pH-Responsive Guest Binding of Polypeptide Containing a Cyclodextrin at the Terminal

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Poly(γ-methyl L-glutamate-*co*-L-glutamic acid) containing a β-cyclodextrin at the terminal (MG/GA-CyD) was prepared and the relationship investigated between the conformation of the polypeptide and inclusion capability of the β-cyclodextrin by circular dichroism and fluorescence spectroscopy in aqueous solution containing 8-anilino-1-naphthalenesulfonate (ANS) as a guest molecule. The terminal β-cyclodextrin of MG/GA-CyD could not include ANS at higher pH, where the polypeptide almost formed a random coil. This was attributed to the terminal β-cyclodextrin being concealed in the random coil of the polypeptide. At low pH, however, random coil-to-α-helix transition of the polypeptide exposed the terminal β-cyclodextrin to the aqueous phase resulting in the ANS-inclusion capability. These results imply that control of the polypeptide conformation leads to conformational switched guest binding of the terminal cyclodextrin.

In many biological systems, conformational changes of proteins are closely related to biological activity.[1—4] It is also desirable in artificial systems that the conformational change of a molecule accompanying an intelligent function can be induced by a specific stimulation.

Recently, M. Mutter et al. showed that α-helix-β-sheet transition of designed oligopeptides can serve as a "conformational switch".[5,6] It was also reported that terminal groups of α-helical polypeptides can be useful for the regulation of their tertiary structure.[7—9] For example, a three-α-helix-bundle was made with the aid of complexation between three terminal bipyridine moieties and a Fe^{2+} ion.[8] An association of two α-helical rods was also induced by the aid of a sandwich-type (2 : 1) complex between terminal crown moieties and K^+ ion.[9]

On the other hand, polypeptides containing glutamic acid residues were found to have α-helix-random coil transitions depending on pH, metal ions, and cationic molecules.[10—18] We expected that such conformational changes of polypeptides might further increase the significance of the role of their terminal groups. That is to say, a function of the moiety arbitrarily selected and fixed at the terminal of polypeptide may be on/off switched by regulating the polypeptide conformation.

First we selected cyclodextrins as a terminal functional group. Cyclodextrin derivatives accommodate a variety of organic compounds as substrates in their central cavities in aqueous solutions.[19] Moreover, some fluorescent probes show that the emission spectra are changed by their inclusion into the hydrophobic cavities.[20—23] In addition, many studies concerned with molecular recognition and artificial enzymes have been done using a variety of cyclodextrin derivatives.[24—31]

We prepared poly(γ-methyl L-glutamate-*co*-L-glutamic acid) containing a β-cyclodextrin at the terminal. The relationship between the conformational change of the polypeptide and inclusion capability of the terminal β-cyclodextrin was investigated by circular dichroism and fluorescence spectroscopy. A possibility of pH-responsive substrate binding by the polypeptide is discussed.

Experimental

Materials. A β-cyclodextrin derivative containing an amino group as an initiater for polymerization of γ-methyl ester of *N*-carboxyl-L-glutamic acid anhydride (MG-NCA) was prepared as follows. The β-cyclodextrin derivative containing an amino group was prepared by the well-established procedure.[26,32—34] β-Cyclodextrin (guaranteed reagent, Nacalai Tesque Co., Ltd.) was mono-*O*-tosylated with 0.5 equivalent of *p*-toluenesulfonyl chloride (guaranteed reagent, Nacalai Tesque Co., Ltd.) in dry pyridine for two hours at room temperature. The resulting mono-*O*-tosylated β-cyclodextrin was purified by recrystallization from water. Moreover, recrystallization from 1-butanol/ethanol/water (volume ratio 5 : 4 : 3) was repeated several times to obtain mono-6-*O*-*p*-toluenesulfonyl-β-cyclodextrin (CyD-OTs). The ^{1}H NMR spectrum (Varian XL-200 spectrometer) of CyD-OTs in deuteriodimethylsulfoxide (DMSO-d_6) was similar to those reported by K. Ishihara et al.[26] The β-cyclodextrin derivative containing an amino group was synthesized by the reaction of CyD-OTs with 10 equivalents of 2-aminoethanethiol (guaranteed reagent, Nacalai Tesque Co., Ltd.) in *N*,*N*-dimethylformamide for 6 h at room temperature. The resulting β-cyclodextrin derivative containing an amino group was purified by reprecipitation with acetone. Reprecipitation of the β-cyclodextrin derivative in methanol/water (3 : 1 v/v) with acetone was repeated several times. As a result, mono-6-deoxy-6-(2-aminoethylthio)-β-cyclodextrin (CyD-NH_2) was obtained. The detosylation and the introduction of an aminoethyl moiety were confirmed from ^{1}H NMR spectra of CyD-NH_2 obtained in DMSO-d_6.[26] The pH titration of CyD-NH_2 in aqueous solution with KOH gave a typical pH titration curve. The pK_a for the conjugate acid of CyD-NH_2 was 8.5 and corresponded to the amino group. In addition, the amount of HCl spent in the neutralization of amino groups of CyD-NH_2 also supported the idea of monosubstitution of β-cyclodextrin. Poly-

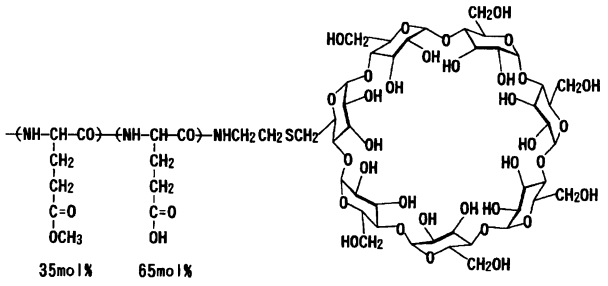
merization of MG-NCA was started with CyD-NH_2. The molar ratio of MG-NCA to CyD-NH_2 was 30. The resulting polypeptide was purified by reprecipitation with methanol. The introduction of β-cyclodextrin to the polypeptide was confirmed by ^{1}H NMR spectra with the peaks at 3.31, 3.66, 4.83, and 5.71 ppm. Saponification of poly(γ-methyl L-glutamate) obtained (PMG-CyD) gave poly(γ-methyl L-glutamate-*co*-L-glutamic acid) containing a β-cyclodextrin at the terminal (Scheme 1, MG/GA-CyD).[35,36] The mean degree of polymerization, $\overline{DP}=80$, was estimated by measurement of polypeptide concentration through a terminal amino group-labeling method using 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl). The content of saponificated residue, 65 mol%, was measured by decrease of a side-chain methoxy proton band (3.88 ppm) in high-resolution ^{1}H NMR spectra of MG/GA-CyD in trifluoroacetic acid.

Measurements. Aqueous solutions of 6.7×10^{-4} base molar MG/GA-CyD containing 1×10^{-3} mol dm^{-3} KCl were used. Circular dichroism (CD) spectra of MG/GA-CyD in 2,2,2-trifluoroethanol (TFE) and aqueous solution were obtained with a spectropolarimeter (JASCO, J-600). Fluorescence spectra of 8-anilino-1-naphthalenesulfonate (ANS) in aqueous solution containing MG/GA-CyD were obtained with a spectrofluorophotometer (Shimadzu, RF-540). The excitation wavelength of ANS was 350 nm. The concentration of ANS was 5.0×10^{-6} mol dm^{-3}. The pH of the solutions was adjusted by the addition of HCl and KOH, and monitored by a pH meter (TOA Electronic Ltd., HM-60S).

Results and Discussion

Conformation of MG/GA-CyD. The CD spectrum of MG/GA-CyD in TFE solution is shown in Fig. 1. MG/GA-CyD indicated an α-helix content of approximately 84%, on the basis of a molecular ellipticity at 222 nm, $[\theta]_{222}$, of completely α-helical poly(γ-methyl L-glutamate). MG/GA-CyD, even connecting to β-cyclodextrin at the terminal, can form α-hexlix structure adequately.

pH-dependent conformational changes of MG/GA-CyD were examined in aqueous solutions containing 1×10^{-3} mol dm^{-1} KCl. α-Helix content estimated from $[\theta]_{222}$ was plotted as a function of pH (Fig. 2). This curve indicated that MG/GA-CyD caused a random coil-α-helix transition similar to poly(L-glutamic acid) (PGA).[10,11,15] However, a small amount of α-helix structure remained even at higher pH, where PGA was in random coil conformation completely. This result



Scheme 1. MG/GA-CyD.

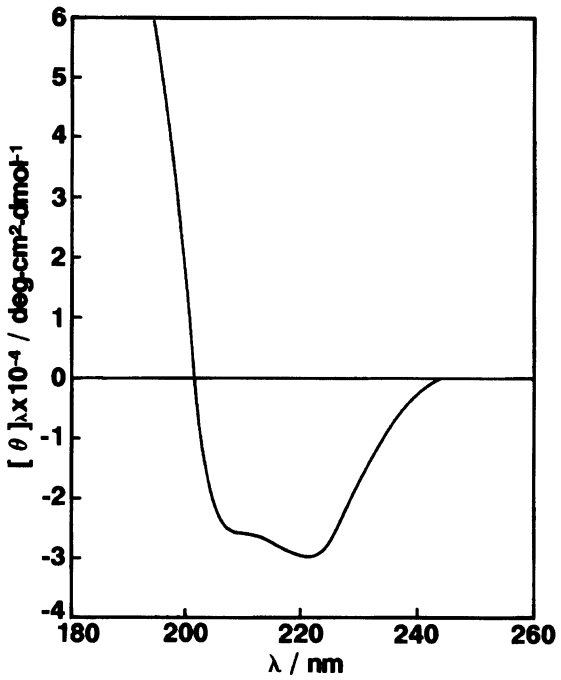


Fig. 1. A circular dichroism spectrum of MG/GA-CyD in TFE solution.

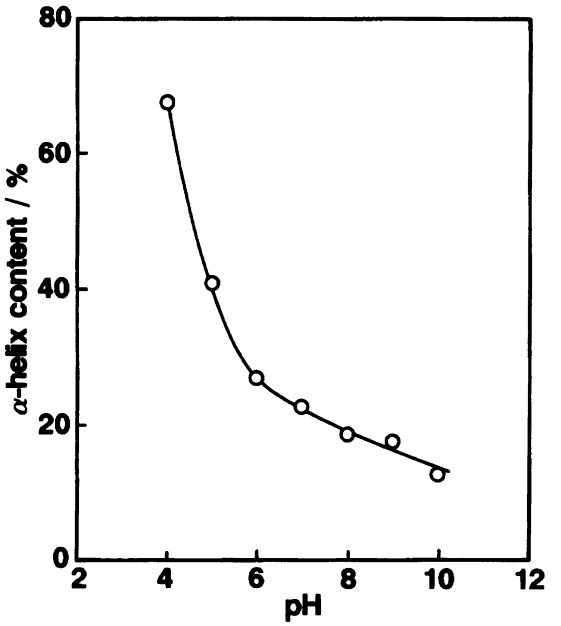


Fig. 2. pH dependence of changes in α-helix content of MG/GA-CyD in aqueous solution.

may be associated with depression of electric repulsion based on the hydrophobic γ-methyl L-glutamate (MG) residues.

Relationship between Conformation of MG/GA-CyD and ANS-Binding to a Terminal β-Cyclodextrin. ANS used as a guest molecule is a fluorescent probe the emission maximum wavelength of which sensitively varies with environmental polarity change.[37] The emission appears around 515 nm in aqueous solu-

tion, while it was shifted to 495 nm in the presence of β-cyclodextrin owing to the binding of ANS in its hydrophobic cavity.[20] Therefore, partition of ANS between the aqueous phase and the cavity of β-cyclodextrin moiety could be monitored easily. Figure 3 shows fluorescence spectra of ANS in aqueous solutions containing MG/GA-CyD at various pHs. At pH 10.0, the emission maximum appeared near 510 nm, indicating that ANS almost existed in aqueous phase. No spectral changes were found between pH 7.0—10.0. At pH 6.0, however, a new shoulder band appeared at shorter wavelengths. Moreover, the emission band clearly appeared near 470 nm at pH 4.0. The emission maximum wavelength of ANS was found to be independent of pH of the polypeptide free aqueous solutions pH range between 4.0—10.0. In addition, no changes in ANS-emission were also confirmed in aqueous solution containing MG/GA without β-cyclodextrin at the terminal. These results indicated the partition of ANS to a less-polar environment, that is, in the central cavity of β-cyclodextrin of MG/GA-CyD. Therefore, these spectra were characterized by the two bands assigned to ANS-emission in aqueous phase (at 510 nm) and in the cavity of β-cyclodextrin moiety (at 470 nm), which could be resolved by Gaussian analysis. Each fluorescence intensity analyzed was plotted as a function of pH (Fig. 4). It was indicated that these spectral changes were based on the changes in partition of ANS between the aqueous phase and the cavity of β-cyclodextrin of MG/GA-CyD, since the intensity increase at 470 nm synchronized with the decrease of intensity at 510 nm.

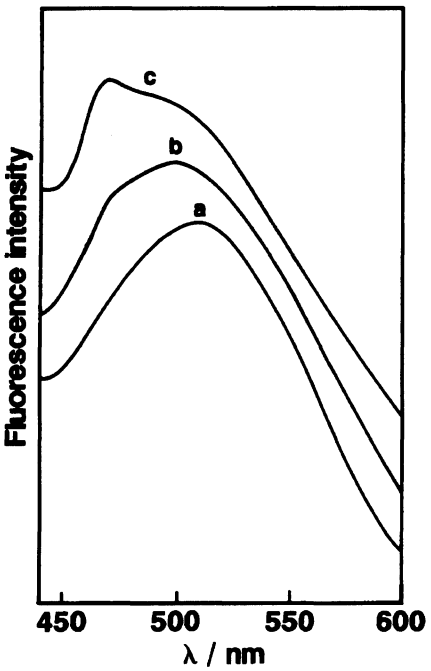


Fig. 3. Fluorescence spectra of ANS in aqueous solutions containing MG/GA-CyD at various pHs. a; pH 10.0, b; pH 5.0, c; pH 4.0.

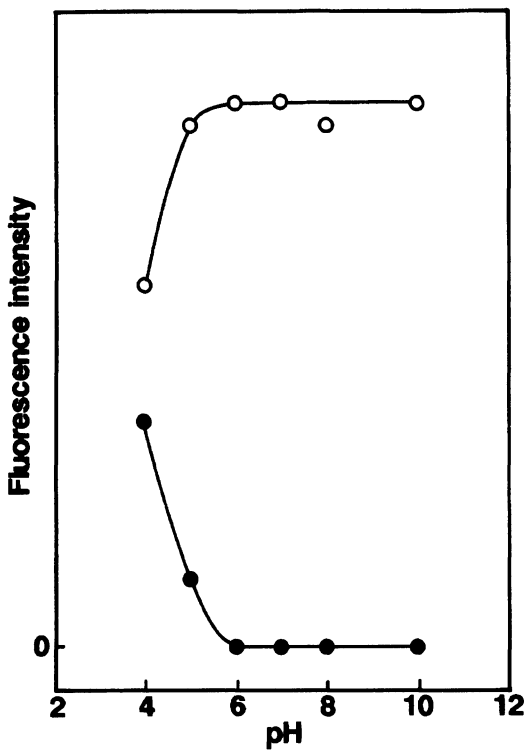


Fig. 4. pH dependence of changes in fluorescence intensity at 510 nm (—○—) and 470 nm (—●—) of ANS in aqueous solutions containing MG/GA-CyD.

To clarify the dependence of the ANS-fluorescence on the polypeptide conformation, CD spectra of MG/GA-CyD in aqueous solutions containing ANS were measured at various pHs. As shown in Fig. 5, a pH-dependent conformational change similar to that of MG/GA-CyD without ANS was found in the CD spectra. The pH dependence of α-helix content estimated from $[\theta]_{222}$ is shown in Fig. 6. This transition behavior corresponded to the changes in fluorescence intensity of ANS. These results support the idea that the conformational transition of MG/GA-CyD led to ANS-fluorescent change. Furthermore, it should be noted that this behavior was found to be reversible, i. e., the ANS at once trapped under the α-helix conformation could be free from β-cyclodextrin again by increasing random coil conformation. The mechanism was considered as follows. At higher pH, MG/GA-CyD had a random coil CD pattern which was somewhat different from that of the common random coil of PGA described above. This result implies that hydrophobic MG residues form a intramacromolecular cluster that binds hydrophobic substrates very efficiently in water similar to the case of amphiphilic polypeptides[38] under the charged coil. It can therefore be presumed that at higher pH MG/GA-CyD forms anionic-coil containing hydrophobic clusters in which the terminal hydrophobic β-cyclodextrin could be incorporated. As a result, anionic ANS could not interact with the terminal β-cyclodextrin moiety by steric

and electrostatic repulsions (Fig. 7). On the other hand, at lower pH where the polypeptide formed α-helix, the terminal β-cyclodextrin could be exposed in the aqueous phase resulting from the conformational change of the backbone to rod-like structure. As a result, ANS could be distributed in the cavity of the β-cyclodextrin (Fig. 7). It was also confirmed that MG/GA without β-cyclodextrin at the terminal provided no change in ANS-emission as noted above. Furthermore, hydrophobic γ-methyl L-glutamate residues may efficiently participate in the reversible behavior.

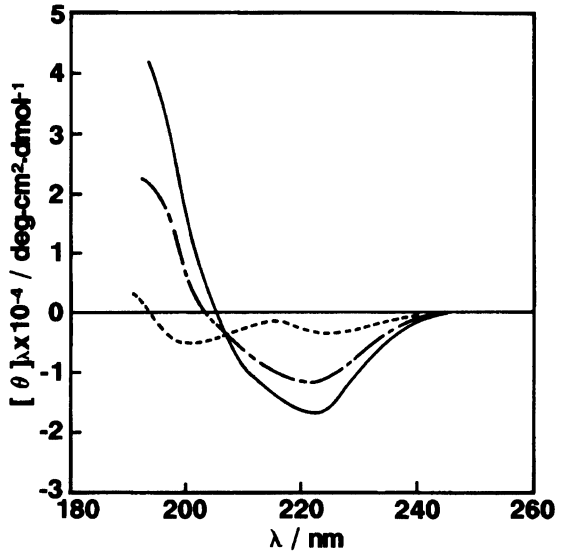


Fig. 5. Circular dichroism spectra of MG/GA-CyD in aqueous solutions containing ANS at various pHs. (---); pH 10.0, (—·—); pH 5.0, (—); pH 4.0.

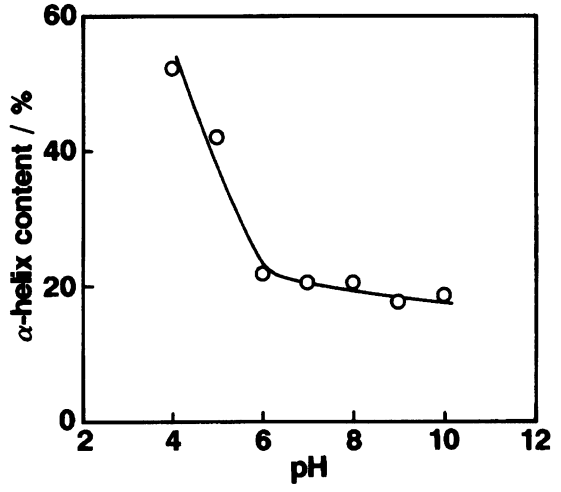


Fig. 6. pH dependence of changes in α-helix content of MG/GA-CyD in aqueous solutions containing ANS.

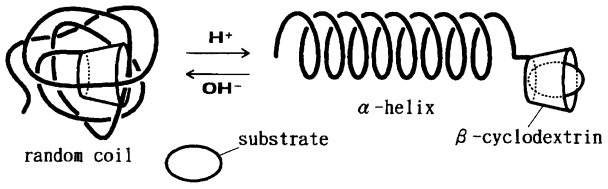


Fig. 7. Schematic picture of anticipated response of MG/GA-CyD.

In conclusion, it was demonstrated that conformational switched guest binding of cyclodextrin was achieved by pH-induced conformational change of the polypeptide. Therefore, the polypeptide containing a functional group at the terminal will be useful as a intelligent molecular system that is applicalbe to a selective stimuli-responsive guest binding, transformation of molecular information, and so on.

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