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Aggregate formation and release behaviour of model substances with block co-polypeptide containing tryptophan

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Abstract

In this study, amphiphilic block co-polypeptide consisting of hydrophilic poly(*N*-hydroxyethyl L-glutamine) (PHEG) and hydrophobic poly(L-tryptophan) (poly(Trp)), PHEG-*block*-poly(Trp)-T, was prepared by aminolysis of poly(γ-benzyl L-glutamate)-*block*-poly(Trp) with 2-amino-1-ethanol and subsequent treatment with trifluoroacetic acid (TFA). By using the block co-polypeptide, aggregate formation and sustained-release behaviour of model substances were investigated. The block co-polypeptide formed aggregates in aqueous medium and showed the ability to uptake hydrophobic substances into their hydrophobic moiety. The block co-polypeptide exhibited pH-response, the critical aggregate concentration of PHEG-*block*-poly(Trp)-T and the sizes of the aggregates depended on pH and decreased at pH 2.0. Moreover, fluorescence studies indicated a loose aggregate structure at pH 2.0 and the release rate of the model substances from the polypeptide aggregates was higher at pH 2.0 than at pH 5.0. These results could be explained by dissociation of Trp residues in the hydrophobic cores of the aggregates.

Keywords: Block co-polypeptide; Tryptophan; Treatment with trifluoroacetic acid

1. Introduction

Synthetic polymers have been widely used as biomaterials and have been developed for use as contact lenses and artificial organs because of their good mechanical properties, transparency, high biocompatibility and so on [1–4]. Increasingly more demanding medical treatments necessitate biomaterials with more controlled properties. There is considerable ongoing research into polymer materials having sensor and actuator functions, so-called intelligent materials, in drug delivery systems (DDS) and artificial muscles [5–7]. DDS is a particularly interesting field, because such systems are not only able to deliver the medicine to the organ which needs it but can improve therapeutic effects and reduce adverse effects [8–11]. Kataoka and co-workers have developed polymeric anti-cancer drugs using polymer micelle drug carrier systems and polyion complex micelles consisting of polyethylene oxide and polypeptides [12–14].

Polypeptides synthesised from amino acids have excellent biocompatibility, because they are composed of the same basic units as those of proteins. Polypeptides also exhibit several conformations such as α-helices, β-sheets and random coils, depending on pH and the kind of amino acid used, and are expected to serve as intelligent materials for DDS and other applications [15,16]. However, applied DDS studies taking advantage of the dissociation or the conformation changes of polypeptides with change in pH have rarely been reported.

In our previous study, it was found that L-tryptophan (Trp) treated with trifluoroacetic acid (TFA) exhibited pH-reversible colour changes from red (below pH 4.0) to yellow (above pH 5.5), and that the colour changes were caused by dissociation of the indole ring of Trp [17]. Moreover, hydrogels, microspheres prepared with co-polypeptides containing Trp, when treated with strong acids, showed a pH response in the degree of swelling, solute permeability, mechanical properties in the pH regions where colour changes took place [17–21]. Trp, having a bulky indole ring, is the most hydrophobic of natural amino acids, and displays hydrophobic interactions in aqueous medium. The aim of the present study was to investigate application possibility for a pH-controlled drug delivery system exploiting the dissociation of Trp in polypeptides treated with TFA. Trp-containing co-polypeptide treated with TFA was expected to exhibit uptake abilities and controlled release of hydrophobic substances by dissociation of Trp.

In this study, block co-polypeptides, PHEG-*block*-poly(Trp), consisting of poly(*N*-hydroxyethyl L-glutamine)

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(PHEG) and poly(Trp), were prepared by aminolysis of poly(γ-benzyl L-glutamate)-*block*-poly(Trp), PBLG-*block*-poly(Trp), with 2-amino-1-ethanol. PHEG-*block*-poly(Trp) was then treated with TFA to obtain the pH-response block co-polypeptide PHEG-*block*-poly(Trp)-T, whose aggregation behaviour, model substance uptake ability, and pH-dependence of sustained-release behaviour were evaluated.

2. Experimental

2.1. Materials

2.1.1. Synthesis of block co-polypeptide, poly(γ-benzyl L-glutamate)-block-poly(Trp)

Block co-polypeptide consisting of poly(γ-benzyl L-glutamate) (PBLG) and poly(L-tryptophan) (poly(Trp)), PBLG-*block*-poly(Trp), was synthesised by two step polymerisation using γ-benzyl L-glutamate (γ-BLG) and L-tryptophan (Trp) *N*-carboxyanhydride (NCA). γ-BLG and Trp NCA were obtained according to the method described in previous paper [17,20]. Each NCA were recrystallisation from ethyl acetate with petroleum ether. First, γ-BLG NCA was dissolved in dioxane (conc. 10 wt%) and then *n*-butylamine (*n*-BA) as an initiater was added at NCA-to-*n*-BA molar ratio of 100 to obtain amine-terminated PBLG (PBLG-NH_2). The PBLG-NH_2 was precipitated in an excess of cold methanol and dried in vacuo.

Second, to prepare PBLG-*block*-poly(Trp), Trp NCA was dissolved in DMF and then the polymerisation of the NCA was initiated with PBLG-NH_2/DMF solution. The concentration of Trp in this polymerisation was 25 mol% (residual molar ratio of γ-BLG/Trp = 68/23). The PBLG-*block*-poly(Trp) was precipitated in a large amount of cold methanol and dried in vacuo. All solvents used in synthesis of PBLG-*block*-poly(Trp) were distilled twice.

2.1.2. Preparation of amphiphilic block co-polypeptide

The amphiphilic block co-polypeptide, poly(*N*-hydroxyethyl L-glutamine)-*block*-poly(Trp), PHEG-*block*-poly(Trp), was prepared by aminolysis of PBLG-*block*-poly(Trp) with 2-amino-1-ethanol (EA) at 50 °C for 5 days. PHEG is water soluble and it seems that PHEG does not affect the aggregates formation of poly(Trp) since the conformation of PHEG in water is random coil [22]. Therefore, PHEG was used as hydrophilic component in amphiphilic block co-polypeptide. The amphiphilic block co-polypeptide was obtained by dialysis of the reaction mixture and lyophilised. Debenzylation of PBLG-*block*-poly(Trp) was confirmed by the disappearance of absorption due to ester groups at 1720 cm^{-1} in FT-IR spectrum.

2.1.3. Preparation of pH-response block co-polypeptide treated with TFA

The amphiphilic block co-polypeptide prepared above were dissolved in TFA with irradiating UV (wavelength 365 nm, intensity 300 μW/cm^2) for 3 days [17]. The amphiphilic block co-polypeptide treated with TFA, PHEG-*block*-poly(Trp)-T was obtained by dialysis of the reaction mixture and lyophilised. Table 1 shows composition of PHEG-*block*-poly(Trp)-T evaluated by ^{1}H NMR in DMSO-d_6. A schematic diagram of preparation of block co-polypeptide treated with TFA is shown in Scheme 1.

Table 1
Molecular characteristics of PHEG-*block*-poly(Trp)-T

Sample	Molecular weight		Trp content (mol%)	M_w/M_n[a]
	PHEG[b]	Poly(Trp)[b]		
PHEG-*block*-poly(Trp)-T	11760 (DP = 68)	4280 (DP = 23)	25	1.4

[a] GPC data.
[b] Calculated from ^{1}H NMR data in DMSO-d_6.

2.1.4. Regents and solvents

All regents and solvents were purchased from Peptide Institute Inc. (amino acids) and Nacalai Tesque, Inc., and were used without further purification except for the solvents used in PBLG-*block*-poly(Trp) synthesis. 1-Anilino-8-naphtalene sulfonic acid magnesium salt (ANS) as an anion model drug, pyrene as a non-ion model drug and 5-fuluorourasil (5-FU) as an anticancer drug were used in this study. 5-FU is known as conventional antimetabolite [23] and produces adverse effects such as dehydration, inflammation of the intestines and so on. Moreover, solubility of 5-FU for water is very low. Therefore, administration of 5-FU by DDS seems to be very significant method [24–26].

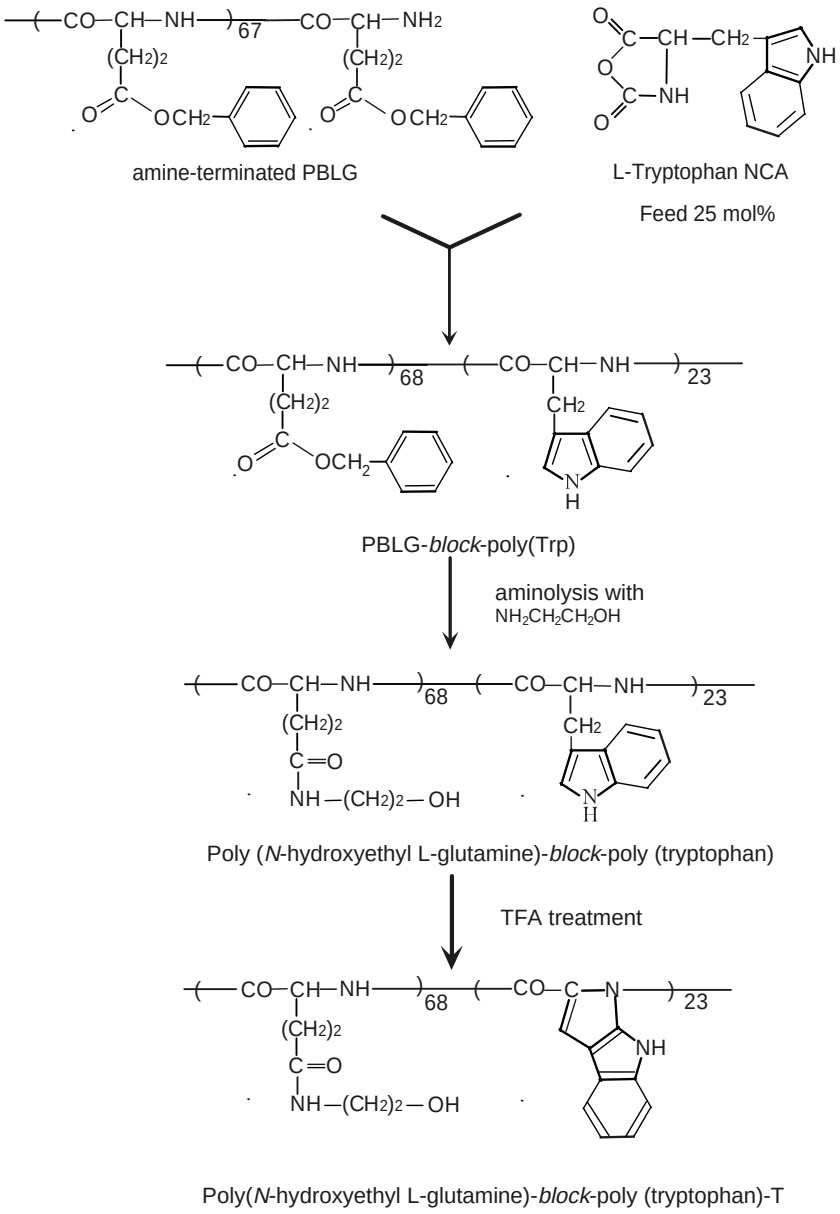
2.1.5. Preparation of block co-polypeptide aggregates

To prepare block co-polypeptide aggregates, PHEG-*block*-poly(Trp) or PHEG-*block*-poly(Trp)-T was dissolved in DMSO and then the block co-polypeptide/DMSO solution was dialyzed in distilled water.

2.2. Measurements

2.2.1. Methods

The composition of amphiphilic block co-polypeptide and aggregates formation of the block co-polypeptide was estimated by ^{1}H NMR (JOEL Model JNM-GX400 spectrometer) measurements. DMSO-d_6 and D_2O were used for the measurement. To confirm debenzylation in aminolysis reaction, FTIR (Nicolet Instruments Model AVATAR 320S FT-IR spectrophotometer) measurements were carried out. FT-IR spectra were measured by the KBr method in the region of 4000–400 cm^{-1}. Gel permeation chromatography (Toso model HLC-8020) was measured with DMF/LiBr as an eluting solvent to estimate molecular weight distribution of block co-polypeptide. To evaluate pH response of co-polypeptide treated with TFA, UV–vis absorption spectra were measured by a JASCO Model V-520 spectrophotometer with a quartz cell having a path length of 1 cm. The pH was adjusted by 1N HCl and 1N NaOH. Fluorescence spectroscopy was performed in a SHIMADZU Model RF-5300FC spectrophotofluorometer to measure the critical aggregate concentration (CAC) and uptake/release behaviour of block co-polypeptides.



Scheme 1. Preparation scheme of pH-response block co-polypeptide.

1-Anilino-8-naphtalene sulfonic acid magnesium salt (ANS) and pyrene (Py) were used as fluorescent probe. The excitation wavelength of 339 and 350 nm were employed for the measurements. To elucidate the conformation of block co-polypeptide, circular dichroism (CD) measurements were carried out with a JASCO Model J-725 CD/ORD spectrometer with a quartz cell having a path length of 0.1 and 1.0 cm. Dynamic light scattering (DLS) measurement (Otsuka Electronic Co., Ltd Model DLS-7000) was carried out with a cylindrical cell having a diameter of 12 mm in order to estimate the size of the aggregates formed by amphiphilic co-polypeptides. Argon laser (488 nm) was used as the light source for the measurement. Transmission electron microscopy (TEM) was performed by using a JEOL JEM-1200EX transmission electron microscope to observe the morphology of the aggregates. The sample was prepared by freezing the aqueous solution including the aggregates on EM-fine grid coated with poly(vinyl formal), and then lyophilised.

2.2.2. Evaluation of azobenzene uptake ability of PHEG-block-poly(Trp)

Ten milligrams of azobenzene was added to the aqueous solution of PHEG-*block*-poly(Trp) adjusted to predetermined concentration and then the mixture was kept at 50 °C to dissolve azobenzene into the aggregates. The azobenzene was used as a model of hydrophobic substance. The insoluble azobenzene was removed by filtration after 3 days and ethanol with the same volume as the filtrate was added. The absorption intensity at 433 nm was measured by using a UV–vis spectrometer to evaluate the amount of dissolved azobenzene in the mixture. The amount of azobenzene was determined with a pre-prepared analytical curve.

2.2.3. Evaluation of uptake ability of TFA-treated block co-polypeptide, PHEG-block-poly(Trp)-T

pH-response amphiphilic block co-polypeptide, PHEG-*block*-poly(Trp)-T was dissolved in DMSO and then a model drug was added to the block co-polypeptide/DMSO solution. The mixed solution was dialyzed in distilled water to introduce a model drug into block co-polypeptide aggregates [27]. The block co-polypeptide aggregates including model drug were obtained by lyophilisation. Fluorescent intensity when the block co-polypeptide aggregates obtained from the above method were re-dissolved in DSMO was measured by using spectrophotofluorometer to evaluate the introduced amount of model drug in the aggregates. The uptake amount of model drug was determined with a pre-prepared analytical curve. Fluorescence intensity of 530 nm (excitation wavelength: 350 nm) and 384 nm (excitation wavelength: 336 nm) were employed for ANS and Py, respectively. In the case of 5-FU, the absorption intensity at 260 nm was measured with UV–vis spectrophotometer.

2.2.4. Evaluation of release behaviour from TFA-treated block co-polypeptide aggregates

The TFA-treated block co-polypeptide, PHEG-*block*-poly(Trp)-T, aggregates including model drug (ANS, Py and 5-FU) were added to 5 ml of buffer solution (pH 2.0 or pH 5.0) and then the solution was poured into a dialysis tube (molecular weight cut off: 8,000) [28]. The tube was immersed into 200 ml of buffer solution (pH 2.0 or pH 5.0) and the released amount of model drug in 3 ml of external solution was measured with time by spectroscopic analytical method. Fluorescence intensity of 530 nm (excitation wavelength: 350 nm) and 384 nm (excitation wavelength: 336 nm) were employed for ANS and Py, respectively. In the case of 5-FU, the absorption intensity at 260 nm was measured with UV–vis spectrophotometer. The released percentage was calculated by following equation:

$$\text{Releasedpercentage}\ (\%) = \frac{C_r}{C_0} \times 100$$

where, C_r is released amount of model drug and C_0 is initial uptake amount of model drug.

3. Results and discussion

3.1. Secondary structure of PHEG-block-poly(Trp)

Conformation of PHEG-*block*-poly(Trp) in aqueous solution was evaluated by CD measurement. The CD spectrum confirms that random coil was dominant for the conformation of PHEG-*block*-poly(Trp). ORD measurement was also carried out to estimate the conformation of poly(Trp) block. The b_0 value in DMF calculated by Moffitt's equation was +495. Fasman et al. performed the conformation analysis of poly (Trp) with a molecular weight 123,000 g/mol, and reported that the poly(Trp) forms a α-helix structure with b_0 value of +570 [29–31]. Although the poly(Trp) used in this study can form α-helix structure, the ordered structure seems to have not been formed at its molecular terminal since the degree of polymerisation is not sufficiently high. It is considered that, as the result of ORD measurement, the α-helix structure of poly(Trp) is stabilised by strong hydrophobic interaction between poly(Trp) molecules.

3.2. Aggregation ability of PHEG-block-poly(Trp)

The aggregation ability of amphiphilic block co-polypeptides was investigated by fluorescence measurements on the magnesium salt of 1-anilino-8-naphthalene sulfonic acid (ANS-Mg). The fluorescence maximum wavelength λ_{max} of ANS-Mg shifts to shorter wavelengths (blue shift) and the fluorescence intensity increases with decreasing solvent polarity. Therefore, the polarity of the microenvironment of this guest molecule (fluorescence probe) can be evaluated by this technique. In fluorescence measurements of ANS-Mg at various PHEG-*block*-poly(Trp) concentrations, the fluorescence intensity increased with increasing concentration of PHEG-*block*-poly(Trp), and the maximum fluorescence wavelength shifted from 535 to 470 nm. These results suggested that in aqueous medium, the poly(Trp) chain formed hydrophobic domains, in which ANS-Mg was taken up. Fig. 1 indicates variation of fluorescence maximum wavelength and intensity as a function of PHEG-*block*-poly(Trp) concentration. The critical aggregation concentration (CAC) obtained from the inflection point of the plot of λ_{max} vs. PHEG-*block*-poly(Trp) concentration was 0.8×10^{-5} mol/l (1.4 mg/l). Fig. 2 shows ^{1}H NMR spectra of PHEG-*block*-poly(Trp) in D_2O and in DMSO-d_6. In DMSO-d_6, signals attributed to indole rings of Trp residues were observed at 7 ppm, whereas these signals disappeared completely in D_2O. The mobility of the poly(Trp) block in PHEG-*block*-poly(Trp) was restricted by hydrophobic aggregation of poly(Trp) in D_2O. On the other hand, hydrophobic aggregation does not occur in DMSO-d_6, a good solvent for PHEG-*block*-poly(Trp). From these ^{1}H NMR results, it was apparent that PHEG-*block*-poly(Trp) formed

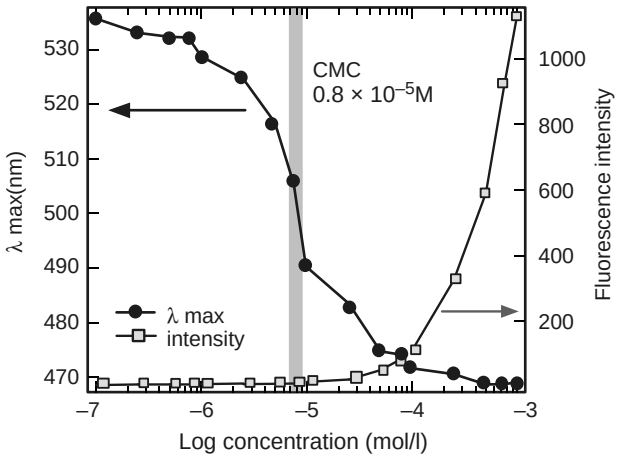


Fig. 1. Plots of the fluorescence maximum wavelength and intensity of ANS as a function of PHEG-*block*-poly(Trp) concentration.

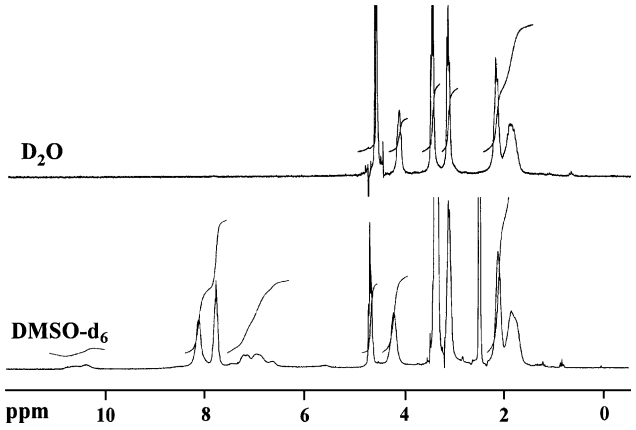


Fig. 2. ^{1}H NMR spectra of PHEG-*block*-poly(Trp) in DMSO-d_6 and D_2O.

aggregates in aqueous medium by compaction of the poly(Trp) block.

3.3. Evaluation of the size of PHEG-block-poly(Trp) aggregates

To evaluate the size of PHEG-*block*-poly(Trp) aggregates, dynamic light scattering (DLS) measurements were performed (Table 2). These measurements revealed that PHEG-*block*-poly(Trp) formed aggregates with an average diameter of 60 nm. Fig. 3 shows a TEM photograph of PHEG-*block*-poly(Trp) aggregates. The TEM photograph showing spherical aggregates confirmed the result of DLS measurements.

3.4. Evaluation of uptake ability of hydrophobic substance for PHEG-block-poly(Trp)

The uptake ability of hydrophobic substance was estimated by measuring the amount of azobenzene dissolved into hydrophobic region formed in PHEG-*block*-poly(Trp) aggregates. Fig. 4 shows the relationship between PHEG-*block*-poly(Trp) concentration and dissolved amount of azobenzene. Since, azobenzene was absorbed in the hydrophobic region formed in PHEG-*block*-poly(Trp), the dissolved amount increased with increasing of PHEG-*block*-poly(Trp) concentration and a good proportionality relationship was observed between the dissolved amount of azobenzene and the concentration of PHEG-*block*-poly(Trp). The uptake amount of azobenzene calculated was 21.3 mg for 1 g of PHEG-*block*-poly(Trp).

3.5. pH response of PHEG-block-poly(Trp) treated with TFA

PHEG-*block*-poly(Trp) treated with TFA, PHEG-*block*-poly(Trp)-T, showed variations in vis spectra in the region

Table 2
Diameter and polydispersity of PHEG-*block*-poly(Trp) aggregate.

Sample name	Diameter (nm)	Polydipersity
PHEG-*block*-poly(trp)	60.3	0.08

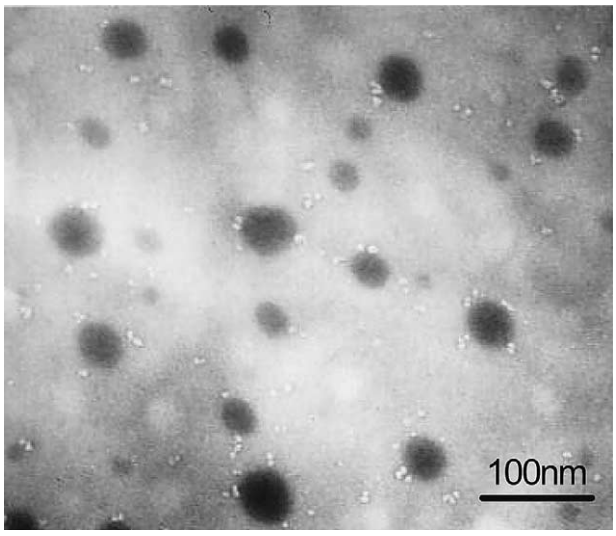


Fig. 3. Transmission electron microscopy photograph of PHEG-*block*-poly(Trp) aggregates.

from pH 2.0 to pH 4.0. On the other hand, pH dependence of vis spectrum and existence of dissociation group were not observed in untreated PHEG-*block*-poly(Trp). In previous study, a TFA-treated random co-polypeptide consisting of *N*-hydroxyethyl L-glutamine and L-Trp showed variation in the vis spectrum in the region from pH 4.0 to pH 5.5, and nonaqueous titration revealed that the colour change was caused by dissociation of indole ring of Trp treated with TFA [17]. It is considered that TFA-treated Trp residues in the block co-polypeptide dissociated below pH 2.0 because PHEG-*block*-poly(Trp) showed similar colour change and pH response by TFA treatment as the TFA-treated random co-polypeptide. The difference in dissociation pH of TFA-treated Trp between random co-polypeptide and block co-polypeptide is attributable to inhibition of protonation of the indole ring by hydrophobic aggregation of the poly(Trp) block.

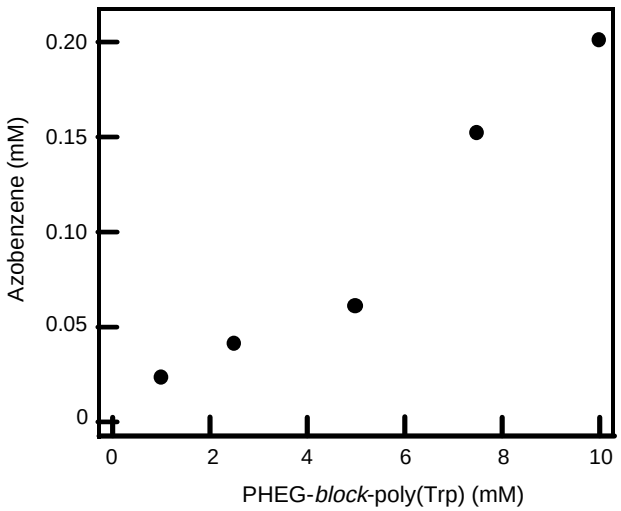


Fig. 4. Relationship between uptake amount of azobenzene and block copolymer concentration.

3.6. Formation of PHEG-block-poly(Trp)-T aggregates

Fig. 5 shows ^{1}H NMR spectra of PHEG-*block*-poly(Trp)-T in D_2O and in DMSO-d_6. In these ^{1}H NMR measurements, solvent-dependent differences were observed, as well as evidence for untreated block co-polypeptide. The peaks due to indole rings in Trp residues were not observed in D_2O, while these peaks appeared at 7 ppm in DMSO-d_6. This confirmed that PHEG-*block*-poly(Trp)-T formed aggregates consisting of a poly(Trp) core and a PHEG outer shell, generated by hydrophobic interactions of the poly(Trp) block. When HCl(aq) was added to PHEG-*block*-poly(Trp)-T in D_2O, ^{1}H NMR spectra showed weak peaks around 7 ppm, attributed to the indole rings in Trp residues. These weak signals meant that the mobility of the poly(Trp) block increased by dissociation of Trp residues treated with TFA, and that the aggregates became unstable by repulsion between dissociated Trp residues. The CAC of PHEG-*block*-poly(Trp)-T was evaluated by fluorescence measurements with pyrene (Py). In the fluorescence spectrum of Py, the intensity at 384 nm (I_3) and 375 nm (I_1) changes with the polarity of the environment and the ratio (I_3/I_1) increases with decreasing polarity [32]. Fig. 6 shows the variation of intensity ratio (I_3/I_1) as a function of PHEG-*block*-poly(Trp)-T concentration at pH 2.0 and at pH 5.0. Under both pH conditions, the ratio initially increased gradually at low PHEG-*block*-poly(Trp) concentrations, and then increased drastically over a certain concentration range, reflecting the formation of PHEG-*block*-poly(Trp)-T aggregates and uptake of Py into their poly(Trp) cores. The CACs obtained from the inflection points in Fig. 6 were 8.0×10^{-5} and 2.5×10^{-4} mol/l at pH 5.0 and pH 2.0, respectively. Thus, PHEG-*block*-poly(Trp)-T exhibited a higher CAC at lower pH. Fig. 7 depicts the pH dependence of the fluorescence intensity ratio (I_3/I_1) of

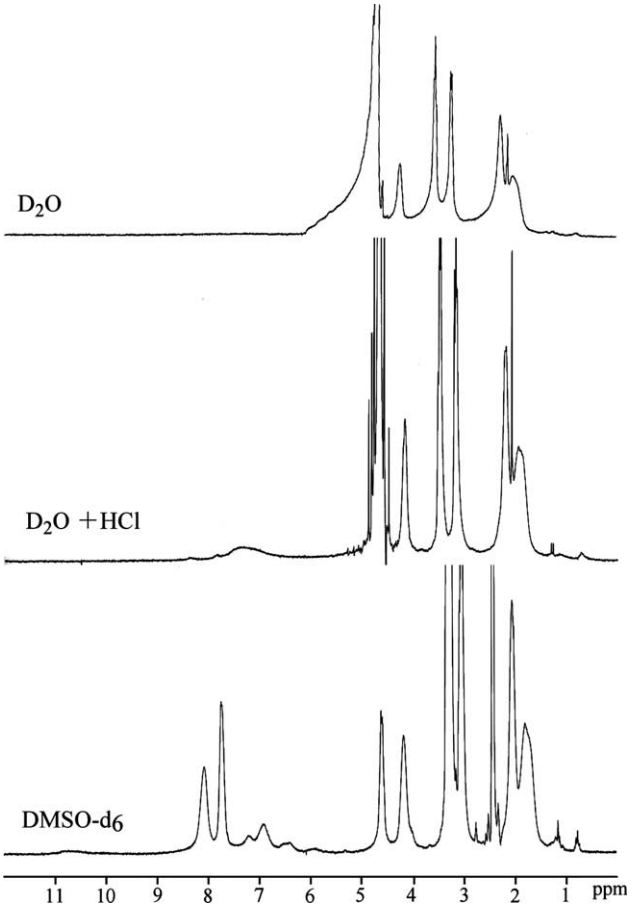


Fig. 5. ^{1}H NMR spectra of PHEG-*block*-poly(Trp)-T in DMSO-d_6 and D_2O.

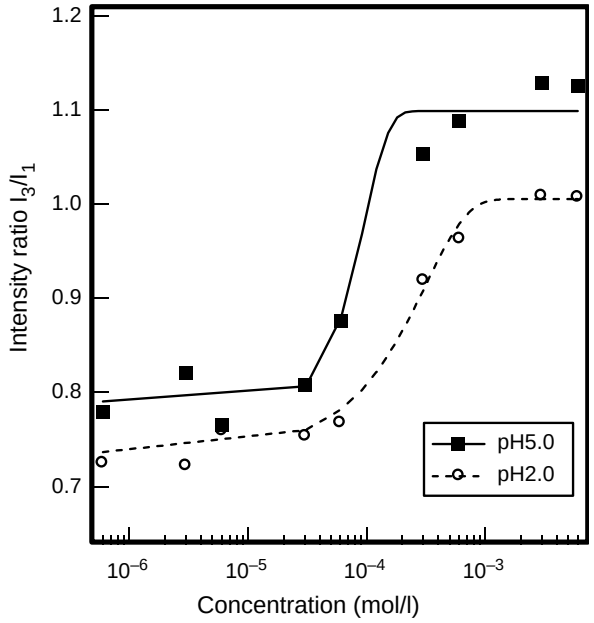


Fig. 6. I_3/I_1 ratio of pyrene as a function of PHEG-*block*-poly(Trp)-T concentration in different pH solutions.

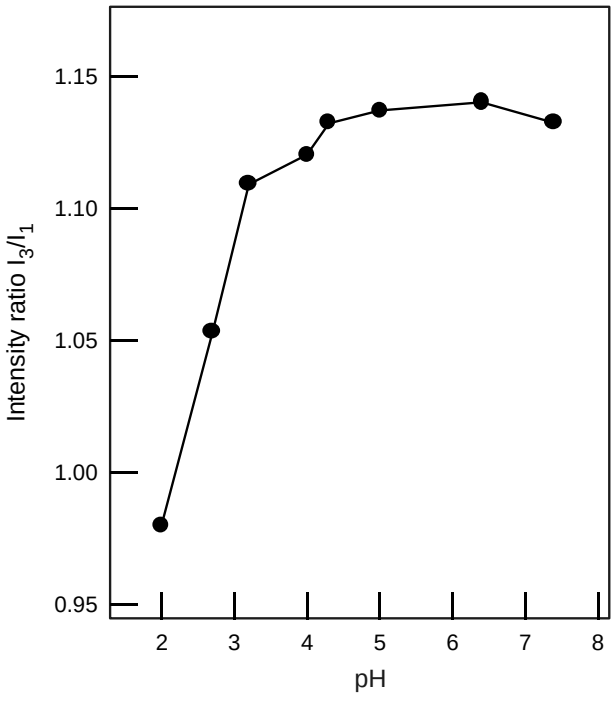


Fig. 7. pH dependence of I3/I1 intensity ratio in PHEG-*block*-poly(Trp)-T solutions, [PHEG-*block*-poly(Trp)-T] = 3.0×10^{-3} mol/l.

Table 3
Aggregate size of PHEG-*block*-poly(Trp)-T in different pH solutions

PH	Diameter (nm)	Polydispersity
5.0	90	0.17
2.0	50	0.25

Py at a concentration above the CAC. The polarity of the environment seemed to increase with lowered pH, because the I_3/I_1 ratio decreased below pH 3. From these results, it was considered that the aggregates of PHEG-*block*-poly(Trp)-T were destabilised by electrostatic repulsion between Trp residues in the aggregates' cores, and by lowering of hydrophobic coagulation power below pH 3.0.

3.7. Evaluation of the size of PHEG-block-poly(Trp)-T aggregates

The pH dependence of aggregate size for PHEG-*block*-poly(Trp)-T was investigated by DLS measurements, and the results are summarised in Table 3. The average diameter of PHEG-*block*-poly(Trp)-T aggregates was ca. 90 nm at pH 5.0 and ca. 50 nm at pH 2.0, and this change in diameter took place immediately upon change in pH. This change in aggregate diameter caused by changing pH originated in dissociation of core Trp residues. It is thought that PHEG-*block*-poly(Trp)-T formed larger aggregates at pH 5.0, where Trp moieties interact more strongly, than at pH 2.0, where TFA-treated Trp dissociates. If the PHEG chain is a random coil in an unperturbed state and the poly(Trp) chain forms an α-helix, the diameter of spherical micelle formed PHEG-*block*-poly(Trp) can be calculated as 30 nm [33]. Therefore, it is not possible to explain the aggregate size at pH 5.0 (90 nm) in terms of a simple core-shell structure. This large increase in aggregate size indicates formation of secondary aggregates, originating from aggregation of hydrophobic domains not covered with hydrophilic PHEG chains. Conversely, the aggregate size decreases at pH 2.0, since the average number of associated molecules decreases because of the disruption of the hydrophobic domains caused by Trp dissociation. The model proposed to account for change in aggregate size is presented in Fig. 8. A TEM micrograph of PHEG-*block*-poly(Trp)-T at pH 5.0 is shown in Fig. 9. Though the presence of aggregates with diameter ca. 100 nm was confirmed by DLS, the aggregates had a wide size distribution; aggregates of ca. 20–50 nm diameter were also observed.

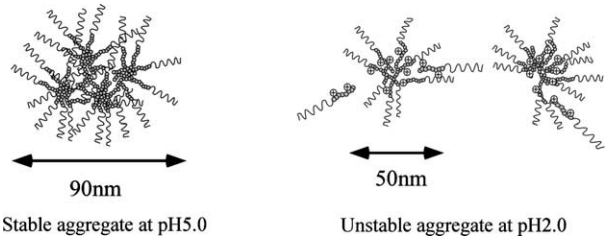


Fig. 8. Presumed model of aggregate size change in PHEG-*block*-poly(Trp)-T.

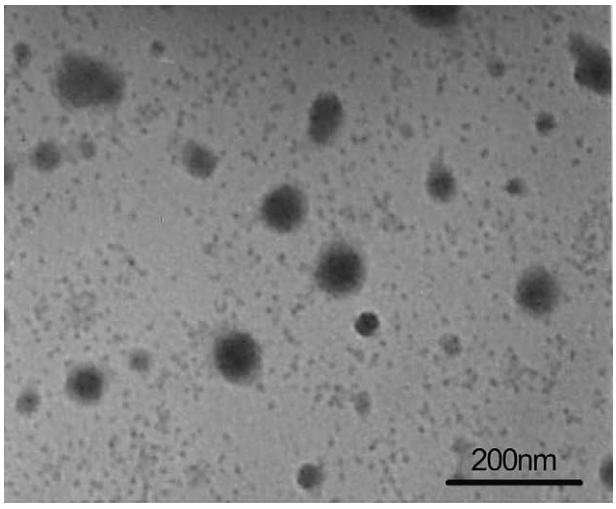


Fig. 9. Transmission electron microscopy photograph of PHEG-*block*-poly(Trp)-T aggregates at pH 5.0.

3.8. Uptake and release behaviour of model substances with PHEG-block-poly(Trp)-T aggregates

The uptake abilities of the aggregates were estimated using model substances (ANS, Py and 5-fluorouracil (5-FU)) by measuring the amount of model substance dissolved into the hydrophobic region of PHEG-*block*-poly(Trp)-T aggregates. Uptake amounts and loading efficiencies for each model substance are summarised in Table 4. The uptake amount (wt%) of each model substance was calculated from the weight of each model substance loaded into aggregates divided by the weight of the loaded aggregates. The loading efficiency (%) was evaluated by dividing the weight of model substance loaded into aggregates by the initial weight of model substance. The uptake amount and efficiency of PHEG-*block*-poly(Trp)-T were the highest for Py (12.8 wt%/84.4%), and decreased in order for 5-FU (3.6 wt%/23.8%) and ANS (0.1 wt%/1.3%). This result indicated that the uptake amount and efficiency of the aggregates strongly depended on the properties of the aggregates (size, stability, etc.) and of the model substance used (charge, hydrophobicity, etc.). For instance, the charge on ANS seems to be the dominant factor accounting for its meager distribution in the aggregates, since its hydrophilicity is high, whereas the hydrophobicity of Py dominates its higher uptake into the aggregates' hydrophobic domains.

Table 4
Uptake amount and loading efficiency of model substances into PHEG-*block*-poly(Trp)-T aggregate

Model substance	Uptake amount[a] (wt%)	Loading efficiency[b] (%)
ANS	0.1	1.3
Py	12.8	84.4
5-FU	3.6	23.8

[a] Uptake amount (wt%) = (amount of model substance in the aggregate/amount of model substance-loaded aggregate) × 100.
[b] Loading efficiency (%) = (amount of model substance in the aggregate/initial amount of model substance) × 100.

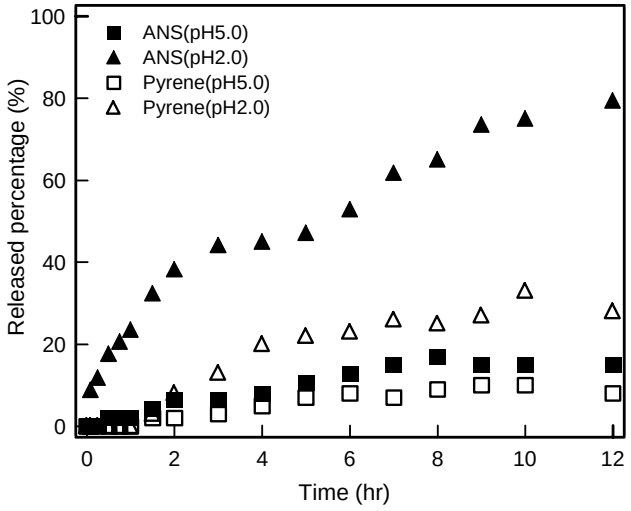


Fig. 10. Release profile of ANS and pyrene from PHEG-*block*-poly(Trp)-T aggregate at different pH.

Fig. 10 shows the release behaviour of ANS and Py from PHEG-*block*-poly(Trp)-T aggregates at pH 2.0 and at pH 5.0. The amount of model substance released was normalised with the total amount of model substance incorporated into PHEG-*block*-poly(Trp)-T aggregates at each pH. After 12 h, about 80% of ANS was released at pH 2.0, and ca. 15% at pH 5.0. The same tendency (more model substance released at lower pH) was observed in measurements on Py. Fig. 11 shows the pH dependence of the release of ANS from PHEG-*block*-poly(Trp)-T aggregates. As can be seen from Fig. 11, the release rate of ANS was accelerated by changing the pH from 5.0 to 2.0, and started to decrease when the pH increased to 5.0 again. This behaviour seems to have been caused by a change in hydrophobicity of poly(Trp) domains in PHEG-*block*-poly(Trp)-T aggregates. The release behaviour of 5-FU from PHEG-*block*-poly(Trp)-T aggregates was also investigated, and its profile (Fig. 12) was similar to those of ANS and Py, i.e. the release rate was lower at pH 5.0 than at pH 2.0. At pH 5.0, the release of 5-FU was negligible. This release increased with decreasing pH, and continuous release was observed at pH 2.0. The release of 5-FU was depressed when the solution pH increased to pH 5.0 again. Thus, although ANS, Py and 5-FU were retained in the hydrophobic regions of the aggregates at pH 5.0, they were released at pH 2.0, since the hydrophobic interaction holding the aggregates together were weakened by dissociation of TFA-treated Trp. The differences in release behaviour of the model substances seemed to originate from differences in hydrophobic interactions between these molecules and Trp.

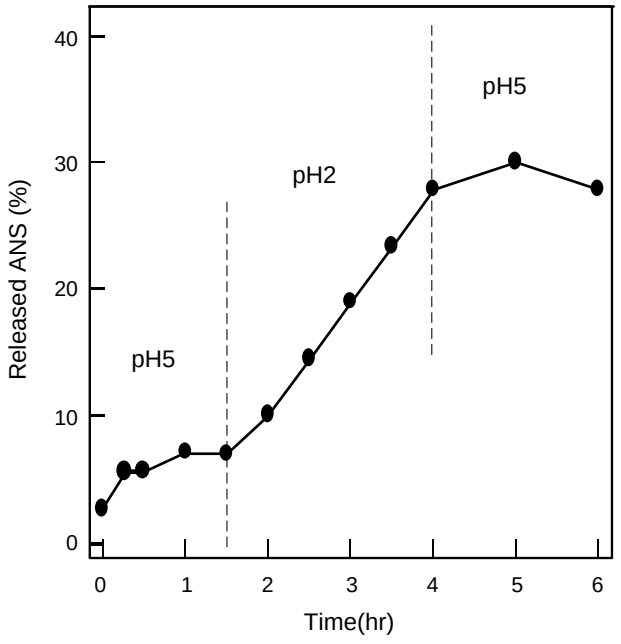


Fig. 11. pH dependence of release behaviour of ANS from PHEG-*block*-poly(Trp)-T aggregates.

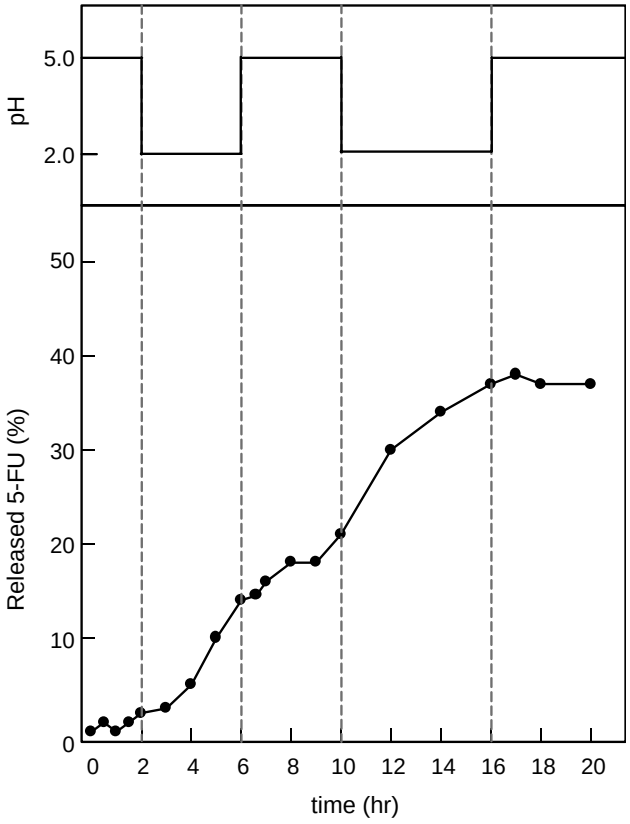


Fig. 12. pH dependence of release behaviour of 5-FU from PHEG-*block*-poly(Trp)-T aggregates.

4. Conclusions

In this study, aggregates of a TFA-treated block copolypeptide consisting of PHEG and poly(Trp) were prepared, and the aggregates' release behaviour was investigated using the model substances Py, ANS and 5-FU. The major conclusions of this investigation were as follows.

(1) PHEG-*block*-poly(Trp) and PHEG-*block*-poly(Trp)-T in aqueous solution formed aggregates containing hydrophobic regions, since poly(Trp) has high hydrophobicity. These aggregates could assimilate model substances into

the hydrophobic region.

(2) In the case of PHEG-*block*-poly(Trp)-T, the block co-polypeptide showed pH dependence of aggregate size: dissociation of Trp residues treated with TFA resulted in a change in average aggregate diameter from ca. 90 nm at pH 5.0 to ca. 50 nm at pH 2.0.

(3) In studies of release behaviour of ANS and 5-FU from PHEG-*block*-poly(Trp)-T, release rates of these model substances were found to be accelerated by decreasing the pH 5.0–2.0, and release rates were depressed when the solution pH rose to 5.0 again.

Thus, novel pH-responsive aggregates were prepared by using a block co-polypeptide treated with TFA. Shifting the pH region where Trp dissociates to neutral pH by changes in composition, etc. and DDS applications will be examined in the future.

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