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Determination of the Cooperativity Parameter σ from Viscometric Data of Poly-L-Glutamic Acid-Na

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SUMMARY

Viscometry of PLGNa solutions was performed for various degrees of neutralization α. The cooperativity parameter σ in the helix-coil transition was estimated using Ptitsyn's method with some modifications. The increase of σ thus obtained with decreasing α is explained in terms of the α dependence of short-range interactions of PLGNa segments.

The helix-coil transition of polypeptides is characterized by two equilibrium constants s and σ ; s applies to the growth of the helical part in the polymer chain and σ to the initiation and is called " cooperativity parameter ". The smaller σ, the sharper becomes the transition and eventually " the all or none " type.

Methods of determining σ have been proposed by many workers (ZIMM et al. 1959, APPLEQUIST 1963 and HAYASHI et al. 1969), and one of these methods (APPLEQUIST 1963) makes use of calorimetric data on the thermally induced transition. In the case of pH induced transitions of ionic polypeptides such as poly(L-glutamic acid)(PLG) or poly(L-lysine)(PLL), a method based on potentiometric titration data has been presented by NAGASAWA and HOLTZER (1964).

PTITSYN et al. (1965) developed a method using viscometric data and applied it to the helix-coil transition of PLGNa and PLL (BYCHKOVA et al. 1971 and BARSKAYA et al. 1971). They introduced Eq.(1) to express $<R^2>$, the mean square end-to-end distance of a helix-containing polypeptide chain, as a function of σ and θ, the helix content (PTITSYN 1967).

$$<R^2> \;=\; (1-\theta)<R^2>_c \;+\; 2Nb_h^2\theta^{3/2}/\sqrt{\sigma(1-\theta)} \qquad (1)$$

where $<R^2>_c$ is the mean square end-to-end distance of the random coil polymer, N the degree of polymerization

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and b_h the helical segment length per monomer unit. Eq.(2) obtained by the rearrangement of Eq.(1) was used to evaluate σ from the slope of the plot of $f(\theta)\alpha_c^2/(1-\theta)$ against $(\theta/1-\theta)^{3/2}$. Here, α_c is the expansion factor for the random coil polymer and b_o is the unperturbed segment length.

$$f(\theta) \equiv \langle R^2\rangle/\langle R^2\rangle_c = 1-\theta + 2b_h^2\theta^{3/2}/\alpha_c^2 b_o^2\sqrt{\sigma(1-\theta)} \qquad (2)$$

$\langle R^2\rangle/\langle R^2\rangle_c$ and α_c^2 were obtained by equating these to $([\eta]/[\eta]_c)^{2/3}$ and $([\eta]_c/[\eta]_o)^{2/3}$, respectively. While $[\eta]_{Cs\to\infty}$, the intrinsic viscosity extrapolated to infinite concentration of added electrolyte, was used for $[\eta]_o$ under theta conditions, this is not appropriate here as pointed out by the present authors because $[\eta]_o$ is greater than $[\eta]_{Cs\to\infty}$ for PLGNa in aq. NaCl solution (SATOH et al.).

In this communication we present the modification of Ptitsyn's method to determine σ from viscometric data.

Viscosity measurements were made to determine the intrinsic viscosity of PLGNa in aq. 0.1M NaCl solution and aq. 0.05M NaCl solution containing 20 vol.% isopropanol at 25 ± 0.05°C. The helix content was estimated by $\theta = -[\Theta]_{222}/40{,}000$ using $[\Theta]_{222}$, the molar ellipticity at 222 nm obtained by circular dichroism measurements. The molecular weights of PLGNa used were 14.7×10^4 and 10.6×10^4. The experimental details are described elsewhere (SATOH et al.). The results are shown in Figs.1 and 2 and TABLE 1.

DISCUSSION

The first problem in Ptitsyn's method is the use of $[\eta]_c$ in the alkaline region to calculate $\langle R^2\rangle_c$ (BYCHKOVA et al. 1971 and BARSKAYA et al. 1971). This is inappropriate since the intrinsic viscosity of PLGNa changes with the charge density of the polymer as shown in Fig.1. The dependence of $[\eta]$ on the degree of neutralization α should be taken into account, because the decrease of α gives rise to the coil-helix transition. $[\eta]$ of PLGNa decreases linearly with α in the random coil region($1.0\geq\alpha>0.7$) and the deviation below $\alpha = 0.7$ should be attributed to the conformational change of the polymer. A hypothetical $[\eta]_c$ must be estimated, therefore, in the transition region. Here we obtain $[\eta]_c$ by assuming a linear dependence in the entire range, even in the transition region. This assumption may be, at least to a certain extent, supported by the fact that the linear part extends to $\alpha = 0.6$ if the transition region is lowered to below $\alpha = 0.6$ by raising the temperature to 50°C (Fig.2).

The second problem concerns the use of Eq.(2) to obtain σ. As α_c and b_c change with α, σ cannot be estimated by the plot of $f(\theta)\alpha_c^2/(1-\theta)$ against $(\theta/1-\theta)^{3/2}$. σ should be evaluated directly from Eq.(1) using the relation $[\eta] = \Phi_0 \langle R^2 \rangle^{3/2}/M$, where Φ_0 is the Flory constant 2.87×10^{21}. The values of σ thus obtained for PLGNa in 0.1M NaCl and 0.05M NaCl-20 vol.% isopropanol are shown in TABLE 1. $\sigma = 2.5 \times 10^{-3}$ for PLGNa(M ≑ 9×10^4) in 0.2M NaCl at 22°C obtained by BYCHKOVA et al. (1971) is compared with our $\sigma = 2.5 \times 10^{-4} \sim 1.4 \times 10^{-3}$,

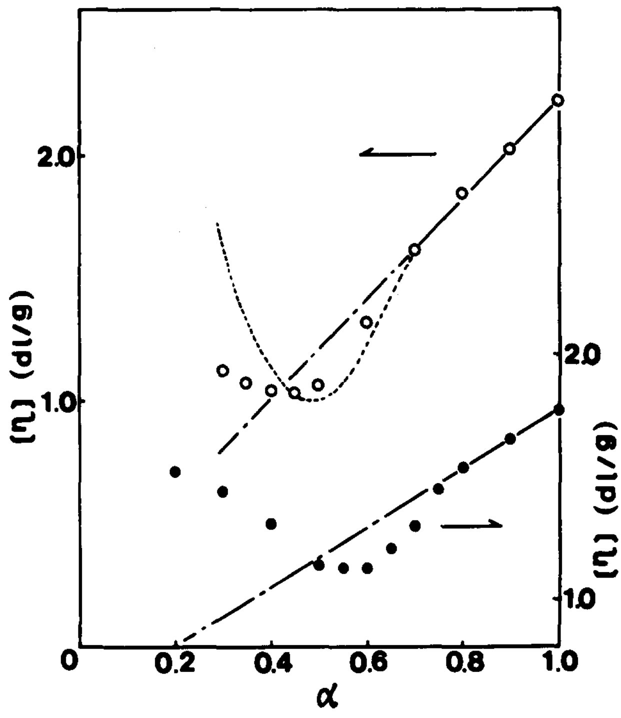

Fig. 1. Plot of [η] vs. α for PLGNa solutions:

○ PLGNa(M = 14.7×10^4) - 0.1 M NaCl
● PLGNa(M = 10.6×10^4) - 0.05 M NaCl - 20% isopropanol

——— : hypothetical $[\eta]_c$

------ : hypothetical [η] calculated with $\sigma = 10^{-3}$

considering the higher molecular weight of our sample. σ obtained as 2.0 x 10^{-4}~ 1.0 x 10^{-3} for PLGNa in 0.05M NaCl-20% isopropanol is also interpreted in the same way.

TABLE 1 shows that in both solvent media σ increases with decreasing α, even in the region $0.1<\theta<0.6$ where Eq.(1) should be valid. Such a dependence of σ on α can be interpreted as being consistent with the results of our previous report (SATOH et al.). The unperturbed segment length b_c of PLGNa decreases with decreasing α, which means that the short-range interaction is under the influence of the charge density. Therefore, it is inferred that the decrease of α would favorably initiate the helix formation through the decrease of the short-range interaction. As shown in Fig.1, hypothetical [η] values for PLGNa in 0.1M NaCl, calculated by Eq.(1) with constant σ, increase sharper below α = 0.45~0.5 and similar to those in aq. organic

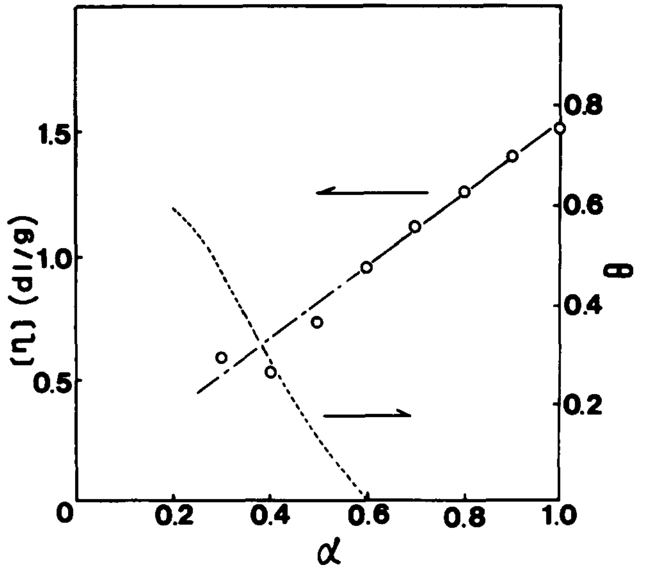

Fig. 2. Plot of [η] and θ vs. α for PLGNa in aq. 0.1 M NaCl solution at 50°C. The molecular weight of PLGNa = 10.6 x 10^4

TABLE 1

The intrinsic viscosity $[\eta]$ and the helix content θ obtained for PLGNa in the two solvents, and the cooperativity parameter σ calculated by Eq.(1)

	PLGNa[a] in 0.1M NaCl			PLGNa[b] in 0.05M NaCl-20 vol.% isopropanol		
α	$[\eta]$ dl/g	θ	$\sigma \times 10^3$	$[\eta]$ dl/g	θ	$\sigma \times 10^3$
1.00	2.22			1.76		
0.90	2.02			1.64		
0.80	1.84			1.53		
0.75				1.44	0.05	0.05
0.70	1.61			1.29	0.14	0.22
0.65				1.20	0.20	0.34
0.60	1.32	0.18	0.28	1.12	0.36	0.57
0.55				1.12	0.52	0.86
0.50	1.06	0.35	0.78	1.13	0.64	1.3
0.45	1.03	0.47	1.0			
0.40	1.04	0.60	1.4	1.30	0.87	3.6
0.35	1.07	0.66	1.7			
0.30	1.12	0.76	2.4	1.43	0.95	9.8
0.20				1.51	1.0	-

[a] Molecular weight of PLGNa = 14.7×10^4

[b] Molecular weight of PLGNa = 10.6×10^4

solvents. However, the increase of σ with decreasing α can not be explained by the slight increase of $[\eta]$ of PLGNa in aq. NaCl solution below $\alpha = 0.45$, because σ in aq. organic solvents is similarly dependent on α (TABLE 1). Explanations for such difference in the $[\eta]$ dependence on α between the two systems are based on the larger σ and the incompleteness of the transition in the aqueous solution. The main cause, however, may be the larger slope of the $[\eta]_c$ dependence on α in aq. NaCl solution, since the dimension of PLGNa in the helix-coil transition region is given by the sum of the contributions of random coil and helical parts as shown by Eq.(1). The larger the decrease of $[\eta]_c$ with decreasing α, the smaller is the contribution of the helical part. In fact, no minimum in the plot of $[\eta]$ against pH is observed for PLGNa in aq. 0.02M NaCl solution where $[\eta]_c$ strongly decreases with α (PTITSYN et al. 1967).

In conclusion, by some improvements of Ptitsyn's method, an increasing trend of σ with decreasing α of PLGNa was observed and which is explained by the decreasing short-range interactions with decreasing α. The dependence of σ on α is not to be considered as a cause of the difference in the α dependence of $[\eta]$ for the two systems.

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