Facile Chemical Modification of the Poly(L-histidine) for a New pH-Sensitive Polypeptide

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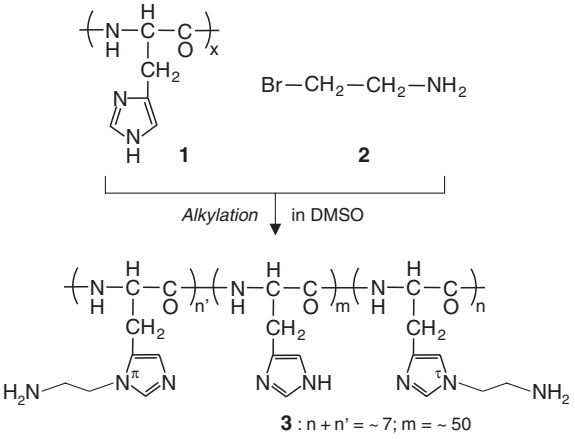
We have synthesized the poly(L-histidine) (PLH) with aliphatic primary amino groups for conjugation as a new pH-sensitive polypeptide. The resulting aminated PLH suddenly precipitated out of the aqueous medium above pH 6.0 owing to the deprotonation of imidazole groups. The aminated PLH was successfully conjugated with maltopentaose by reductive amination. The resulting PLH-maltopentaose conjugate improved its water solubility at physiological pH.

Physiological pH in biological systems is not often preserved. During the internalization of proteins into cells via receptor-mediated endocytosis, for example, the pH value of the resulting endosome decreased up to around 6.0.[1] Such slight change of pH has led us to design the new pH-sensitive polypeptide for biochemical and pharmaceutical application such as drug delivery systems.[2,3]

A polypeptide, poly(L-histidine) (PLH), has many imidazole groups with a pK_a around 6.0. The protonation of imidazole groups triggers off membrane fusion as pH of the environment decreased.[4,5] However, the PLH has no functional groups for easy modification, except an amino terminal as nucleophile, so that the application of the PLH has been limited. Here we describe the facile chemical modification of the PLH for conjugation with biologically active molecules, i.e., the synthesis of "precursory polypeptide" for making pH-sensitive devices.

To conjugate biomolecules with the PLH, we attempted to introduce aliphatic primary amino groups into the PLH. These primary amino groups are widely used for chemical modification,[6] i.e., ε-amino groups of lysine residues of a protein. As shown in Scheme 1, PLH (**1**) was reacted with 2-bromoethylamine (**2**) (hydrobromide) for one-step introduction of amino groups.[7] The GPC profile of the resulting polypeptide (**3**) indicated that unreacted 2-bromoethylamine reagents were removed by the purification process, including dialysis. The number-average molecular weight (M_n) of the isolated polypeptide determined by GPC was about 9.0×10^3 ($M_w/M_n = 1.5$). ^{1}H NMR spectra (Figure 1) of the resulting polypeptide showed the characteristic signals of both PLH and aminoethyl moieties: δ 2.8–3.0 (methylene protons of aminoethyl group), 3.0–3.2 (β-methylene protons of histidine), 4.6 (α-methine protons of histidine), 7.0–7.2/7.6/8.2–8.4 (imidazole protons of histidine). From the signal ratio, the content of aminoethyl histidine was estimated to be about 15 mol %. Seven aminoethyl groups were, on average, conjugated per PLH macromolecule. Furthermore, the amination rate was dependent on the reaction conditions.



Scheme 1. Synthesis of aminated PLH.

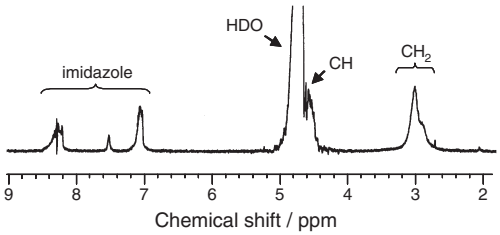


Figure 1. ^{1}H NMR spectrum of aminated PLH in D_2O.

To examine whether the properties of imidazole and amino groups were preserved, we carried out the acid–base titration of the polymer solution, as shown in Figure 2. The imidazole protons of PLH were dissociated around pH 6.0, while the resulting polypeptide exhibited two-step proton dissociation. First and second proton dissociation was attributed to that of imidazole (pH 6) and amino (pH 10) groups, respectively. The dissociation profile varied according to the amination rate; namely, the titration curve of the resulting polypeptide was almost consistent with the content of introduced aminoethyl groups determined by ^{1}H NMR. Thus, we have easily prepared the PLH with ali-

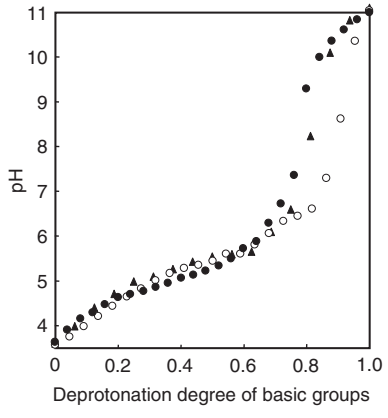


Figure 2. Acid–base titration curves of polypeptides: (●) 15 mol % aminated PLH, (▲) 13 mol % aminated PLH, and (○) PLH. Acidic polymer solutions were titrated with the stepwise addition of 0.5 M NaOH. The horizontal axis is normalized as the total basic groups of each polypeptide.

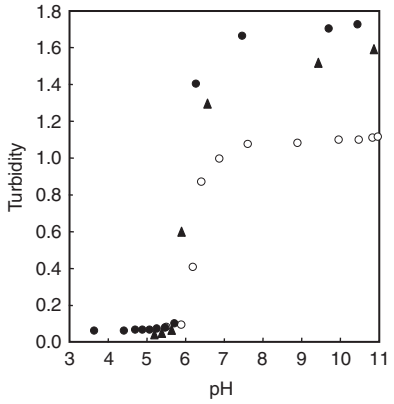


Figure 3. Effect of pH on the solubility of polypeptides in water: (●) 15 mol % aminated PLH (3.4 mg/mL), (▲) 13 mol % aminated PLH (4.0 mg/mL), and (○) PLH (3.5 mg/mL). The turbidity was measured by monitoring the absorbance at 500 nm of the polypeptide aqueous solution during the acid–base titration.

phatic primary amino groups, i.e., "aminated PLH."

Furthermore, the solution behavior of the aminated PLH was examined. As shown in Figure 3, the PLH exhibited precipitation above pH 6.0 owing to the deprotonation of the imidazole groups. The precipitation was also seen in the aminated PLH. Furthermore, the aggregation tendency was also influenced by the amination ratio. These results suggest that the pH-dependent property of PLH was preserved in spite of the amination under the experimental conditions.

To synthesize new pH-sensitive drug carriers, we subsequently attempted to conjugate aminated PLH with carbohydrates. A hydrophilic oligosaccharide, maltopentaose, was chosen as carbohydrate for the improvement of the water solubility at physiological pH. The maltopentaose was reacted with aminated (15 mol %) PLH by reductive amination between the reducing end of the carbohydrate and amino groups of the polypeptide using $NaBH_3CN$ as a reducing agent.[8] The GPC profile indicates that the polypeptide was isolated from unreacted maltopentaose by dialysis and that no intermolecular side reaction occurred. The ^1H NMR spectra (Figure 4) of the polypeptide showed the characteristic signals of maltopentaose moieties as well as aminated PLH: maltopentaose, δ 3.4–4.0 (H-2,3,4,5,6); aminated PLH, δ 2.8–3.2 (methylene protons), 4.6 (α-methine protons), 7.0–7.2/7.6/8.2–8.4 (imidazole protons). From the signal ratio of the maltopentaose to PLH backbone, the content of the carbohydrate in the conjugate was estimated to be about 1 mol %. On average, one maltopentaose molecule was conjugated per PLH macromolecule.

We examined how the conjugated hydrophilic maltopentaose influenced pH-dependent behavior of aminated PLH in water. Figure 5 shows the UV–vis spectra of aminated PLH and

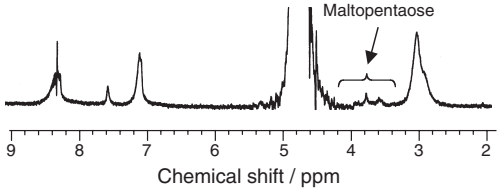


Figure 4. ^1H NMR spectrum of PLH-maltopentaose conjugate in D_2O.

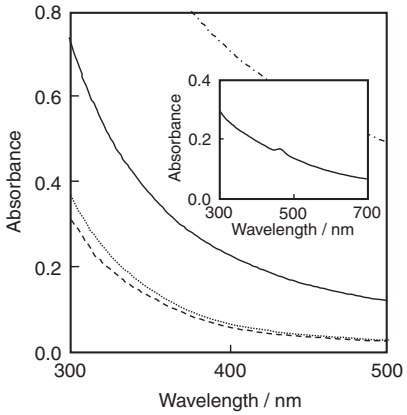


Figure 5. Effect of pH on the UV–vis spectra of polypeptides in water (1 mg/mL): (PLH-maltopentaose) pH 9.6 (——), and pH 5.2 (· · · · ·); (aminated PLH) pH 7.5 (– · –), and pH 5.0 (– –). The inset shows the spectra of the mixture of PLH-maltopentaose and drugs after removal of free drugs at pH 7.5.

PLH-maltopentaose conjugate, respectively, under various pH conditions. The almost same spectra at pH 5 indicated that both polypeptides were soluble in the same concentration because maltopentaose has no absorbance from 300 to 500 nm. At higher pH, the both baselines went up, owing to the turbidity appearance (cf. Figure 3). It should be noted that the resulting conjugate exhibited less turbidity even at pH 10, compared with aminated PLH. These results suggest that the hydrophilic carbohydrate increased the water solubility of aminated PLH at physiological pH. Furthermore, the appearance of the absorbance derived from drugs (manganese porphyrin) suggests that the PLH-maltopentaose conjugate formed the complex with the drugs (Figure 5, inset).

In conclusion, we have synthesized aminated PLH for conjugation with biomolecules. The efficient conjugation of various functional biomolecules with amino groups of PLH can offer unique pH-sensitive devices for biomedical fields.

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References and Notes

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7 A typical procedure is as follows: PLH hydrochloride (50 mg) and 2-bromoethylamine hydrobromide (3.75 g) were dissolved in 12.5 mL of dimethyl sulfoxide (DMSO). The reaction mixture was incubated at 40 °C for 6 days, followed by dialysis against distilled water using a Spectra/Por 7 membrane (molecular weight cutoff = 10^3) to remove unreacted 2-bromoethylamine. The resulting polypeptide was obtained by freeze-drying.
8 A typical procedure is as follows: $NaBH_3CN$ (17 mg) was added to the mixture of aminated PLH (10 mg) and maltopentaose (100 mg) in 2.5 mL of DMSO in the presence of 50 µL of 1 M HCl after the preincubation at 40 °C for 3 days. The reaction mixture was incubated at 40 °C for 1 week, followed by dialysis against distilled water using a Spectra/Por 7 membrane (molecular weight cutoff = 10^4) to remove unreacted maltopentaose. The resulting polypeptide was obtained by freeze-drying.

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