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Conformational Changes of the Poly(α-L-glutamic acid)-Cu(II) Macromolecular Complexes in the pH Range 4—7. A Comparative Study by Means of Viscosity and Circular Dichroism[1)]

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Intrinsic viscosity, [η], and far-UV circular dichroism, CD, were measured at 25 °C for poly(α-L-glutamic acid), poly(Glu), solutions in 7.5 mM and 150 mM NaCl in the presence and the absence of copper(II) ions over the pH range 4—7, where poly(Glu) undergoes two conformational transitions. The first is the well-known helix-coil transition and the second, which is followed by completion of the helix, was attributed to the formation of aggregates. The pH dependence of [η] and molar ellipticity was analyzed with the two-state model. The helix-coil transition can not be represented by the all-or-none process. The aggregation mode of helices is predominantly the side-by-side type in 7.5 mM NaCl, but probably a mixture of the side-by-side and the head-to-tail types in 150 mM NaCl. The hydrodynamic shapes of poly(Glu) and its Cu(II)-complexes were calculated with ellipsoid, cylinder, and straight chain element models. The so-called random-coil form is an extended-coil in 7.5 mM NaCl at higher pH. The poly(Glu) helix is probably slightly shortened as compared with the α-helix.

Among the transition metal ions which bind to poly(α-L-glutamic acid), poly(Glu), the copper ion is unique in that it induces the conformational change of poly(Glu).[2—18)] Our recent studies[11—14)] have clarified that three classes of the Cu(II)–Glu residue complexes are present, and that the formation of these complexes is related to the conformational change of the poly(Glu) backbone. A light scattering study has revealed the formation of poly(Glu) aggregates in both the absence and the presence of Cu^{2+} ions in the low pH region.[18)] While the viscosity of poly(Glu) has often been measured,[19—24)] few attempts were made to interpret the conformational changes in terms of the hydrodynamic shape. For example, Go *et al.* have noted, on a qualitative basis, that the variation of the intrinsic viscosity, [η], of poly(Glu) with pH reflects several distinct changes in the backbone conformation.[20,21)] The present viscometric work aims at the quantitative interpretation of the overall conformational changes of poly(Glu) in the absence and the presence of Cu^{2+} ions. For this purpose, the variations of [η] were correlated with changes of the far-UV circular dichroism, CD, under closely paralleled conditions. Thus, the overall hydrodynamic shapes of poly(Glu) in the absence and the presence of Cu^{2+} ions could be compared with optically determined conformational changes. With reference to the previous light scattering results, we present some quantitative discussions on the axial ratio of the rodlike poly(Glu) helix, the possible aggregation mode, and the effects of the concentrations of Cu^{2+} ions and sodium chloride.

Experimental

Materials and Preparations of Sample Solutions. The sodium salt of poly(Glu), hereafter denoted simply as poly(Glu), was described elsewhere.[11)] The degree of polymerization, DP_w, of the sample for viscosity was 708,[18)] and the one for CD was nominally 610.[14)] Other chemicals were of reagent grade and used without further purification. In a given poly(Glu) or poly(Glu)–Cu(II) solution, the final concentration of HCl plus NaCl was kept constant at 7.5 mM (1 M = 1 mol dm^{-3}) or 150 mM. The mixing ratio, R, was defined in the same manner as before and adjusted by changing the amount of Cu^{2+}.[11)]

Measurements. A modified Ubbelohde-type viscometer was used at 25±0.01 °C. The flow times were 264.1 and 265.9 s, respectively, for the 7.5 and 150 mM NaCl solutions which were used as solvents. A sample poly(Glu) solution (5 ml, [Glu] = 8 mM) was diluted up to twentyfold by the stepwise addition of the NaCl solution to the viscometer. The initial concentration of [Glu] was reduced to 1 mM at pH values lower than 4.6 to avoid precipitation. Since no buffer reagent was used, values of pH of a given polymer solution vary slightly upon dilution. The relation between the shift of pH and the dilution of the poly(Glu) concentration was predetermined outside the viscometer, and utilized to correct the observed values of the reduced viscosity, η_{sp}/c, for an initially set pH, where c is the mass concentration of poly(Glu) in g/100 cm^3. The pH-corrected values of η_{sp}/c were then extrapolated to zero concentration to obtain [η] at the given initial pH as

$$\eta_{sp}/c = [\eta] + k_2c. \qquad (1)$$

Linear extrapolations by the least-square method were possible in all cases, except for the poly(Glu) solutions in 7.5 mM NaCl at pH higher than 5, for which the Fouss plot[25)] also produced nonlinear curves. In these cases, the most likely extrapolated values were taken for [η]. The pH of sample solutions was measured on a Hitachi-Horiba N-5 pH meter under the same conditions as used for the viscosity; the measurement of the CD spectra has been described elsewhere.[11)]

Partial Specific Volume. The partial specific volume of the poly(Glu) in the presence and the absence of Cu^{2+} ions, $\bar{v}$, in the 150 mM NaCl solution was calculated with the aid of Eq. 2[26)] by using the value of the refractive index increment, dn/dc, at 436 nm.[18)]

$$\bar{v} = \left(\frac{n_1^2+2}{n_1^2-1}\right)k - \frac{6n_1}{(n_1^2+2)(n_1^2+1)}\left(\frac{dn}{dc}\right), \qquad (2)$$

where n_1 is a refractive index increment of the poly(Glu) solution in 150 mM NaCl and k is the specific refraction of the solute prior to mixing with solvent. The value of k was determined to be 0.223 from Eq. 2 with a value of 0.604 for $\bar{v}$ of the poly(Glu) solution in 200 mM NaCl at a pH of 4.42[23)] and with a value of 0.1734 for dn/dc of the poly(Glu) solution

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in 150 mM NaCl at the same pH.[18] The value of k was assumed to be constant at other R and pH values. The dependence of $\bar{v}$ on pH was assumed to be the same for the two poly(Glu) solutions, in 7.5 mM and 150 mM NaCl, for a particular value of R.

Results and Discussion

Dependence of Viscosity on pH. Figure 1 summarizes the intrinsic viscosity and the coefficient k_2 for the poly(Glu) solutions in the presence and the absence of Cu^{2+} ions. When the plot of η_{sp}/c *vs.* c is curved, k_2 was determined from the initial slope of the curved line. Values of $[\eta]$ and k_2 in 7.5 mM NaCl decrease sharply with the decrease in pH. The large value of k_2 indicates that the electrostatic interaction between ionized poly(Glu) ions is poorly suppressed at the low ionic strength; hence, the polyion may be in the highly extended-coil form in the neutral pH range. Qualitatively, the sharp decrease of $[\eta]$ in the pH region between 7 and 5.6 may be ascribed to the change from the extended- to the less extended-coil, mainly because of charge neutralization by added hydrogen ions.[20,21] An apparent plateau region for $[\eta]$ between pH 5.6 and 5.1 may also be attributed to the formation of helix for poly(Glu).[20,21] These views must, however, be verified on a quantitative basis (see below). A further decrease in $[\eta]$ after the completion of the helix (pH 5.1) may reflect the formation of aggregates.[17,18,27–30] The higher contents of Cu^{2+} ions ($R=8$) result in the lower values of $[\eta]$ over the entire pH region. This decrease should be an effect of the bound Cu(II), because almost all Cu^{2+} ions are bound to poly(Glu) at pH higher than 5.[14]

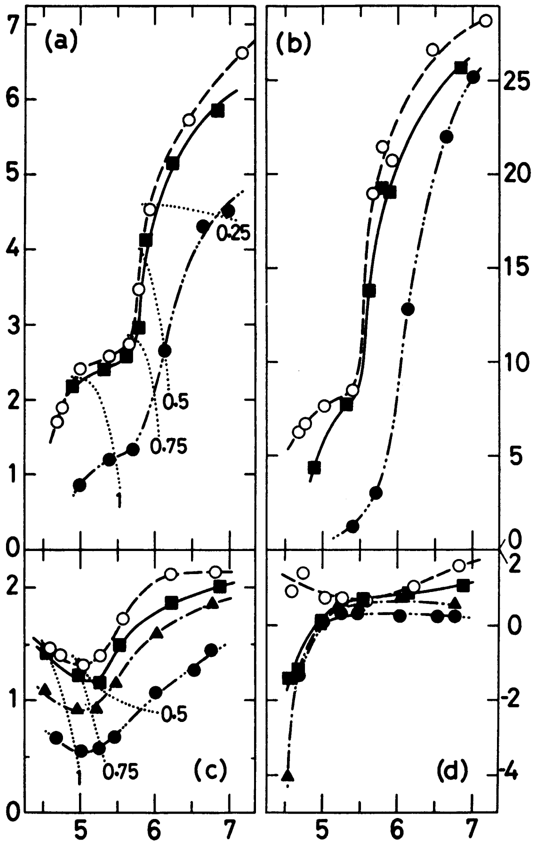

Fig. 1. The pH dependence of the intrinsic viscosity, $[\eta]$, and the increment of reduced viscosity, k_2, for poly(Glu) and poly(Glu)–Cu(II). (a) and (b) at 7.5 mM NaCl and (c) and (d) at 150 mM NaCl. Values of R are ∞ (poly(Glu) only) (——○), 32 (——■), 16 (– –▲), and 8 (—·—●). Dotted curves indicate the helix fraction f_h as given by numerals.

For poly(Glu) solutions in 150 mM NaCl, the behavior of $[\eta]$ is quite different from the above.[20–22] The expansion of the poly(Glu) backbone chain by the charge repulsion of ionized Glu residues is less marked at the higher ionic strength. Values of $[\eta]$ and k_2 are small, k_2 being nearly constant over the entire pH region. Each $[\eta]$ *vs.* pH curve shows a minimum at pH about 5. The curves are similar to each other in the presence and the absence of Cu^{2+} ions, but values of $[\eta]$ decrease with the decrease in R.

Far-UV CD of Poly(Glu) and Its Cu(II)-Complexes. Figure 2 shows typical far-UV CD spectra of poly(Glu) in 150 mM NaCl solutions in the presence and the absence of Cu^{2+} ions. The general characteristics are nearly the same as those in 7.5 mM NaCl solutions.[11] The CD spectra of poly(Glu) show an isoelliptic point at 203.5 nm in the pH range 7.76—4.35; however, they

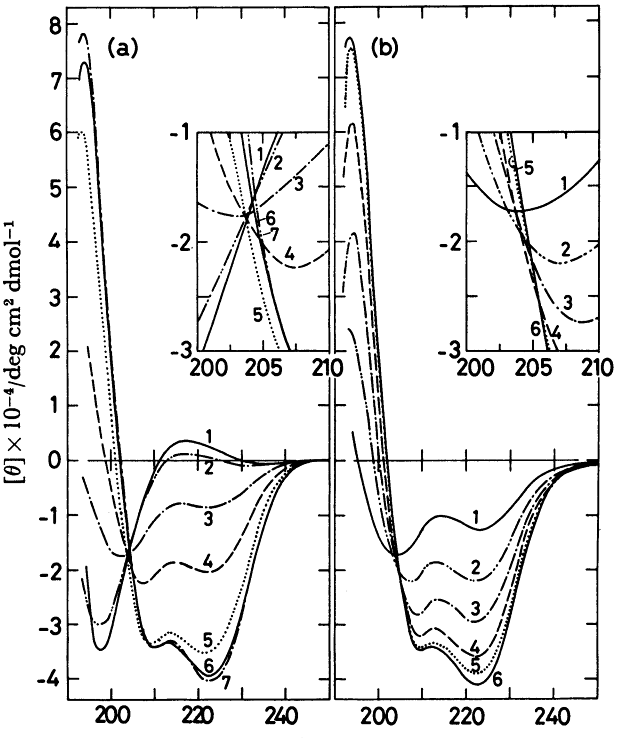

Fig. 2. The far-UV circular dichroism spectra of poly(Glu) at various pH in the absence (a) and the presence ($R=8$) of Cu^{2+} ions (b) at 150 mM NaCl. Values of pH are: (a) 7.76 for 1, 5.68 for 2, 5.21 for 3, 5.07 for 4, 4.57 for 5, 4.28 for 6, and 4.19 for 7; (b) 6.40 for 1, 5.53 for 2, 5.16 for 3, 4.90 for 4, 4.71 for 5, and 4.48 for 6. Inserts are the enlargement of the $[\theta]$ *vs.* wavelength plots in the neighborhood of isoelliptic points.

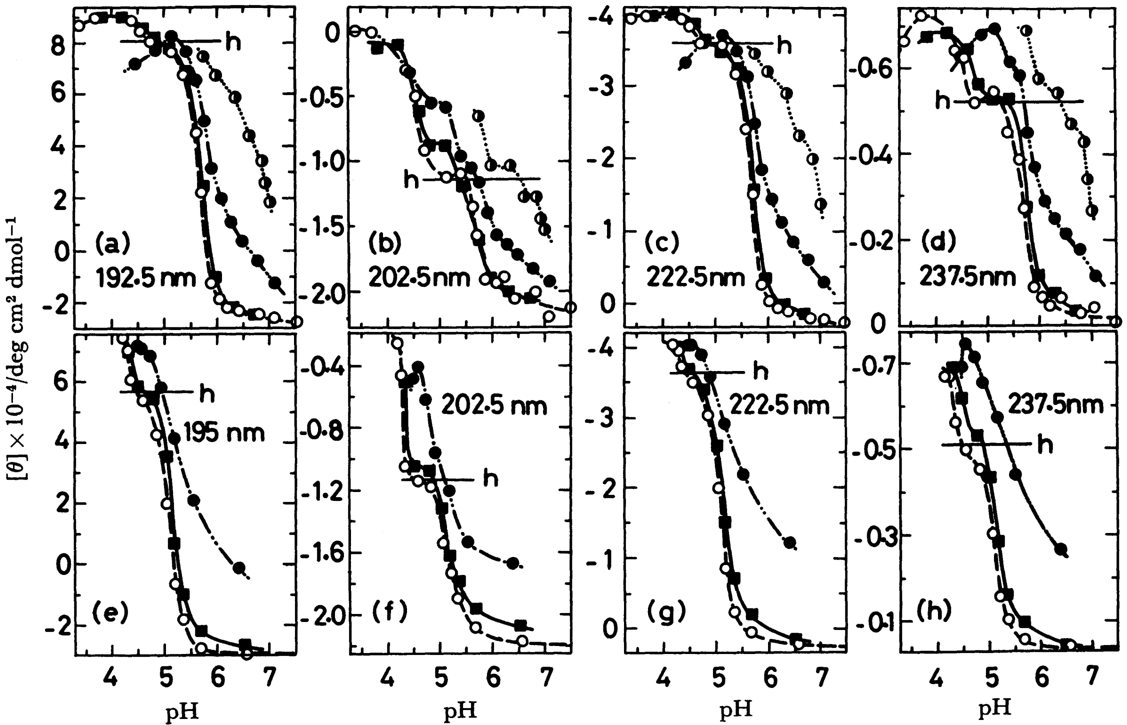

Fig. 3. The pH dependence of the molar ellipticities of poly(Glu) and its Cu(II)-complexes at the selected wavelengths at 7.5 mM ((a)—(d)) and 150 mM ((e)—(h)) NaCl. Values of R are ∞ (— — ○), 32 (——■),8 (——●), and 4 (······◑). The horizontal line in each figure is the value of [θ] for which $f_h=1$.

deviate from this point at pH's lower than 4.3, showing a larger negative peak at 222.5 nm. The CD spectra of poly(Glu)–Cu(II) complexes show the isoelliptic point only in a limited pH range above 4.5 ($R=32$) or 5.16 ($R=8$). The presence of an isoelliptic point in a family of CD spectra indicates that each observed spectrum can be decomposed into two component-spectra: the helix and the coil spectra in this case.[31] The steady deviation of the isoelliptic point to higher wavelengths indicates that another conformational change is likely to occur at pH lower than 4.28 for poly(Glu) and lower than 5.6 for poly(Glu)–Cu(II). It is now clear that this spectral feature is related to the formation of aggregate helices, as observed by light scattering measurements.[18] The possible contribution of the extrinsic CD band of the bound Cu(II) to the observed molar ellipticity, [θ], is ruled out, since the CD spectra of poly(Glu) alone show the same kind of deviation.[11]

The dependence of [θ] on pH was examined at some selected wavelengths to estimate the fraction of helix formed in the poly(Glu) backbone in the presence and the absence of Cu^{2+} ions. Figure 3 shows the plots of [θ] *vs.* pH for poly(Glu) and its Cu(II)–complexes in 7.5 mM and 150 mM NaCl solutions. A two-step change in [θ] is clearly observed at each selected wavelength. (The choice of a wavelength often enlarges or conceals the stepwise change.) These two stepwise changes are less overlapped for solutions of lower ionic strength and higher R, but tend to merge to each other, as the amounts of Na^+ and Cu^{2+} ions increase.

The helix fraction, f_h, for poly(Glu) at a given pH may be calculated by the conventional method as[11,14]

$$f_h = \frac{[\theta]^{c}_{\lambda}-[\theta]_{\lambda}}{[\theta]^{c}_{\lambda}-[\theta]^{h}_{\lambda}}, \qquad (3)$$

where $[\theta]^{c}_{\lambda}$ and $[\theta]^{h}_{\lambda}$ are the molar ellipticities of the pure coil and the pure helix states at the wavelength, λ, and $[\theta]_{\lambda}$ is the observed molar ellipticity of the poly(Glu) solution at a given pH. It has been amply documented in the literature that unique assignments of values for $[\theta]^{h}_{\lambda}$ and $[\theta]^{c}_{\lambda}$ are difficult even for the standard polypeptides, poly(Glu) and poly(L-lysine);[32] however, to retain internal consistency throughout, we selected the molar ellipticity at the onset of the second transition (the one on the lower pH side) for the value of $[\theta]^{h}_{\lambda}$ and the molar ellipticity at the highest observed pH 7.76 for $[\theta]^{c}_{\lambda}$. Values of $[\theta]^{h}_{\lambda}$ are indicated by the horizontal lines in Fig. 3. The helix fraction for the poly(Glu)–Cu(II) complex was also calculated with the above values of $[\theta]^{h}_{\lambda}$ and $[\theta]^{c}_{\lambda}$. The values of pH, for which $f_h=1$, 0.75, 0.5, 0.25, and 0, were evaluated from the f_h *vs.* pH plots at several wavelengths and averaged for poly(Glu) solutions in 7.5 mM and 150 mM NaCl in the absence and the presence of Cu^{2+} ions.

In order to compare the CD data with the intrinsic viscosity, the f_h *vs.* pH plots must be evaluated at infinitely diluted poly(Glu) concentrations. This was done by making use of a previous report by Nitta *et al.*[33] Their results showed that the f_h *vs.* pH plots shift toward the higher pH side by 0.14 in pH-unit for poly(Glu) in 7.5 mM NaCl, but remain unshifted in 150 mM NaCl. In Fig. 1, the helix fractions thus estimated at infinite dilution (dotted lines) are indicated; hence, comparison

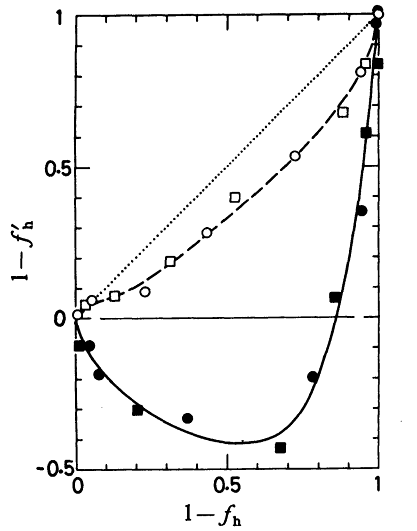

Fig. 4. Comparison of the helix fraction, f'_h, from the intrinsic viscosity with the helix fraction, f_h, from CD for poly(Glu) and poly(Glu)–Cu(II) at $R=\infty$ and 32 and at 7.5 mM (a) and 150 mM NaCl (b). For (a), $[\eta]_h=2.40$ and $[\eta]_c=6.50$ ($R=\infty$, ○) and $[\eta]_h=2.25$ and $[\eta]_c=6.50$ ($R=32$, □). For (b), $[\eta]_h=1.52$ and $[\eta]_c=2.13$ ($R=\infty$, ●) and $[\eta]_h=1.45$ and $[\eta]_c=2.13$ ($R=32$, ■). $[\eta]$'s are in 100 cm^3 g^{-1}.

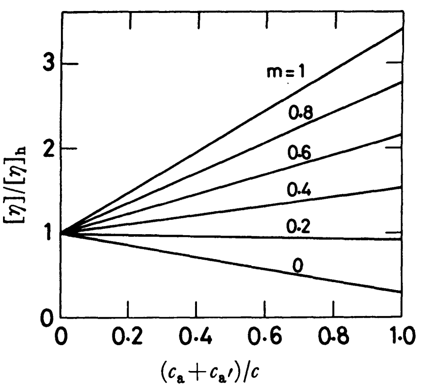

Fig. 5. The theoretical variations of $[\eta]/[\eta]_h$ with the ratio of concentrations of aggregated helices, $c_a+c_{a'}$, to the total concentration of helices, c. The parameter m is $c_{a'}/(c_a+c_{a'})$. For $m=1$ (the head-to-tail type), $L_{a'}=2124$ Å and $2b_{a'}=2b=13$ Å, and for $m=0$ (the side-by-side type), $L_a=1062$ Å and $2b_a=26$ Å, and for unaggregated helix, $L=1062$ Å and $2b=13$ Å being assumed.

of the helix fraction with the intrinsic viscosity is now possible for poly(Glu) and poly(Glu)–Cu(II) systems.

Helix Fraction and Hydrodynamic Behavior. *(i) Helix-coil Transition and Viscosity*: If a poly(Glu) solution contains only pure helix-polyions (the mass concentration of c_h) and pure coil-polyions (c_c), the observed reduced viscosity, $\langle\eta_{sp}\rangle/c$, where $c=c_h+c_c$, and the intrinsic viscosity, $\langle[\eta]\rangle$, may be written as[34]

$$\frac{\langle\eta_{sp}\rangle}{c} = \frac{[\eta]_h c_h + (k_2)_h c_h^2}{c} + \frac{[\eta]_c c_c + (k_2)_c c_c^2}{c}, \tag{4}$$

and
$$\lim_{c\to 0}\frac{\langle\eta_{sp}\rangle}{c} = \langle[\eta]\rangle = f'_h([\eta]_h-[\eta]_c)+[\eta]_c, \tag{5}$$

where $f'_h=c_h/c$, and $[\eta]_h$ and $[\eta]_c$ are the intrinsic viscosities of the unaggregated (single-stranded) helix-polyion and the extended coil-polyion, respectively, while $(k_2)_h$ and $(k_2)_c$ are the corresponding coefficients in Eq. 1. Values of $(1-f'_h)$ were calculated with $[\eta]_h$, $[\eta]_c$, and $\langle[\eta]\rangle$, and are plotted against $(1-f_h)$ in Fig. 4, values of $(1-f_h)$ being calculated from Eq. 3. The values of $(1-f'_h)$ for poly(Glu) and poly(Glu)–Cu(II) do not fit those of $(1-f_h)$ over the entire helix-coil transition region. This result confirms that the helix-coil transition cannot be represented *hydrodynamically* by the all-or-none process of the two-state model. Hence, both partly helical and partly coil portions are contained in each polyion chain in this transition region.

(ii) Viscosity and Aggregation Process of Helices: Light scattering studies have shown that the molecular weight of poly(Glu) and poly(Glu)–Cu(II) increases very rapidly with lowering pH,[18,28] while the viscosity of poly(Glu) tends to increase or decrease in the same pH range depending on ionic strength (Fig. 1). Therefore, it is now beyond doubt that some aggregation process is involved in the pH region where the formation of helix is nearly completed. Aggregated helices appear to be quite cohesive against dilution, since extrapolation of scattered light intensity[18] or reduced viscosity to zero concentration of poly(Glu) exhibits no strong tendency of molecular dissociation. The mode of aggregation should be reflected on the pH dependence of $\langle[\eta]\rangle$ or $\langle\eta_{sp}\rangle/c$. Two extreme modes may be represented by the side-by-side and the head-to-tail association schemes. Here, the observed intrinsic viscosity, $\langle[\eta]\rangle$, is given as

$$\frac{\langle[\eta]\rangle}{[\eta]_h} = \frac{c_h}{c} + \frac{c_a}{c}\cdot\frac{[\eta]_a}{[\eta]_h} + \frac{c_{a'}}{c}\cdot\frac{[\eta]_{a'}}{[\eta]_h}, \tag{6}$$

where $[\eta]_a$ and $[\eta]_{a'}$, are the intrinsic viscosities for the aggregated helices of the side-by-side (unprimed) and the head-to-tail (primed) types ($c=c_h+c_a+c_{a'}$). For the rigid rodlike aggregated helix (the *n*-mer) consisting of n single-stranded helices (monomers), the values of $[\eta]_a$ or $[\eta]_{a'}$ may be calculated with Eq. 7 (see the later sections for detail), together with the appropriate numerical values for the length L and the diameter $2b$.

In order to illustrate the viscometric behavior in the helix-aggregate region (Fig. 1), we utilize Eq. 6 for an assumed simple system of the monomer-dimer equilibrium. Figure 5 shows that the quantity $\langle[\eta]\rangle/[\eta]_h$ decreases with formation of the side-by-side aggregates, but increases with formation of the head-to-tail aggreates. This result is qualitatively in accord with the observed relationship between $[\eta]$ and pH in Fig. 1, indicating that the aggregated poly(Glu) or poly(Glu)–Cu(II) helices are predominantly the side-by-side type in 7.5 mM NaCl, but probably a mixture of both this and the head-to-tail type with a slight chain overlap in 150 mM NaCl. Interchain hydrogen bonds of the acetate dimer type, chelation by Cu(II),[14] and hydrophobic bonds between undissociated side-chain carboxyl groups belonging to different poly(Glu) chains are all attractive forces, favoring formation of the aggregated helices. Indeed, these structures are supported by CPK atomic model building. We are at present unable to specify if the aggregation is the all-or-none process, as assumed in the present discussion, or if it resumes the open association process, in which aggregated helices elongate lengthwise and become thicker in diameter simultaneously, with lowering of the pH. This is partly because the fractional change of formed aggregates with pH cannot be evaluated by the CD method, in contrast with the result for the helix-coil region (Fig. 4).

Hydrodynamic Shape of Poly(Glu) and Cu(II)-Complexes. Since the two-state model is inadequate for the pH-variation of $[\eta]$, the hydrodynamic shape of poly(Glu) in the presence and the absence of Cu^{2+} ions must be estimated, by use of appropriate models, from values of $[\eta]$ and $\bar{v}$. In the following calculation, the poly(Glu) sample is assumed to be monodisperse as regards the molecular weight.[35]

(i) Undeformable Ellipsoid and Cylindrical Models: As a coarse approximation, the shapes of the poly(Glu) and its Cu(II)-complexes may be assumed by the undeformable prolate ellipsoid of revolution with the axial ratio $p(=a/b)$, where a is the major semi-axis and b is the minor semi-axis. Simha's equation may be employed:[36]

$$[\eta] = \bar{v}\left\{\frac{p^2}{15[\ln(2p)-m]} + \frac{p^2}{5[\ln(2p)-m+1]} + \frac{14}{15}\right\}, \qquad (7)$$

where $m=1.5$ for the ellipsoid of revolution, and $m=1.8$ for a long cylinder. Since the results for values of p are almost the same, only the case of ellipsoid is shown in Fig. 6. The apparent axial ratios decrease with the increase in helix fraction. Thus, the hydrodynamic shape of poly(Glu) may be interpreted as being changed from the extended-coil ($p \simeq 160$) to the less extended-coil in the 7.5 mM NaCl solutions and from the less extended- to the shrunk- or compact-coil in 150 mM NaCl solutions, probably because of charge neutralization by added protons and Cu^{2+} ions (especially, at low values of R), and because of charge suppression by added Na^+ ions (in the case of 150 mM NaCl).

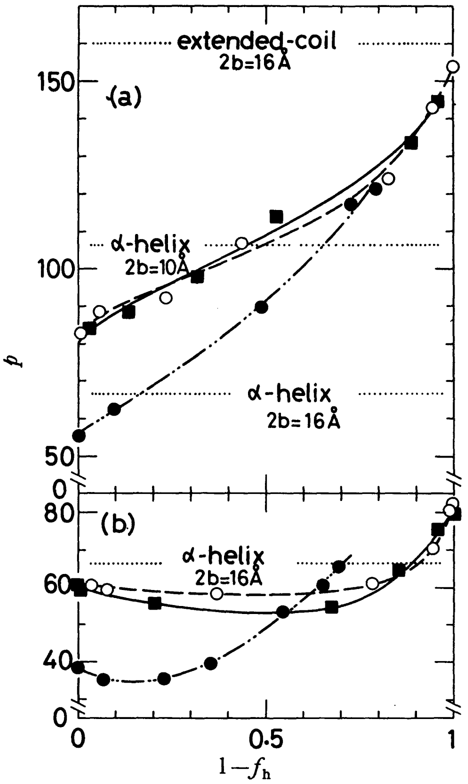

Fig. 6. The dependence of the axial ratio p on the helix fraction f_h of poly(Glu) and poly(Glu)–Cu(II) at 7.5 mM (a) and 150 mM NaCl (b). Values of R are ∞ (— —○), 32 (——■), and 8 (–·–●).

If an appropriate value is assigned to b, the length of the cylindrical or ellipsoidal macroion, L, may be estimated. Two extreme values of 10 Å and 16 Å are used for $2b$ in the present calculation. (The distance between the α-carbon and the oxygen of the side chain carboxylate is about 8 Å.) Figure 7 summarizes the results, in which "extended-coil" indicates the upper limit of the length of the polyion in its most extended-form (2560 Å). This limiting value is given by the length of a Glu residue (3.62 Å) multiplied by the weight-average degree of polymerization (708), which was obtained from light scattering.[18] The lengths of the poly(Glu) coil in 7.5 mM NaCl and at neutral pH are in the range of 1500—2400 Å, depending on the hydrodynamic diameter $2b$. This result indicates that the backbone chain is rather elongated in 7.5 mM NaCl, and that the poly(Glu) ions are not in the so-called random-coil state. (This is the reason why we use the term extended-coil). In 150 mM NaCl solution, the

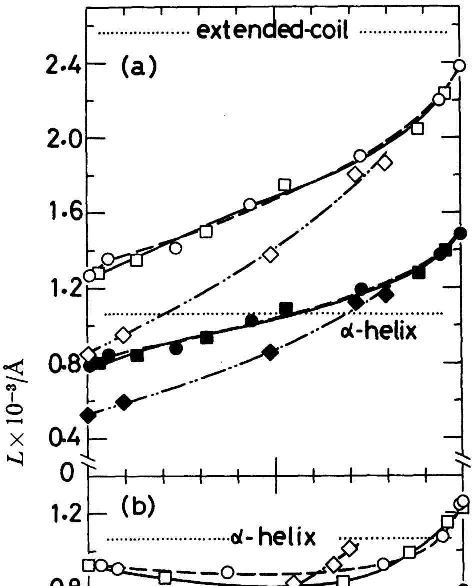

Fig. 7. The dependence of the length of poly(Glu) and poly(Glu)–Cu(II), L, calculated with the cylindrical model on the helix fraction f_h at 7.5 mM (a) and 150 mM NaCl (b). Values of R are ∞ (circles), 32 (squares), and 8 (diamonds). Two extreme values of 10 Å (filled symbols) and 16 Å (open symbols) are assumed for $2b$. The dashed lines indicate the length of α-helix (1062 Å) and that of extended-coil (2560 Å), these lengths being independent of values of $2b$.

poly(Glu) coil is much less extended even at neutral pH.

The possible length for the α-helix is also indicated in Fig. 7 with the horizontal lines, by using a value of 1.5 Å per Glu residue for the axial translation. It is clear that the overall length of the poly(Glu) chain calculated from the viscometric data critically depends on the choice of values of $2b$.[35] If the unhydrated and unaggregated α-helix is assumed for the conformation of poly(Glu), the value of $2b$ would be 13 ± 1 Å in 7.5 mM NaCl, but it would be an unrealistically large value of 20 ± 1 Å in 150 mM NaCl (Fig. 7). On the other hand, the most likely value of $2b$ is probably 11 ± 1 Å, as judged from CPK atomic model building for the unhydrated α-helix. Hence, if poly(Glu) or its Cu(II)-complexes are rigid and rodlike in shape at or near the completion of helix ($f_h=0.9-1.0$), the helical conformation should not be perfectly α-helical. It should be a slightly shortened helix in the low salt concentration, the length per residue, L/res, being about 1.3 Å instead of 1.5 Å. A more distinct deviation from the α-helix is evident in 150 mM NaCl, the L/res being about 1.0 Å. The effect of bound Cu(II) on the L/res value is also quite large. An alternative interpretation of the results in the helix region (f_h=0.9—1.0) is given as follows: The conformation of poly(Glu) and its Cu(II)-complexes is not perfectly rigid, but is partially broken or semi-flexible even at or near the 100% helix content in aqueous solutions.

(ii) Wormlike Model: As a typical example, Kuhn's straight element model is examined:[37]

$$[\eta]=\frac{A_m d^2 N_A}{M_g}\cdot\frac{0.43\,Z}{-1.6+2.3\log(A_m/d_h)+\sqrt{d/A_m}\sqrt{Z}},\qquad(8)$$

where A_m is the length of the straight line chain element, d and M_g are, respectively, the actual length and the molecular weight of the monomeric unit, Z is the degree of polymerization, d_h is the hydrodynamic thickness, and N_A is Avogadro's number. The value of d_h was again assumed to be 10—16 Å under the same consideration as above. The value of d in Å is given by Eq. 9 as a function of helix fraction; the α-helix (at $f_h=1$) and the most extended chain (at $f_h=0$) are assumed.

$$d=1.5f_h+3.62(1-f_h).\qquad(9)$$

The dependence of A_m on f_h is shown in Fig. 8. In the coil region, the length of each straight line chain element is about 200 Å at 7.5 mM NaCl, whereas it is about 80 Å at 150 mM NaCl. The extension of the coil conformation due to the charge repulsion of ionized carboxylate groups is suppressed by the excess salt ions. The smaller value of A_m at $R=8$ may be explained as being due to the bound Cu(II) folding the backbone chain by the charge suppression and chelation.[11–14] For the $R=\infty$ and 32 solutions, the shortest straight chain elements are 180—190 Å, a value about 15-fold smaller than the contour length, in the region where $0.1<f_h<0.4$, and for the $R=8$ solution the A_m value is about 150 Å in $0.6<f_h<0.8$ region. Values of A_m depend on the salt conditions greatly. This result should be taken as indicating that the formation of partial helices in the helix-coil region causes a poly(Glu) chain to contract, and that each straight chain element becomes shorter with more of such elements at a higher salt concentration. As the longer helices are formed at higher f_h, values of A_m increase; however, these values should be only nominal in the high f_h region where this model is unsuitable.

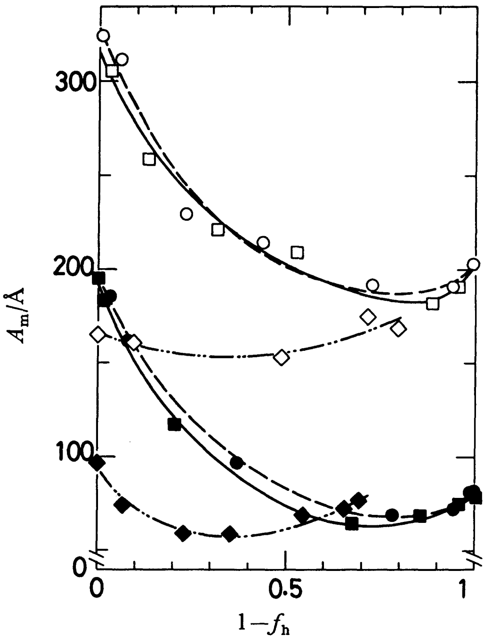

Fig. 8. The dependence of the straight chain element A_m of poly(Glu) chain on pH at 7.5 mM (open symbols) and 150 mM NaCl (filled symbols). Values of R are ∞ (circles), 32 (squares), and 8 (diamonds). The d_h value of 10 Å was used in these calculations.

Conclusion

The helix fraction estimated from the far-UV CD data does not correspond linearly to the helix fraction evaluated from viscometric data. Thus the all-or-none conformational transition is unlikely to occur in aqueous added-salt solutions of poly(Glu) and its Cu(II) complexes. At the same helix fraction, the overall conformations of poly(Glu) and poly(Glu)–Cu(II) are not necessarily the same, but depend on pH and the concentrations of Na^+ and Cu^{2+} ions. At a low concentration of salt, the poly(Glu) chain is in an extended-coil in the neutral pH region and the aggregated helices are likely to be of the side-by-side type in the low pH region. At a high salt concentration, the conformation of poly(Glu) seems to be compact in the helix-coil intermediate region. The added Cu^{2+} ions also accelerate the shrinking of poly(Glu) conformation by inducing helix fragments. This shrinking effect should result from intra- and inter-molecular chelations of Cu(II),[14,18] and thus it can be correlated to the appearance of the characteristic absorption band in the helix-coil intermediate region.[11–14]

References

1) Macromolecule-Metal Ion Complexes. IX. For the preceding paper, see Ref. 14.

2) A. L. Jacobson, *Biopolymers*, **2**, 207 (1964).

3) H. Takesada, H. Yamazaki, and A. Wada, *Biopolymers*, **4**, 713 (1966).

4) Y. Hibino and S. Sugai, *Rep. Prog. Polym. Phys. Jpn.*, **11**, 513 (1968).

5) H. Sigel and G. Blauer, *Helv. Chim. Acta*, **51**, 1246 (1968).

6) S. Inoue, K. Yamaoka, and M. Miura, *Bull. Chem. Soc. Jpn.*, **45**, 1314 (1972).

7) S. Inoue, K. Yamaoka, and M. Miura, *J. Sci. Hiroshima Univ., Ser. A*, **39**, 27 (1975).

8) S. Yamashoji, H. Yoshida, and G. Kajimoto, *Yukagaku*, **25**, 128 (1976).

9) J. A. Marinsky, *Coord. Chem. Rev.*, **19**, 125 (1976).

10) T. Hiraoki, M. Kaneko, and K. Hikichi, *Polym. J.*, **11**, 397 (1979).

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

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

13) S. Noji and K. Yamaoka, *Macromolecules*, **12**, 1110 (1979).

14) T. Masujima and K. Yamaoka, *Biopolymers*, **19**, 477 (1980).

15) N. Imai and J. A. Marinsky, *Macromolecules*, **13**, 275 (1980).

16) M. Koide and E. Tsuchida, *Makromol. Chem.*, **182**, 359 (1981).

17) M. Koide and E. Tsuchida, *Makromol. Chem.*, **182**, 367 (1981).

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

19) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J, Polym. Sci.*, **23**, 851 (1957).

20) Y. Go, Y. Kondo, and K. Aoyama, *Kobunshi Kagaku*, **25**, 344 (1968).

21) Y. Go, Y. Kondo, E. Yamabe, and T. Okumura, *Kobunshi Kagaku*, **25**, 477; 548 (1968).

22) V. E. Bychkova, O. B. Ptitsyn, and T. V. Barokaya, *Biopolymers*, **10**, 2161 (1971).

23) Y. Tadokoro, K. Kawahara, H. Ochiai, and I. Murakami, *Rep. Prog. Polym. Phys. Jpn*, **21**, 585 (1978).

24) H. Kanehiro, J. Komiyama, M. Satoh, and T. Iijima, *Nippon Kagaku Kaishi*, **1980**, 254.

25) R. M. Fouss and U. P. Strauss, *J. Polym. Sci.*, **3**, 246; 602 (1948).

26) W. Heller, *J. Phys. Chem.*, **76**, 2437 (1972).

27) T. M. Schuster, *Biopolymers*, **3**, 681 (1965).

28) Y. Tomimatsu, L. Vitello, and W. Gaffield, *Biopolymers*, **4**, 653 (1966).

29) J. Y. Cassim and J. T. Yang, *Biochem. Biophys. Res. Commun.*, **26**, 58 (1967).

30) B. R. Jennings, G. Spach, and T. M. Schuster, *Biopolymers*, **6**, 635 (1968).

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

32) N. Greenfield, B. Davidson, and D. Fasman, *Biochemistry*, **6**, 1630 (1967); J. Y. Cassim and J. T. Yang, *Biopolymers*, **9**, 1475 (1970); J. R. Parrish, Jr., and E. R. Blout, *ibid.*, **10**, 1491 (1971); Y-H. Chen, J. T. Yang, and H. M. Martinez, *Biochemistry*, **11**, 4120 (1972).

33) K. Nitta, M. Yoneyama, and N. Ohno, *Biophys. Chem.*, **3**, 323 (1975).

34) C. Wolff, A. Silberberg, Z. Priel, and M. N. Layec-Raphalen, *Polymer*, **20**, 281 (1979).

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

36) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

37) H. Kuhn, W. Kuhn, and A. Silberberg, *J. Polym. Sci.*, **14**, 193 (1953).