of Bg·HOAc is larger than that of guanidinium acetate. The order of log $\gamma_\pm$ is consistent with the order predicted by the cosphere overlap model on the assumption that the biguanide ion is a structure breaker and cation-anion interaction predominate over the other interactions. The differences of log $\gamma_\pm$ values between biguanide and guanidinium salts imply that the biguanide ion breaks water structure more significantly than the guanidinium ion does. Butyl Bg·HCl shows the lowest value among the four salts. On the basis of the cosphere overlap model, this result can be attributed to the hydrophobic cation-cation interaction. As the concentration increases and two butylbiguanide ions approach each other, the water-structure-enforced ion pairing would occur to compensate the decrease of the entropy based on the hydrophobic hydration, leading to the decrease of $\gamma_\pm$ value. Therefore cation-cation interaction is the most predominant factor for the decrease of $\gamma_\pm$ value of butylbiguanide hydrochloride, while cation-anion interactions for the other three salts.

The molar conductivities of biguanide salts and ions at infinite dilution are shown in Table 3. Using the Λ° values in Table 3, the Stokes' radius (γ_s) of

Table 1. Molality of isopiestic solutions

m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HCl)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HCl)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HCl)
0.1214	0.1229	0.5891	0.6384	0.9658	1.0986
0.2031	0.2085	0.6785	0.7440	0.9895	1.1262
0.3328	0.3488	0.7071	0.7808	1.0092	1.1524
0.3487	0.3666	0.7777	0.8639	1.0255	1.1747
0.4383	0.4657	0.8101	0.9047	1.0578	1.2153
0.5267	0.5669	0.9370	1.0615	1.3766	1.6380
m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HBr)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HBr)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HBr)
0.1214	0.1229	0.5891	0.6469	0.9895	1.1536
0.2031	0.2085	0.6785	0.7551	1.0092	1.1797
0.3328	0.3486	0.7074	0.7940	1.0255	1.2006
0.4054	0.4346	0.7777	0.8795	1.0578	1.2457
0.4383	0.4709	0.8101	0.9204	1.2384	1.4954
0.5267	0.5723	0.9370	1.0853	1.3766	1.6917
0.5514	0.6040	0.9658	1.1261		
m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HOAc)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HOAc)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (Bg·HOAc)
0.1214	0.1214	0.6785	0.6914	0.9895	1.0138
0.2031	0.2040	0.7074	0.7207	1.0092	1.0350
0.3328	0.3367	0.7777	0.7932	1.0578	1.0862
0.4383	0.4425	0.8101	0.8272	1.2384	1.2764
0.5514	0.5595	0.9370	0.9614	1.3766	1.4223
0.5891	0.5971	0.9658	0.9923		
m/mol kg^{-1} (KCl)	m/mol kg^{-1} (ButylBg·HCl)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (ButylBg·HCl)	m/mol kg^{-1} (KCl)	m/mol kg^{-1} (ButylBg·HCl)
0.1319	0.1354	0.6785	0.7780	0.9658	1.1774
0.2031	0.2117	0.7074	0.8200	0.9895	1.2088
0.3328	0.3579	0.7777	0.9123	1.0092	1.2371
0.4383	0.4797	0.8101	0.9557	1.0578	1.3086
0.5514	0.6207	0.9370	1.1338	1.3766	1.8094

Table 2. Activity and osmotic coefficients of biguanide salts at 25 °C

m/mol kg^{-1}	Bg·HCl		Bg·HBr		Bg·HOAc		ButylBg·HCl	
	ϕ	$\gamma_\pm$	ϕ	$\gamma_\pm$	ϕ	$\gamma_\pm$	ϕ	$\gamma_\pm$
0.1	0.760	0.918	0.753	0.914	0.771	0.926	0.744	0.908
0.2	0.693	0.891	0.684	0.885	0.714	0.908	0.670	0.876
0.3	0.649	0.872	0.635	0.864	0.681	0.898	0.619	0.853
0.4	0.614	0.856	0.601	0.847	0.655	0.892	0.581	0.834
0.5	0.585	0.843	0.571	0.836	0.637	0.887	0.550	0.817
0.6	0.561	0.832	0.547	0.822	0.621	0.884	0.524	0.802
0.7	0.541	0.822	0.526	0.811	0.608	0.881	0.501	0.789
0.8	0.524	0.813	0.508	0.801	0.597	0.879	0.480	0.777
0.9	0.508	0.804	0.491	0.792	0.587	0.877	0.462	0.766
1.0	0.493	0.797	0.475	0.783	0.579	0.876	0.446	0.756
1.2	0.468	0.782	0.450	0.767	0.565	0.874	0.418	0.736
1.4	0.447	0.770	0.426	0.752	0.553	0.872	0.393	0.718
1.6	0.429	0.759	0.406	0.739	0.543	0.871	0.372	0.701
1.8	0.412	0.748	0.389	0.726	0.531	0.871	0.344	0.678
2.0					0.528	0.871		

each biguanide ion and butylbiguanide ion calculated from Eq. 2 were 2.57 and 3.52 Å.

$$\gamma_s = \frac{0.82|z|}{\Lambda^\circ \eta_0} \quad (2)$$

where z and η_0 are the valency of ion and the viscosity of water, respectively. Partial molar volumes at infinite dilution of biguanide ion and butylbiguanide ion were 69.8 and 137.1 cm^3/mol at 25 °C, respectively.[3] Assuming that these ions are spherical, the ionic radii of biguanide ion and butylbiguanide ion

Table 3. Molar conductivities of biguanide ions and salts at 25 °C

Salts or ions	Λ° / cm^2 Ω^{-1} mol^{-1}
Bg·HCl	112.4
Bg·HBr	114.3
Bg·HOAc	76.2
ButylBg·HCl	102.5
Bg·H$^+$	35.8
ButylBg·H$^+$	26.2

are calculated to be 2.55 and 3.20 Å, respectively. The ionic radius of the biguanide ion obtained from the partial molar volume is almost the same as that obtained from Eq. 2. But as for the butylbiguanide ion, the former is smaller than the latter indicating the hydrophobic hydration of butyl group.

The denaturation abilities for the three biguanide salts lie in the order $Br^- > Cl^- > OAc^-$, which is the reverse order of $\gamma_\pm$ values. This fact supports that the structure breaking actions of the biguanide salts contribute to the denaturation of protein in aqueous solutions. As reported previously,[3] butyl Bg·HCl has the strongest denaturation ability, but the denaturation mechanism may be different from the other three salts on the basis of the consideration of the mean activity coefficients. In other word, the hydrophobic interaction between the butyl group of butylbiguanide and the hydrophobic moiety of protein may play an important role as observed in the interaction of tetraalkylammonium salts and DNA.[6]

From the analysis of viscosity B-coefficients, the biguanide ion was concluded to be neither a structure maker nor a structure breaker.[3] But thermodynamically the biguanide ion should be a structure breaker. We have no idea to account for the discrepancy at the present stage. Now we are planning NMR study for solving this problem.

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