Polymer Journal, Vol. 15, No. 6, pp 481—484 (1983)

NOTE

Macromolecule–Metal Ion Complexes X. Temperature-Dependent Hysteresis in Formation of Poly(α-L-glutamic acid)–Cu(II) Macromolecular Complex

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(Received December 13, 1983)

KEY WORDS Poly(α-L-glutamic acid)–Cu(II) Complex / Hysteresis / Absorption / Optical Rotatory Dispersion / Helix-Coil Transition

Interactions between poly(α-L-glutamic acid), poly(Glu), and Cu^{2+} ion have been widely studied.[1-13] For example, we reported that three classes of Glu residue–Cu(II) complexes are formed in the poly(Glu)–Cu(II) system in the pH range 4—7.[4,6-8] In the course of these studies, we noted that some poly(Glu)–Cu(II) samples, once stored at 4°C, remained a deeper blue color at 25°C than those stored at the same room temperature, and also that irreversible changes are associated with a temperature-cycle between 4 and 70°C.[4] In order to clarify the conditions for a stable poly(Glu)–Cu(II) complex solution and call attention to the implication of irreversible changes in color, we now carried out a further study on the effect of temperature-cycles on absorption and optical rotatory dispersion, ORD, of poly(Glu) solutions in the presence and absence of Cu^{2+} ions.

EXPERIMENTAL

The sodium salt of poly(α-L-glutamic acid), poly(Glu), the preparation of poly(Glu)–Cu(II) solutions, and the measurements of absorption spectra and ORD curves were described previously.[4,7] The mixing ratio, R, of Glu residue to Cu^{2+} was 16 in poly(Glu)–Cu(II) solutions containing sodium chloride (7.5 and 150 mmol dm^{-3}) as the neutral salt.[7] The temperature-cycles of the poly(Glu)–Cu(II) solution were performed in thermostated quartz cells. The temperature, measured by a thermistor thermometer inside the cell, was changed at the rate of 0.6—0.8°C min^{-1} both in ascending and descending processes, and was cycled by starting first from 25°C toward the high temperature side (90—95°C), returning to the low side (5—7°C), and finally backing again up to 25°C. Some sample solutions were subjected to an additional cycle to test irreversibility. A 1—5 minute standing period was allowed at each temperature before absorption and ORD measurements in order to attain equilibrium for the complex formation. In some cases, solutions were stored overnight at given temperatures. Neither the fibrils of poly(Glu) and poly(Glu)–Cu(II) nor a change in absorption was found, *e.g.*, at pH 4.2 and at 25°C or 4°C. The pH of a sample was always measured at room temperature on a Hitachi-Horiba type N-5 pH meter after completion of the temperature-cycle. A difference in pH within 0.1 pH unit was observed before and after a temperature-cycle.

RESULTS AND DISCUSSION

Absorption Spectra and ORD Curves

Figure 1 shows the absorption spectra and ORD curves of poly(Glu)–Cu(II) at various temperatures in the presence of 7.5 mM NaCl. The ORD curve at

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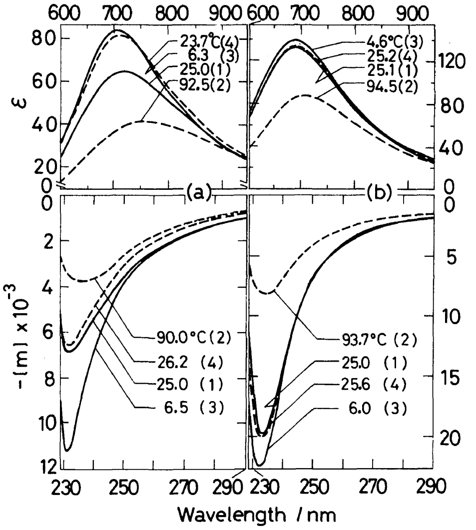

Figure 1. Absorption spectra (upper) and ORD curves (lower) of poly(Glu)–Cu(II) complexes (R = 16) at various temperatures in the course of a temperature-cycle. The number in parentheses refers to the sampling order. (a) pH 5.8 and (b) pH 4.2. The ε_{680} is expressed in $\mathrm{mol^{-1}\,dm^3\,cm^{-1}}$ and the $[m]_{233}$ in $\mathrm{deg\,cm^{-1}\,mol^{-1}\,dm^3}$.

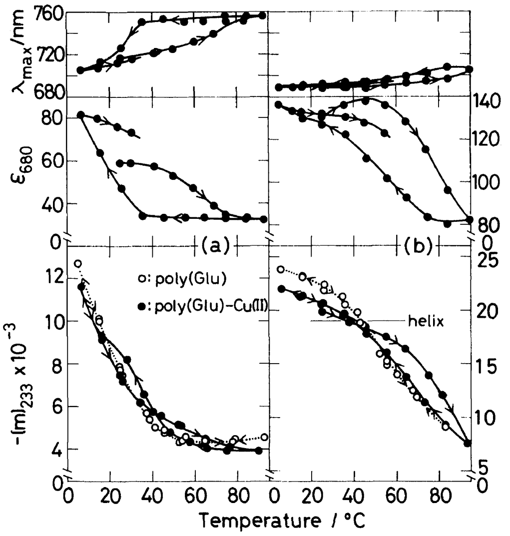

Figure 2. Variation in the molar absorption coefficient of the total Cu^{2+} ion at 680 nm, ε_{680}, the peak wavelength, λ_{max}, and the molar rotation at 233 nm, $[m]_{233}$, of the poly(Glu)–Cu(II) samples (at R = 16 and at 7.5 mM NaCl) in the temperature-cycle. Arrows indicate the direction of the change in temperature. (a) pH 5.8 and (b) pH 4.2. The temperature variations in $[m]_{233}$ of poly(Glu) without Cu^{2+} ions (dotted lines) are also included for comparison. Since the λ_{max} does not shift toward 800 nm (λ_{max} of unbound Cu^{2+}) with a change in temperature, the contribution of the unbound Cu^{2+}, whose ε_{680} is only about 8 and whose presence is 1—3% of the total Cu^{2+} at most, to the total ε_{680} value is negligible.

25°C shows a negative trough at about 233 nm, reflecting the helix formation.[7] Since the ORD curve of poly(Glu)–Cu(II) in the 230—300 nm region can be superimposed upon that of poly(Glu), the contribution of the Cotton effect associated with a charge-transfer band of the bound Cu(II) near 260 nm is negligible.[7] At pH 5.8 (Figure 1a) where the coil-to-helix transition occurs, the helical content in this temperature-cycle was estimated to be 34% (25°C), 14% (90°C), 58% (6.5°C), and 31% (26.2°C) from the observed rotation at 233 nm, $[m]_{233}$. The value of $[m]_{233}$ was assumed to be -19000 for the complete helix, and that of -1500 for the complete random coil.[7] The absorption band of poly(Glu)–Cu(II) showed a red shift at high temperatures but a blue shift at low temperatures. The molar absorption coefficients at 680 nm, ε_{680}, were 64.5 (25°C) before the temperature-cycle and 82.0 (23.7°C) afterwards, a difference of 17.5 being a strong indication of some irreversible process.[4] At pH 4.2 (Figure 1b) where aggregated helices are formed at 25°C,[13] the values of $[m]_{233}$ at lower temperatures (<40°C) exceeded -19000, revealing the formation of aggregates. The effect of the temperature-cycle on ε and $[m]$ was more pronounced at elevated temperatures (*cf.* Figure 2). According to our previous studies,[4,6–8] the observed absorption change by the temperature-cycle may be assigned to the interconversion of Glu residue–Cu(II) complexes[15]: complex II (25°C)→I (high temperature)→II (low temperature) at pH 5.8, whereas complex III (25°C)→II (high temperature)→III (low temperature) at pH 4.2. These complexes were defined previously as follows: Complex I is formed between Cu(II) and the nearest neighbor carboxyl groups (with and without an amide group) on a single chain of extended random-coil or helix; complex II results from the intramolecular chelation of Cu(II) with two or more of isolated carboxyl groups on a single polymer chain, and predominates in the helix-coil transition region; complex III results from the intermolecular chelation of Cu(II) between two or more carboxyl groups on two or more different helices, and predominates in the helix-aggregation region.[4,6–8,12,13] These three types are characterized typically by the values of $\lambda_{\max}$ and ε_{680}: 755 ± 5 nm and 32 (complex I), 710 ± 5 nm and 148 (complex II), and 685 ± 5 nm and 158 (complex III).[8]

Temperature-Cycles of Absorption and ORD

Figure 2 shows the variations of absorption (specified by the ε_{680} and $\lambda_{\max}$ in the visible region) and of the molar rotation at 233 nm with the rise and fall of temperatures in a temperature-cycle. In the absence of Cu^{2+} ions, the $[m]_{233}$ *vs.* T curves of poly(Glu) in 7.5 mM NaCl (dotted lines) show none of hysteresis loops at pH 4.2, 5.8, and 7.3 (not shown). This indicates that the conformational changes of poly(Glu) induced by the temperature-cycle are reversible at least in the pH range 4.2—7.3 and in 7.5 mM NaCl. (It has been reported that the hysteresis appears in the aggregation process for poly(Glu) solutions containing 0.1 M sodium acetate in the pH range 4.1—4.5.[14]) In the presence of Cu^{2+} ions, however, both absorption and ORD change irreversibly with temperature over a wide pH range.

At pH 5.8 (Figure 2a), the $[m]_{233}$ *vs.* T curve for the poly(Glu)–Cu(II) solution showed a small 8-shaped, knotted hysteresis loop. The bound Cu(II) seems to prevent the helix-coil intermediate state from shifting to the random-coil state against the rise of temperature (50→90°C), but it does not seem to promote the regeneration of the helix state in the reversing process from 90°C to lower temperatures. The absorption of bound Cu(II) also showed a large hysteresis loop. The Glu residue–Cu(II) complex formed at 25°C (complex II)[8] seems to be stable in the forward path (25→40°C), but it gradually transforms to another complex (complex I) at higher temperatures. Once fully formed at 92.5°C, complex I cannot be reconverted easily to the original complex II until 35°C in the descending process. The Glu residue–Cu(II) complex formed at the lowest temperature (6.3°C) is again stable against the second rise to room temperature. (This is the peculiar observation described in ref 4.) When the lowest turn-back temperature is set higher than 6.3°C, the difference of ε_{680} between first and second temperature-cycles becomes small, and a single hysteresis loop appears.

At pH 4.2 (Figure 2b) where poly(Glu) helices form aggregates at 25°C, the $[m]_{233}$ *vs.* T curve again showed a hysteresis loop above 45°C, where the helix-to-coil transition occurs. The bound Cu(II) seems to interfere with the thermal disruption of the helix, but the returning process from 95°C indicates that, once the helices are broken, their regeneration is nearly independent of Cu^{2+} ions. The ε_{680} *vs.* T curve shows a large hysteresis loop above 25°C with a maximum at 45°C. At pH 7.3 where poly(Glu) is in the random-coil form, the $[m]_{233}$– and ε_{680}–T curves showed no hysteresis loop on the temperature-cycle in either the presence or absence of Cu^{2+} ions. The closely related hysteresis phenomena were always observed in poly(Glu)–Cu(II) solutions containing 150 mM NaCl, but were not reproducible because of precipitates at high temperature.

It is now quite evident that hysteresis is associated with the macromolecular complex formation between poly(Glu) and Cu^{2+} ions, particularly, in the helix-coil intermediate region. Once the helical segments in a complexed poly(Glu) molecule(s) are destroyed with an increase in temperature, the original poly(Glu)–Cu(II) or Glu residue–Cu(II) complexes are not necessarily regenerated on the backward, cooling process of the temperature-cycle. We should then be aware that the increase in helical content by complexing with Cu^{2+} at room temperature results from a binding reaction favoring local or microscopical interactions, but such a reaction may well lead to an unstable or, at least a metastable equilibrium from the overall macromolecular point of view. The various hysteresis phenomena probably occur in many polymer–metal ion and –dye macromolecular complexes. We should exercise the utmost caution in preparing and treating these complexes, since, as clearly shown in this Note, their conformations are highly dependent on the history or *status quo ante* of the polymer *as such*, with which the small-molecular-weight ligands interact.

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