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Some Problems Associated with the Exact Solution Conformation of Poly(α-L-glutamic acid) as Determined by Optical Methods

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Synopsis. By combining the experimental data on circular dichroism and light scattering, the helical conformation of poly(Glu) in 0.15 mol dm^{-3} NaCl solutions was estimated. The axial translation per residue was probably in the range of 1.2—1.3 Å for the unaggregated helix, while the persistence length was *ca.* 120 Å for the random-coil.

The conformation of poly(α-L-glutamic acid), poly(Glu), in aqueous solutions has been studied widely, since the optical and other physical properties are considered to be the primary reference for the α-helix and the random coil.[1] In order to determine an exact helical conformation, light scattering is a convenient means which gives both molecular weight M_r and overall length L.[2] Yet, there remain three major obstacles to identifying the conformation: (1) The pH region for the completion of helix formation should be known, (2) the α-helix is one of several helical conformations which poly(Glu) may take, and (3) a poly(Glu) sample is generally polydisperse with M_r and L. During the investigation of the macromolecular complex between poly(Glu) and Cu^{2+} ion, poly(Glu) was also studied by circular dichroism (CD), light scattering, viscosity, and other methods.[3–8] Based on these results, some hitherto mostly ignored difficulties, which are associated with the optical determination for the helical conformation of poly(Glu) in aqueous solutions, will be discussed.

Results and Discussion

Estimation of the Helix Fraction. Figure 1 shows the pH dependence of the molar ellipticity at 222.5 nm, $[\theta]_{222.5}$, for a poly(Glu) sample in 150 mM (1 M=1 mol dm^{-3}) NaCl solution. (Descriptions were given elsewhere on CD[3,8] and light scattering.[7]) Stepwise changes have been observed beyond experimental errors both in CD and ORD (at 233 nm) for poly(Glu) samples, which are derived either from poly(γ-benzyl L-glutamate)[3,8] or from poly(γ-methyl L-glutamate),[4,6] in solutions with and without added NaCl. (A similar change in CD was recently detected with a multipurpose technique.[9]) The helix fraction,[8] $f_h=([\theta]^c-[\theta])/([\theta]^c-[\theta]^h)$, can be evaluated from the CD data, where $[\theta]^c$ and $[\theta]^h$ are, respectively, the molar ellipticities for the states of coil and helix. This two-state expression has been verified by the principal-component-analysis method[5] and also by the presence of an isoelliptic point in the CD spectra of poly(Glu).[5,8] It should be noted that f_h does not necessarily denote the fraction of unaggregated helix molecules in a given poly(Glu) solution, because the CD is a short-range phenomenon and does not reflect the overall molecular shape.[1,10]

The first problem is the assignment of an appropriate value to $[\theta]^h$, since the formation of aggregates overlaps with the completion of monomeric helices.[7,8] A value of -36300 (pH=4.42) was used in a previous work,[8] by considering that the perfect helix is completed at point A, the onset of the second transition. However, the helix formation may as well be completed at the midpoint of the second transition (point B), for which $[\theta]^h=-38100$ and pH=4.31.[5] The oft-observed highest value of -40600 may equally be assigned to $[\theta]^h$ (pH=4.10), *i.e.*, point C.[1] This last procedure tacitly accepts the notion that the helix formation is not completed until aggregates are formed. The value of $[\theta]^c$ is 2650 (point D).

Helix Fraction and Root-mean-square Radius of Gyration. In Fig. 1, the square root of the *z*-average of the mean-square radius of gyration $\langle s^2\rangle_z^{1/2}$, obtained from light scattering,[7] is plotted against the helix fraction obtained from CD. Regardless of the choice of the point for completion of helix (A, B, or C), the value of $\langle s^2\rangle_z^{1/2}$ becomes minimum in the $(1-f_h)$ range of 0.7—0.8. The plots indicate that both helical and coiled segments coexist in a single poly(Glu) chain, excluding the possibility that completely helical and completely coiled molecules exist in varying equilibria in the coil-to-helix transition process (otherwise, the $\langle s^2\rangle_z^{1/2}$ *vs.* $(1-f_h)$ plot would be linear).[8]

The second problem is the assignment of a value of $\langle s^2\rangle_z^{1/2}$ to the perfect helix. As the helix formation is nearly completed (f_h=0.9), observed values of

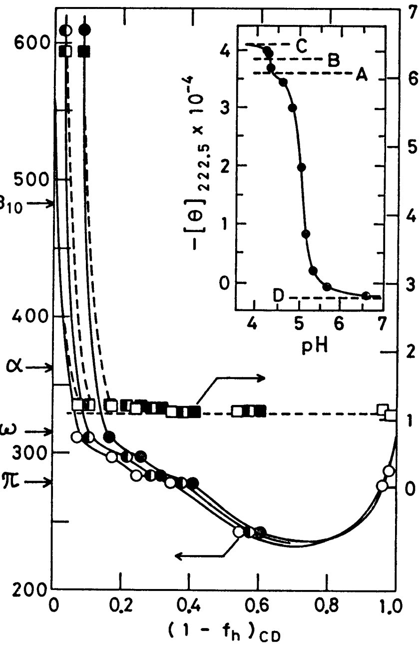

Fig. 1. Dependence of the square root of the *z*-average of the mean-square radius of gyration, $\langle s^2\rangle_z^{1/2}$, (circles) and the weight-average molecular weight, M_w, (squares) on the fraction of the poly(Glu) coil, $(1-f_h)_{CD}$, in the 150 mM NaCl solutions at 25 °C. The helical conformation is assumed to be completed at point A (open symbols), B (half-closed), or C (closed). Insert: the pH dependence of the molar ellipticity (deg cm^2 dmol^{-1}) at 222.5 nm.

$\langle s^2\rangle_z^{1/2}$ range between 305 and 450 Å, depending on the choice of point A, B, or C (Fig. 1). However, no definite value can be specified for the monomeric, perfect helix ($f_h=1$), because of a concomitant formation of aggregates, as envisaged in Fig. 1 by the change in weight-average molecular weights, M_w, which remain nearly constant at a value of 1.07×10^5 (pH>*ca.* 5) but sharply increase with a decrease in pH.[7] Nevertheless, the limiting value of $\langle s^2\rangle_z^{1/2}$ ($f_h\rightarrow 1$) is most likely between 280 and 320 Å.

Value of $\langle s^2\rangle_z^{1/2}$ *and Conformation of Helix.* The third problem is the estimation of the axial translation per residue, h, which defines the helical conformation (h=2.0 Å for the 3_{10}-, 1.5 A for the α-, 1.3 Å for the ω-, and 1.15 Å for the π-helix). For a long rodlike molecule with length L, $\langle s^2\rangle=L^2/12$,[2] but the poly(Glu) sample is polydisperse. Therefore, $\langle s^2\rangle_z$ must be divided by the z-average of the square of degree of polymerization, $\langle DP^2\rangle_z$, not by the weight-average $\langle DP^2\rangle_w$ (an oft-neglected fact), to calculate values of h. If $\langle s^2\rangle_z^{1/2}$ is divided by $\langle DP\rangle_w$, the value of h would be erroneously large. For a polydisperse sample in which each molecule is a rigid perfect helix,

$$h^2 = \langle L^2\rangle_z/\langle DP^2\rangle_z = 12\langle s^2\rangle_z/\langle DP^2\rangle_z$$
$$= 12m_0^2\langle s^2\rangle_z/M_{z+1}\cdot M_z, \tag{1}$$

where m_0 is the residue weight, M_{z+1} and M_z are the (z+1)- and z-average molecular weights. From electric birefringence on the same poly(Glu) sample,[11] the polydispersity, M_w/M_n, was estimated to be 1.12 for the unaggregated α-helix by assuming the Lansing-Kraemer distribution, from which the relation $(M_{z+1}\times M_z)^{1/2}=M_w(M_w/M_n)^{3/2}$ results.[12] In Fig. 1, the values of $\langle s^2\rangle_z^{1/2}$ calculated from Eq. 1 are indicated by arrows for four typical helices. The conformation of poly(Glu) now appears closer to the ω-helix (315 Å) rather than to the π-helix (270 Å) or the α-helix (363 Å). A viscometric study has led to the similar conclusion about the conformation.[8]

Persistence Length and Chain Flexibility. The persistence length q is a measure for the chain flexibility and is related to the radius of gyration.[13] According to Kratky and Porod,[14]

$$\langle s^2\rangle_i = 2qL_i[(1/6)-(q/2L_i)+(q/L_i)^2-(q/L_i)^3(1-e^{-L_i/q})] \tag{2}$$

for the ith molecule of contour length L_i. Since $\langle s^2\rangle_z=\sum N_iM_i^2\langle s^2\rangle_i/\sum N_iM_i^2$, it is a complicated average of the contour length.[15] As a first approximation, the z-average length L_z is utilized in place of L_i,[16] together with a simplified relation: $L_z=[h\times f_h+3.62\times(1-f_h)]\langle DP\rangle_z$.[8] Values of q and q/L_z were calculated at various helix fractions for different helical conformations (Table 1). The contour length L_z for the completely coiled poly(Glu) is 2870 Å (=3.62×708×1.12); yet, q is only *ca.* 120 Å, indicating that the poly(Glu) chain is highly coiled in the 150 mM NaCl (20-fold in excess of Glu residues) in contrast to the 7.5 mM NaCl solution.[8] The chain becomes very contracted at the onset of the coil-to-helix transition ($f_h\approx 0.2$), q being only *ca.* 75 Å, but it becomes stiffer as the helix fraction increases.

TABLE 1. THE PERSISTENCE LENGTH POLY(Glu) IN 150 mM NaCl[a)]

$(f_h)_{CD}$ $\langle s^2\rangle_z^{1/2}$/Å		0 320[b)]	0.2 240	0.4 237	0.6 268	0.8 290
q/L_z	α-[c)]	0.0420	0.0294	0.0391	0.0777	0.172
	ω-	0.0420	0.0301	0.0418	0.0890	0.234
	π-	0.0420	0.0308	0.0440	0.0990	0.316
q/Å	α-	120.6	74.5	86.0	144.7	262.4
	ω-	120.6	75.3	89.2	157.3	327.3
	π-	120.6	76.4	91.8	167.9	412.0

a) The helical structure was assumed to be completed at point A. b) A extrapolated to $(1-f_h)_{CD}\rightarrow 1$. c) Three different helical conformations.

The fourth and barely touched problem is the possibility of kinked or broken points in a long helical backbone. For example, seven Glu residues forming a coil-segment in a poly(Glu) helix correspond to the 99% helix content. A 1% difference is utterly impossible to detect by optical methods, but such a single break may lower the overall chain length or the value of $\langle s^2\rangle_z^{1/2}$ considerably, making the shorter helix conformations only apparent. For a perfect helix, values of q/L_i should be infinitely large, but this can never be confirmed because of the exponential increase in length and molecular weight of aggregated helices.

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References

1) R. W. Woody, *J. Polym. Sci., Macromol. Rev. Ed.*, **12**, 181 (1977).

2) C. Tanford, "Physical Chemistry of Macromolecules," Wiley, New York, (1961), Chaps. 3 and 5.

3) K. Yamaoka and T. Masujima, *Bull. Chem. Soc. Jpn.*, **52**, 1286 (1979).

4) K. Yamaoka and T. Masujima, *Polym. J.*, **11**, 889 (1979).

5) K. Yamaoka, T. Matsuda, and M. Takatsuki, *Bull. Chem. Soc. Jpn.*, **53**, 968 (1980).

6) S. Noji, T. Nomura, and K. Yamaoka, *Macromolecules*, **13**, 1114 (1980).

7) T. Masujima, *Bull. Chem. Soc. Jpn.*, **56**, 838 (1983).

8) T. Masujima, K. Yamaoka, and J. Hori, *Bull. Chem. Soc. Jpn.*, **56**, 1030 (1983).

9) H. Hayashi and A. Wada, *Biopolymers*, **20**, 695 (1981). Earlier reports on the stepwise change were cited in Ref. 8.

10) M. Rinaudo and A. Domard, *J. Am. Chem. Soc.*, **98**, 6360 (1976).

11) K. Yamaoka and K. Ueda, *J. Phys. Chem.*, **86**, 406 (1982).

12) W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

13) H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).

14) O. Kratky and G. Porod, *Recl. Trav. Chim. Pay-Bas*, **68**, 1106 (1949).

15) From Eq. 2, the following relation results: $\langle s^2\rangle_z=(qL_z/3)-q^2+(2q^3/L_w)-(2q^4/L_w\cdot L_n)(1-\langle e^{-L/q}\rangle_n)$.

16) M. Mandel and J. Schouten, *Macromolecules*, **13**, 1247 (1980).