Rheological Study of Lysozyme in Dimethyl Sulfoxide + Water Solution at 298.15 K

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The viscosities, η, of ternary mixtures of water (1) + dimethyl sulfoxide (2) + lysozyme (3) were measured, and the intrinsic viscosities of lysozyme were determined by extrapolating the reduced viscosities to infinitely dilute lysozyme concentration at 298.15 K. A binary mixture of water + dimethyl sulfoxide had a maximum deviation of viscosity at approximately 0.33 mol fraction of dimethyl sulfoxide (x_2). Lysozyme unfolded and aggregated with an accompanying increase in the intrinsic viscosity of lysozyme at $x_2 =$ 0.3−0.4. These results indicated that the strong interaction between water and dimethyl sulfoxide might influence the conformation of lysozyme by interfering interactions between water and lysozyme.

Introduction

Dimethyl sulfoxide (DMSO), a typical aprotic solvent having both polar and nonpolar groups, is an important solvent in chemistry, biotechnology, and medicine for the dissolution of various substances and as an antifreezing agent of living cells. Many thermodynamic studies that have been conducted to determine the properties of DMSO aqueous solutions indicate a strong interaction between water and DMSO molecules.[1−3] There is an interest in studying how such a strong interaction can influence protein conformation in this solution.

A study of proteins in the DMSO aqueous solution is very important for elucidating the mechanism of protein folding because the native conformation of protein in solution is produced by a delicate balance between covalent and noncovalent bonds such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions. We determined that lysozyme denatured and aggregated in DMSO aqueous solutions by strong interaction between water and DMSO as shown by fluorescence spectra and partial specific volumes of lysozyme.[4]

The viscosity is a property of a solution that is one of the important thermodynamic parameters characterizing the solution. The intrinsic viscosity of protein in solution can sensitively reflect protein conformation.[5]

To determine the rheological properties of ternary mixture solutions (water + DMSO + lysozyme) and the effects of binary mixture solutions (water + DMSO) on the conformation of lysozyme, aqueous solutions of lysozyme in various DMSO concentrations were measured by viscometry at 298.15 K, and the intrinsic viscosities were determined in various aqueous DMSO solutions.

Experimental Section

Materials. Hen egg white lysozyme was purchased from Sigma (six times recrystallized, lot no. 90K1922). Dimethyl sulfoxide (DMSO, Kanto Kagaku, spectroscopy grade) was distilled over freshly activated molecular sieves 4A under reduced pressure of 0.3 kPa at 327 K. Ultrapure-grade guanidine hydrochloride was purchased from Kishida Chemical Co., and water was distilled twice.

Sample Preparation. In this study, mole fractions of DMSO (x_2) were calculated assuming two components (water (1) + DMSO (2)) because the mole of lysozyme (3) was negligible compared to the mole of DMSO and water. All solvents and solutions were prepared according to previously mentioned procedures.[4] Precise x_2 values for the solvents (water + DMSO) and solutions (water + DMSO + lysozyme) were respectively calculated using the weight of DMSO and water. The differences in x_2 between the solvents and the solutions were accurate to within 10^{-4}. The concentrations of lysozyme were determined using dilution factors obtained from gravimetric and density data for the solvents and solutions. Solvents and solutions in guanidine hydrochloride aqueous solutions (5.5 M, with 0.1 mM HCl) were prepared according to the same procedure. The x_2 values were determined with an uncertainty of 10^{-4}, and the concentrations of lysozyme were calculated with an uncertainty of 10^{-6} g·cm^{-3}.

Experimental Techniques. Viscosities of solutions were measured using a heart-shaped viscometer in our custom-made thermostat that was maintained at 298.15 K with an uncertainty of 0.001 K. The viscometer had a flow time of approximately 100 s and 200 s for water and DMSO, respectively, at 298.15 K, and the repeatability was ±0.05 s. The density data used in this study were from our previous results with an uncertainty of 10^{-6} cm^3·g^{-1}.[4]

Calculations of Hydrodynamic Parameters. In this study, absolute viscosity of a solution, η, is given by the following equation

$$\eta = \frac{\rho t}{\rho_1 t_1}\eta_1 \qquad (1)$$

where ρ and t are the density and the flow time of solution and ρ_1, t_1, and η_1 are the density, flow time, and absolute viscosity of water (0.890 25 mPa·s)[6] at 298.15 K, respectively. The η values of solutions were determined according to eq 1 with an uncertainty of 10^{-4} mPa·s.

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10.1021/je049928n CCC: $27.50
Published on Web 08/12/2004

Table 1. Viscosities (η) of Ternary Solutions (Water (1) + DMSO (2) + Lysozyme (3)) in Various Concentrations of Lysozyme (c_3) and Various Mole Fractions of DMSO (x_2) at 298.15 K

c_3/mg·cm^{-3}	η/mPa·s	c_3/mg·cm^{-3}	η/mPa·s	c_3/mg·cm^{-3}	η/mPa·s	c_3/mg·cm^{-3}	η/mPa·s
$x_2 = 0.0000$		$x_2 = 0.0249$		$x_2 = 0.0499$		$x_2 = 0.1298$	
0.000	0.8903	0.000	1.0954	0.000	1.3238	0.000	2.1919
2.018	0.8958	2.073	1.1039	2.074	1.3263	2.175	2.2152
3.099	0.8979	3.128	1.1076	3.115	1.3275	3.260	2.2165
4.151	0.9026	4.205	1.1110	4.108	1.3287	5.368	2.2344
5.209	0.9061	5.264	1.1126	5.152	1.3300	6.458	2.2447
6.274	0.9078	6.321	1.1155	6.178	1.3313	7.551	2.2533
7.232	0.9108	7.379	1.1183	7.232	1.3326		
$x_2 = 0.1996$		$x_2 = 0.2497$		$x_2 = 0.2994$		$x_2 = 0.3483$	
0.000	3.0140	0.000	3.4089	0.000	3.6708	0.000	3.7192
4.000	3.0689	2.217	3.4431	2.208	3.7111	2.143	3.7885
6.200	3.0882	2.951	3.4513	3.296	3.7256	3.211	3.7975
8.000	3.1051	4.409	3.4694	4.386	3.7430	4.271	3.8094
8.300	3.1179	5.521	3.4828	5.623	3.7566	5.387	3.8193
9.500	3.1229	6.621	3.4916	6.737	3.7653	6.471	3.8341
10.000	3.1326	7.719	3.5019	7.867	3.7775	7.540	3.8481
						8.613	3.8791
$x_2 = 0.3696$		$x_2 = 0.3896$		$x_2 = 0.4296$		$x_2 = 0.4993$	
0.000	3.7200	0.000	3.7041	0.000	3.6224	0.000	3.3669
2.194	3.7851	2.183	3.7820	2.445	3.6958	2.134	3.4289
3.267	3.8048	3.275	3.7942	3.703	3.7246	3.224	3.4573
4.340	3.8258	4.526	3.8221	4.953	3.7496	4.324	3.4817
5.410	3.8534	5.643	3.8483	6.220	3.7738	5.456	3.5055
6.503	3.8745	6.748	3.8759	7.499	3.8015	6.572	3.5323
7.619	3.8923	7.862	3.9006				
$x_2 = 0.5971$		$x_2 = 0.6993$		$x_2 = 0.7495$		$x_2 = 0.7992$	
0.000	3.0536	0.000	2.5501	0.000	2.4440	0.000	2.3328
2.149	3.1124	2.061	2.5999	2.216	2.5056	2.133	2.3897
3.265	3.1282	3.045	2.6195	3.334	2.5230	3.239	2.4020
4.400	3.1518	4.037	2.6379	4.414	2.5414	4.370	2.4210
5.509	3.1728	5.008	2.6593	5.531	2.5607	5.482	2.4358
6.654	3.1889	5.995	2.6795	6.646	2.5767	6.575	2.4548
		7.064	2.6970	7.781	2.5931	7.671	2.4694
$x_2 = 0.8978$		$x_2 = 1.0000$		Gdn-HCl (5.5 M)			
0.000	2.1350	0.000	1.9960	0.000	1.3485		
2.293	2.1815			2.308	1.3772		
3.426	2.1976			3.461	1.3912		
4.579	2.2164			5.769	1.4100		
5.747	2.2306			6.923	1.4180		
				8.076	1.4251		
				9.230	1.4371		

The intrinsic viscosity of lysozyme, [η], is given by the following equation[5]

$$\eta_{sp} = \frac{\eta}{\eta_0} - 1 \tag{2}$$

$$\frac{\eta_{sp}}{c_3} = [\eta] + k[\eta]^2 c_3 \tag{3}$$

where η_{sp} is the specific viscosity of the solution, η_0 is the absolute viscosity of the solvent obtained from eq 1, c_3 is the concentration of lysozyme (cm^3·g^{-1}), and k is the Huggins coefficient. In this study, the reduced viscosity values, η_{sp}/c_3, were determined with an uncertainty of 10^{-4} cm^3·g^{-1} and the [η] values with an uncertainty of 10^{-2} cm^3·g^{-1} due to the comprehensive uncertainties in the various measurements such as sample preparation, determination of concentration, and measurements of flow time.

Results and Discussion

The η values of ternary mixture solutions (water + DMSO + lysozyme) at various x_2 and various concentrations of lysozyme are listed in Table 1. These results were averaged values whose errors were within 0.004 mPa·s. Figure 1 shows the deviations of viscosity, $\delta\eta$ (= $\eta - \eta_{app}$), of binary solvents (water + DMSO) compared to the apparent average viscosities of solvents, η_{app}, which were calculated by[7] $\ln \eta_{app} = (1 - x_2) \ln \eta_1 + x_2 \ln \eta_2$. The maximum $\delta\eta$ value occurred at approximately $x_2 = 0.33$ as in a ref 8, indicating a strong interaction of water with

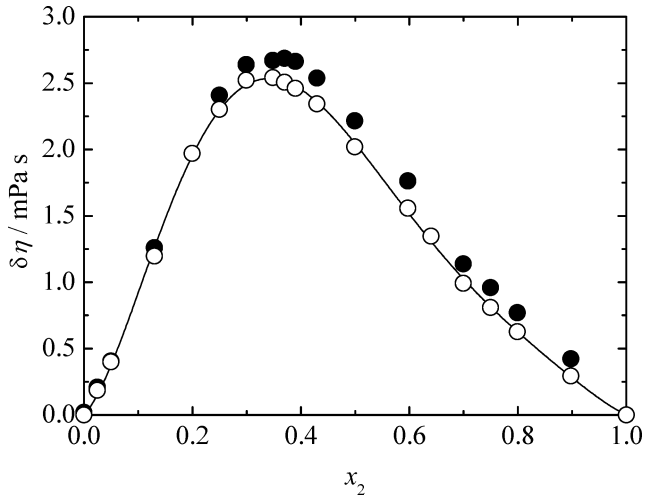

Figure 1. Deviations of viscosity ($\delta\eta$) of a solvent and solution depending on the mole fraction of dimethyl sulfoxide (x_2) at 298.15 K: ○, solvent; ●, solution (c_3 = 7.0 mg·cm^{-3}); solid line, approximated curve obtained by the Redrich−Kister equation.

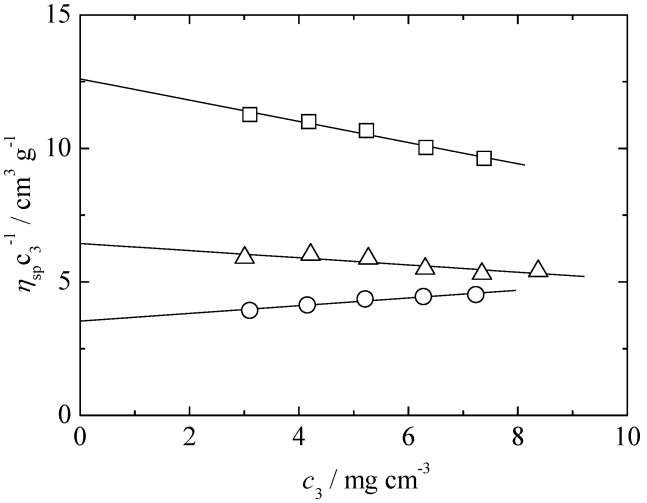

Figure 2. Dependence of η_{sp}/c_3 on concentration of lysozyme at typical mole fractions of DMSO (x_2): ○, $x_2 = 0.000$; △, $x_2 = 0.2497$; □, $x_2 = 0.5971$.

Table 2. Intrinsic Viscosities and Huggins Coefficients of Lysozyme in Various DMSO Aqueous Solutions at 298.15 K

x_2	$[\eta]$/cm^3·g^{-1}	k	SD
0.0000	2.84 ± 0.43	7.52 ± 4.47	0.27
0.0249	3.90 ± 0.17	−12.38 ± 1.47	0.99
0.0499	3.98 ± 0.21	−2.28 ± 3.88	0.26
0.1298	4.02 ± 0.29	−8.98 ± 7.73	0.56
0.1998	3.84 ± 0.16	−6.75 ± 2.80	0.21
0.2497	4.22 ± 0.20	−9.44 ± 0.91	0.08
0.2994	5.54 ± 0.15	−7.26 ± 3.26	0.09
0.3483	6.41 ± 0.70	−4.01 ± 2.43	0.34
0.3696	7.25 ± 0.29	−5.61 ± 1.34	0.32
0.3896	7.96 ± 0.14	−2.13 ± 0.48	0.11
0.4296	8.81 ± 0.24	−4.37 ± 0.63	0.20
0.4993	9.26 ± 0.16	−3.24 ± 0.39	0.12
0.5971	8.82 ± 0.13	−3.45 ± 0.51	0.09
0.6993	9.59 ± 0.11	−2.56 ± 0.36	0.17
0.7495	10.6 ± 0.20	−3.64 ± 0.28	0.11
0.7992	10.3 ± 0.32	−3.18 ± 0.43	0.16
0.8978	9.97 ± 0.28	−4.68 ± 0.86	0.22
1.0000	*a*		

[a] Not measured.

DMSO as also observed in many previous measurements of DMSO aqueous solutions.[1−4,8] The $\delta\eta$ of ternary mixture solutions at 7.0 mg·cm^{-3} of lysozyme compared to the η_{app} of solvents are shown in Figure 1. It should be noted that the $\delta\eta$ of ternary solutions were more greater compared to binary solvents in all x_2, and such increases were significantly dependent on x_2. These increases and dependence could reflect conformational changes of lysozyme with accompanying changes in volume and accessible surface area of lysozyme.

Rheological properties of lysozyme can be reflected by the intrinsic viscosities in DMSO aqueous solutions. Figure 2 shows the dependence of η_{sp}/c_3 on the concentration of lysozyme at a few typical mole fractions of DMSO. Intrinsic viscosities were determined by the extrapolation of the reduced viscosities to infinitely dilute lysozyme concentration according to eq 3. The intrinsic viscosities and Huggins coefficients listed in Table 2 were obtained by the linear least-squares method and are shown with standard deviations.

Figure 3 shows the dependence of the intrinsic viscosity on x_2 (A) together with our previous results of fluorescence spectra and partial specific volumes of lysozyme (B).[4] Our previous results show that there are at least three states

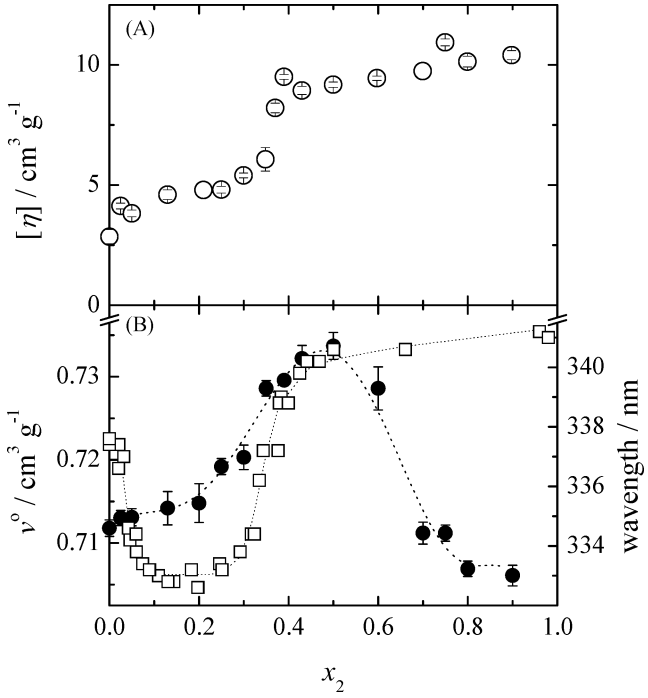

Figure 3. (A) Intrinsic viscosities, ○, (B) partial specific volumes, ●, and wavelength of maximum fluorescence emission, □, for lysozyme in various mole fractions of DMSO. Dotted lines are fitted lines.

in the denaturation process of lysozyme in DMSO: the native state (in water), the intermediate state ($x_2 = 0.05-0.2$), and the denatured-aggregated state ($x_2 > 0.4$). It is clear that the intrinsic viscosity changed in these three states as was indicated by the results of fluorescence measurement in the denaturation process. The intrinsic viscosity of lysozyme at the native state was (2.84 ± 0.43) cm^3·g^{-1} in water, consistent with the literature values 2.66 cm^3·g^{-1}.[9] The intrinsic viscosity of lysozyme at the intermediate state in $x_2 = 0.1998$ was (3.84 ± 0.16) cm^3·g^{-1} and at the denatured-aggregated state in $x_2 = 0.3896$ was (7.96 ± 0.14) cm^3·g^{-1}. The intrinsic viscosity of lysozyme at the denatured state produced by a strong denaturant, guanidine hydrochloride (5.5 M, with 0.1 mM HCl aq), was (6.60 ± 0.16) cm^3·g^{-1}, which was consistent with the literature values, 6.5 and 5.3 cm^3·g^{-1}.[5,10] The fact that the intrinsic viscosity of the denatured state in DMSO was considerably greater than in guanidine hydrochloride indicates that lysozyme was both denatured and aggregated in DMSO aqueous solution as observed by changes in light scattering[11] and partial specific volumes.[4]

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Received for review February 15, 2004. Accepted July 1, 2004.

JE049928N