

Calorimetric Studies on the Coil-Helix Transition of Poly(L-Lysine) in Water-Organic Solvent Mixtures

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By combining the heat of dilution of solution of poly(L-lysine) in water-organic solvent mixtures with corresponding heat of mixing of water-organic solvent system, the integrated heats of the coil-helix transition of poly(L-lysine) were estimated to be about -6.8 kJ in ethanol, -16.0 kJ in isopropanol, and 10.5 kJ per mole of constitutional repeating unit in N,N-dimethylformamide (DMF), respectively. The solvent composition at which the transition occurs was determined to be about 0.74 of volume fraction of ethanol, 0.74 of volume fraction of isopropanol, and 0.84 of volume fraction of DMF, respectively. Those results are in good agreement with those obtained by optical rotatory dispersion (ORD). In addition, the solvent composition at the transition point of poly(L-lysine) in water-N,N-dimethylacetamide (DMAc) solvent mixtures was also determined to be about 0.83 of volume fraction of DMAc by ORD.

1. Introduction

In the previous paper¹⁾, we measured the heat of coil-helix transition of poly(L-lysine) and poly(L-ornithine) in water-methanol solvent mixtures by using a twin microcalorimeter at 298.15 K. The solvent composition at which the transition occurs was determined to be about 0.85 and 0.94 of the volume fraction of methanol for poly(L-lysine) and poly(L-ornithine), respectively. These results obtained were in good agreement with those obtained by optical rotatory dispersion (ORD).

On the other hand, the heats of coil-helix transition of poly(L-lysine) and poly(L-ornithine) seem to be comparable with those determined from pH change by Scheraga *et al.*²⁾.

In order to obtain further information about the solution properties of poly(L-lysine) in other organic solvents than methanol, we will report the heat of coil-helix transition and the composition of transition of poly(L-lysine) in water-ethanol, -isopropanol, -N,N-dimethylformamide, and -N,N-

dimethylacetamide solvent mixtures with the help of optical rotatory dispersion.

2. Experimental

2.1 Materials

Poly(L-lysine): poly{imino[1-(2-benzyloxycarbonyl ethyl)-2-oxoethylene]} samples with molecular weights (M_w) of 8×10^4 and 30×10^4 , respectively used in this study were purchased from Sigma Co. Ltd. (U.S.A.).

The solvents used were water, ethanol, isopropanol, N,N-dimethylformamide (DMF), and N,N-dimethylacetamide (DMAc). Those organic solvents were analytical reagent grade of commercial products and water was purified by standard method.

2.2 Apparatus and Procedure

The microcalorimeter used in this study and its measuring procedure have been described previously¹⁾.

The optical rotatory dispersion (ORD) data were measured by using a JASCO J-20.

The concentration of poly(L-lysine) used in the calorimetric study was 0.20 unit mol^{*} dm⁻³, and that used in the optical rotatory dispersion meas-

* Unit mol = mol of constitutional repeating unit.

urement was about 4.5×10^{-3} unit mol^{*} dm⁻³.

3. Results and Discussion

3.1 Heat of Dilution of Ternary Solution

The heat of dilution of poly(L-lysine) solution in the mixed solvents such as water-ethanol, -isopropanol, and -DMF systems was measured by using a microcalorimeter at 298.15 ± 0.001 K, according to the same experimental procedure as described previously¹⁾.

The heat of dilution of poly(L-lysine)-water-ethanol system proved to be exothermic, except for the region from 0.51 to 0.80 in volume fraction of ethanol, and that of poly(L-lysine)-water-isopropanol system was endothermic in all dilution process. On the other hand, the heat of dilution of poly(L-lysine)-water-DMF system was exothermic, except for the region from 0.88 to 0.90 in volume fraction of DMF. The results obtained were listed as ΔH_{obs} in the second column of Table 1.

The heat of dilution of ternary system as seen in Table 1 is considered usually to consist of the following three factors: the first is the heat of coil-helix transition of poly(L-lysine) from the coil state to the helix one in dilution process. The second is the heat of dilution of poly(L-lysine) solution. This kind of heat, however, has been experimentally confirmed to be very small in comparison with that caused by the conformational change such as the transition or dissociation as pointed out in the previous papers^{3,4)}. Then, this heat may be neglected at the present work. The final heat is the heat of mixing of the two solvents. Therefore, in order to obtain the net heat of coil-helix transition of poly(L-lysine) in the solution, this heat of mixing of two solvents must be exactly determined.

3.2 Heat of Mixing of Two Solvents

The heats of mixing of the water-ethanol, -isopropanol, and -DMF systems were measured by successive additions of a pure organic solvent to water under the same experimental conditions as those in the measurement of the heats of dilution of ternary solutions. The results obtained are also summarized as ΔH_{12} in the third column of Table 1.

3.3 The Differential Heat of the Coil - Helix Transition

Subtraction of the corresponding heat of mixing from ΔH_{obs} for each dilution process will lead to the differential heat of coil-helix transition of poly(L-lysine), which is listed as ΔH_t in the fourth of Table 1.

Table 1 shows that the differential heat of coil-helix transition, ΔH_t , for poly(L-lysine)-water-alcohol systems is exothermic, but, ΔH_t for poly(L-lysine)-water-DMF system is endothermic. This suggests that the later system undergoes so-called reversed transition as reported previously⁴⁾.

The plots of ΔH_t against volume fraction of helix solvent are shown in Figs. 1 and 2, together with that of methanol system reported previously¹⁾. ΔH_t at first increases, and then sharply decreases, after passing through maximum, as volume fraction of helix solvent increases. The volume fraction, at which ΔH_t shows maximum, corresponds to the solvent composition of transition point of poly(L-lysine) in mixed solvents. The transition of poly(L-lysine) takes place in the neighborhood of 0.74 of volume fraction of ethanol, 0.74 of volume fraction of isopropanol and 0.84 of volume fraction of DMF in mixed solvents, respectively.

3.4 Optical Rotatory Dispersion Measurement

In order to confirm the solvent composition of transition of poly(L-lysine) in mixed solvents estimated from calorimetric work, the optical rotatory dispersion (ORD) was measured by using a spectropolarimeter under the same experimental conditions as those for the calorimetric works.

The results obtained are shown in Figs. 1 and 2, where the helical contents calculated according to Yang's equation⁵⁾ (alcohol-water system) and Moffit-Yang's equation (DMF-water system), respectively, are plotted against the volume fraction of alcohol and/or DMF in the mixed solvents, together with the differential heat of transition obtained from calorimetric works.

From Figs. 1 and 2, the solvent composition at which the transition takes place sharply for poly(L-lysine) in the mixed solvents are 0.77 of volume fraction of ethanol, 0.75 of volume fraction of isopropanol, and 0.85 of volume fraction of DMF. The results obtained are listed in the last column of Table 2, together with those obtained from

Table 1 Heats Liberated when Aqueous Poly(L-lysine) are Diluted with Organic Solvent at 298.15 K.

vol. fraction of organic solvent	observed heat of dilution			integrated heat of transition
	ΔH_{obs} (J)	ΔH_{12} (J)	ΔH_1 (J)	ΔH (kJ/unit mol)
water - ethanol solvent mixture				
0.341	-11.887	-11.887	0.000	0.000
0.437	-4.950	-4.935	0.015	-0.080
0.508	0.728	0.751	-0.023	-0.201
0.564	1.905	1.931	-0.026	-0.338
0.608	2.128	2.181	-0.053	-0.617
0.644	1.679	1.769	-0.090	-1.091
0.674	1.332	1.473	-0.141	-1.833
0.699	1.238	1.398	-0.160	-2.675
0.721	0.799	0.999	-0.200	-3.728
0.740	0.483	0.638	-0.156	-4.549
0.756	0.326	0.541	-0.215	-5.681
0.771	0.288	0.361	-0.073	-6.065
0.784	0.110	0.201	-0.091	-6.544
0.795	0.043	0.082	-0.039	-6.749
0.805	-0.056	-0.046	-0.010	-6.802
0.815	-0.063	-0.063	0.000	-6.802
0.823	-0.211	-0.211	0.000	-6.802
0.838	-0.680	-0.680	0.000	-6.802
water - isopropanol solvent mixture				
0.424	6.040	6.040	0.000	0.000
0.496	12.279	12.279	0.000	0.000
0.551	13.895	13.895	0.000	0.000
0.596	14.307	14.330	-0.023	-0.128
0.632	14.040	14.190	-0.150	-0.961
0.663	13.740	13.877	-0.137	-1.722
0.689	13.594	13.784	-0.190	-2.778
0.711	13.112	13.327	-0.215	-3.972
0.730	12.379	12.760	-0.385	-6.111
0.747	11.818	12.261	-0.443	-8.572
0.762	11.217	11.575	-0.358	-10.561
0.775	10.676	10.942	-0.266	-12.036
0.787	10.183	10.444	-0.261	-13.489
0.797	9.688	9.838	-0.150	-14.322
0.807	9.052	9.322	-0.270	-15.822
0.816	8.916	8.935	-0.019	-15.928
0.824	8.345	8.367	-0.022	-16.050
0.831	7.929	7.929	0.000	-16.050
0.838	7.535	7.535	0.000	16.050
water - DMF solvent mixture				
0.791	5.870	5.870	0.000	0.000
0.809	4.087	4.196	0.109	1.199
0.825	2.655	2.841	0.186	3.245
0.835	1.609	1.927	0.318	6.743
0.850	-1.062	1.240	0.178	8.701
0.860	0.694	0.796	0.102	9.823
0.869	0.367	0.417	0.050	10.373
0.876	0.139	0.139	0.000	10.373
0.883	0.031	0.031	0.000	10.373
0.889	0.195	0.195	0.000	10.373
0.895	0.282	0.282	0.000	10.373
0.900	0.316	0.316	0.000	10.373

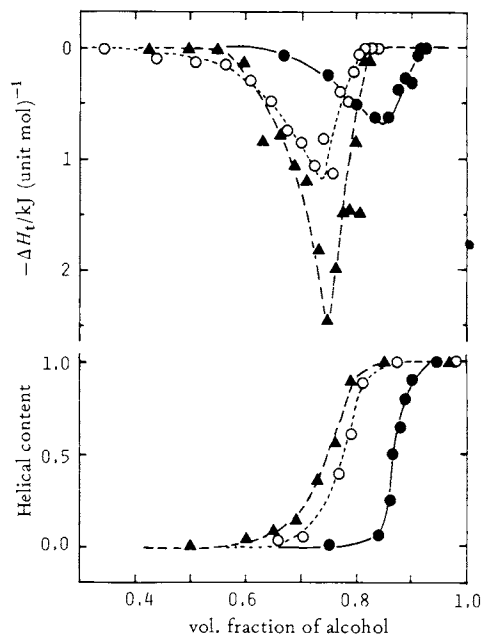


Fig. 1 Plots of differential heat of transition, ΔH_t , and helical content against volume fraction of alcohol for poly(L-lysine) in water-alcohol solvent mixtures; ●: methanol (ref. 1), ○: ethanol, ▲: isopropanol.

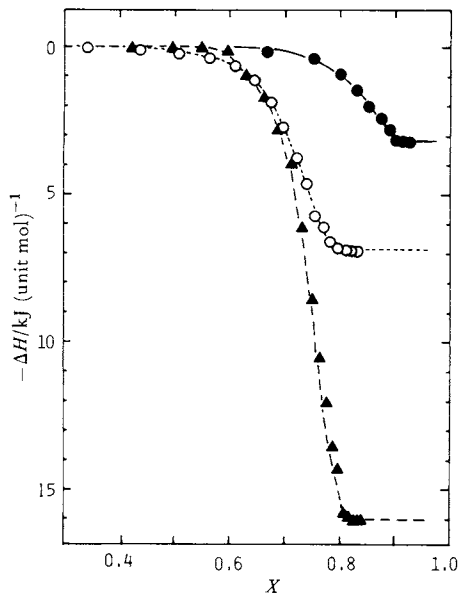


Fig. 3 Plots of integrated heat of transition, ΔH , against volume fraction, X , of alcohol for poly(L-lysine) in water-alcohol solvent mixtures; ●: methanol (ref. 1), ○: ethanol, ▲: isopropanol.

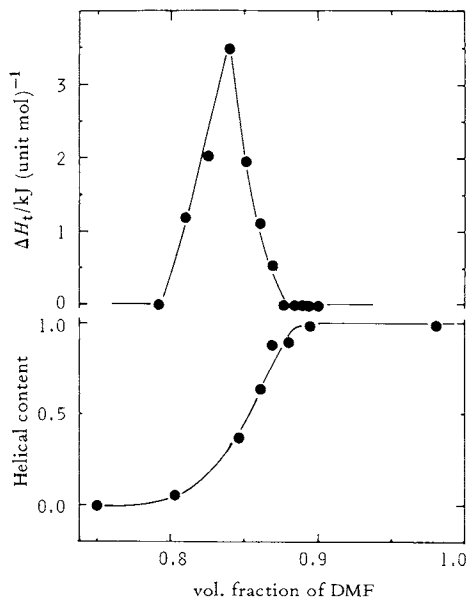


Fig. 2 Plots of differential heat of transition, ΔH_t , and helical content against volume fraction of DMF for poly(L-lysine) in water-DMF solvent mixtures.

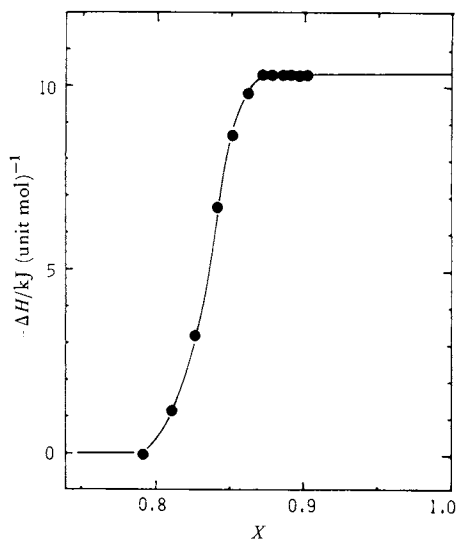


Fig. 4 Plots of integrated heat of transition, ΔH , against volume fraction, X , of DMF for poly(L-lysine) in water-DMF solvent mixtures.

Table 2 The Heats of Coil - Helix Transition, $\lim_{X \rightarrow 1.0} \Delta H$, of Poly(L-lysine) at 298.15 K.

$M_w \times 10^{-5}$	solvent	$\lim_{X \rightarrow 1.0} \Delta H$ (kJ/unit mol)	composition of transition	method
0.7	H ₂ O - CH ₃ OH	-3.1 ^{a)}	0.85* 0.86**	solvent composition change
0.8	H ₂ O - C ₂ H ₅ OH	-6.8	0.74* 0.77**	
3.0	H ₂ O - (CH ₃) ₂ CHOH	-16.0	0.74* 0.75**	
3.0	H ₂ O - DMF	10.5	0.84* 0.85**	
3.0	H ₂ O - DMAc		0.83**	
1.1	0.1 N KCl	-5.1 ^{b)}		pH change

* calorimetric measurement

** ORD measurement

a) ref. 1

b) ref. 2

calorimetric measurements. The solvent composition of transition obtained by the optical method seems to be in good agreement with that determined from calorimetric measurement.

Inspection of Figs. 1 and 2 lead to the conclusion that the solvent composition at the transition of poly(L-lysine) in the aqueous alcohol solutions shifts to the lower volume fraction of alcohol as the chain length of alcohol increases.

3.5 The Net Heat of Coil - Helix Transition

The integrated heat of coil-helix transition per unit mole of poly(L-lysine) can be easily derived by successive addition of the differential heat of coil-helix transition for each dilution process and the results are listed as ΔH in the last column of Table 1.

In Figs. 3 and 4, the integrated heat of coil-helix transition, ΔH , is plotted against the volume fraction, X , of alcohol and/or DMF in the mixed solvents, together with that of methanol system as reported previously¹⁾.

The ΔH shows the sigmoidal curves and reaches definite values as X approaches 1.0. The $\lim_{X \rightarrow 1.0} \Delta H$ may correspond to the net heat of coil-helix transition of poly(L-lysine) in the solutions and are collected in Table 2 together with those obtained by Scheraga *et al.*²⁾.

Table 2 shows that the values of the net heat of coil-helix transition of poly(L-lysine) in the

aqueous alcohol solutions seem to depend on the chain length of alcohol and increase as chain length of alcohol increases. While, solvent composition at which the transition takes place for poly(L-lysine) in mixed solvents moves to the lower volume fraction of alcohol as chain length of alcohol increases. The transition of poly(L-lysine) for ethanol and isopropanol may play an important role as a helicogenic solvent by bringing about more destruction of three dimensional structure of water with the increasing of chain length of alcohol molecules. Further detailed study on those factors, however, will be needed.

It should be noted here that poly(L-lysine) in water-DMF system has a positive $\lim_{X \rightarrow 1.0} \Delta H$ -value and hence undergoes a reversed transition in a similar manner to that of poly- γ -benzyl-L-glutamate in DCA-DCE systems^{3,4)}. This suggests that DMF may be an anomalous solvent. To clarify this anomalous behavior of DMF system is beyond the present study.

The $\lim_{X \rightarrow 1.0} \Delta H$ -values estimated in this study are slightly larger than those determined by pH change by Scheraga *et al.*²⁾. But, this slight difference can be well explained if the molecular weight dependence of the heat of transition of poly(L-lysine) and the effect of microscopic medium are existing.

In addition, we measured the optical rotatory dispersion of poly(L-lysine)-water-N,N-dimethyl-

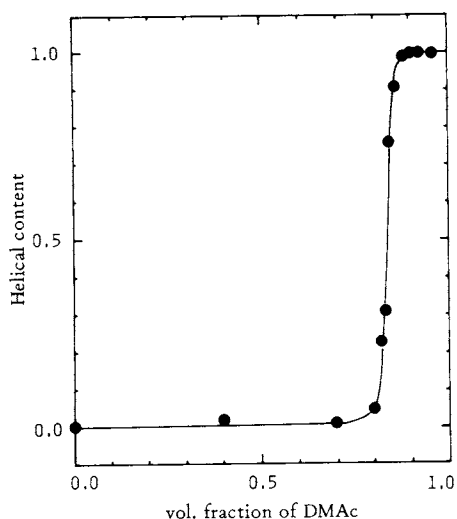


Fig. 5 Plots of helical content against volume fraction of DMAc for poly(L-lysine) in water-DMAc solvent mixtures.

acetamide (DMAc) system and the results are shown in Fig. 5, where the helical content is plotted against the volume fraction of DMAc. Fig. 5 shows that coil-helix transition exists in this system also. Furthermore, calorimetric study will be needed.

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(阪大理 崎山 稔)