Controlled Association of Hydrophobized Polysaccharide by Cyclodextrin

Kazunari Akiyoshi,* Yoshihiro Sasaki, Kenichi Kuroda, and Junzo Sunamoto*
Department of Synthetic Chemistry & Biological Chemistry, Graduate School of Engineering, Kyoto University
Yoshida Hommachi, Sakyo-ku, Kyoto 606-01

(Received September 24, 1997; CL-970737)

The self-aggregate of cholesterol-bearing pullulan (CHP) dissociated by complexation with β-cyclodextrin (β-CD) to yield a dis-aggregated CHP-CD complex, in which the cholesteryl group was a suitable guest to β-CD. The monodisperse nanoparticles of the CHP self-aggregate were regenerated by addition of 1-adamantancarboxylic acid (ADC), which is the better guest molecule to β-CD than cholesterol. CD regulated the association and dissociation of hydrophobized polysaccharides in water.

Most of biopolymers self-assemble and form functional nano-organized systems. Dynamics of the assembly of the macromolecules are often controlled by binding of a suitable effector molecule. For example, in molecular chaperon system, which assists a protein folding, ATP regulates formations of the assembly between folding protein intermediates as a guest and chaperon as a host.[1] In artificial system, however, it is still difficult to control the associations between macromolecules in water. Recently, we developed monodisperse hydrogel nanoparticles, which are formed in water by self-aggregation of hydrophobized polysaccharides such as cholesterol-bearing pullulan (CHP).[2,3] The domains of the associated cholesteryl groups of CHP provide noncovalent cross-linking points of the gel structure. The size and the density of the hydrogel nanoparticle are controlled by changing the substitution degree of the cholesteryl groups of CHP.[4] The nanoparticle complexed with various soluble proteins inside the hydrogel depending on the size of the proteins.[4-6] This is an example of well-controlled association between two different macromolecules. In this paper, we describe the control of association of the hydrophobized polysaccharide by cyclodextrin (CD).

Cholesterol-bearing pullulan (CHP-108-0.9: 0.9 cholesterol group per 100 anhydroglucoside unit was substituted to pullulan of Mw 108000)[3] was dissolved in DMSO, dialyzed against Milli-Q water and then 50 mM Tris buffer (pH 7.5). After dialysis, the suspension was sonicated using a probe type sonifier (TOMY, UR-200P) at 40 W for 5 min at room temperature. Static light scattering measurement (DLS-700, Otsuka Electronics, equipped with a vertically polarized 5-mW He-Ne laser, 633 nm) showed that the nanoparticle of the CHP-108-0.9 self-aggregate consists of about 7 molecules of CHP. The hydrodynamic radius (Rh) of the nanoparticle is 16 nm by dynamic light scattering (Spectra-Physics Series 2000 argon ion laser, which was operated at 488 nm and 200 mW with a Brookhaven BI-2030 256 channel digital correlator). The aggregation number of the cholesteryl moieties in one hydrophobic domain was 3.7±0.5, which was estimated by the fluorescence quenching method.[3]

The main driving force of self-aggregation of CHP is the association of the hydrophobic cholesterol groups of CHP in water. CD solubilizes various hydrophobic compounds in water by the incorporation into the hydrophobic cavity.[7] Especially, β-CD effectively complexes a cholesterol.[7] After addition of β-CD solution (final concentration 3mM) to CHP self-aggregate suspension (final concentration 2 μM), the intensity of light scattering immediately decreased. This would be due to the

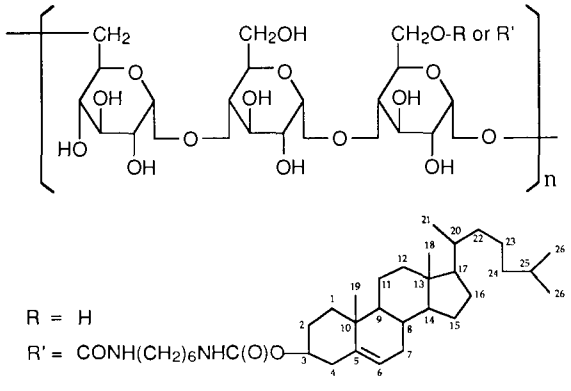

Figure 1. Structures of cholesterol-bearing pullulan (CHP).

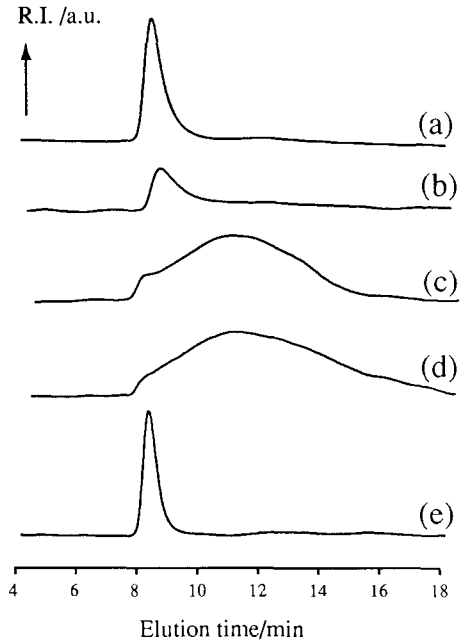

Figure 2. Chromatogram of HPSEC. (a) CHP self-aggregate, (b) 2 μM CHP self-aggregate + 10 mM β-CD, (c) 2 μM CHP self-aggregate + 10 mM β-CD, eluting buffer contains 10 mM β-CD, (d) parent pullulan unmodified, (e) 2 μM CHP self-aggregate + 10 mM β-CD after the addtion of 1 mM ADC.

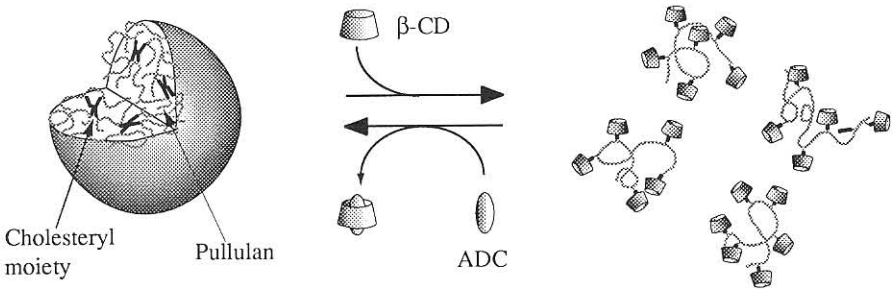

Figure 3. Schematic representation of dissociation and association of CHP upon competitive complexation.

dissociation of the self-aggregate. The interaction between the CHP self-aggregate and β–CD was further confirmed by a high performance size exclusion chromatography (HPSEC: Pharmacia, Superdex 200HR column, ø = 10 × 30 (mmID × cm)) (Figure 2). The sonicated sample of the dilute suspension of CHP gave a single peak (Figure 2a) on the chromatogram reported previously.[3] When a mixture of the CHP self-aggregate (2 μM) and β–CD (10 mM) was applied to the same column through a membrane filter (pore size 0.45 mm), the peak of the CHP self-aggregate decreased (Figure 2b). However, no peak attributable to the complex between CHP and β–CD appeared. This is due to the adsorption of the CHP-CD complex to the membrane filter or the column. To avoid the adsorption to the column, 50 mM Tris-sulfate buffer containing 10 mM β–CD was used as an eluent. In this case, a broad peak newly appeared (Figure 2c). The peak was similar to that of the parent pullulan unmodified (Figure 2d). The results suggest that the CHP self-aggregate certainly dissociated by complexation with β–CD.

To obtain a direct evidence of the complexation between β–CD and the cholesteryl group of CHP, ^{1}H-NMR (JEOL AA-400 FT-NMR) was measured. The signals of the cholesteryl group of CHP (1.5 mg/ml) in DMSO$_{d6}$, which is a good solvent of CHP, were observed in the region from 0.06 ppm to 2.0 ppm at 30 °C. In water, the cholesteryl group signals were broaden by the restriction of the molecular motion upon the association.[2] When β–CD (5 mM) was added, however, the signals of the cholesteryl group became sharper. The chemical shift of the angular methyl signal (δ 0.73 ppm) at C18 of the cholesterol skeleton (Figure 1) largely sifted to downfield (approx. +0.08 ppm) compared with the case of CHP in DMSO$_{d6}$. Moreover, the terminal methyl signals (δ 0.77 ppm) at C26 of the cholesteryl group shifted upfield (approx. - 0.07 ppm). The similar chemical shift changes were reported in the complex of bile acid with β–CD.[8] Harada et. al. reported the complexation of CD with alkyl chain conjugated to polyacrylamide in water.[9] The cholesteryl group of CHP certainly complexed with β–CD in water.

The effect of 1-adamantancarboxylic acid (ADC) on the re-aggregation of CHP was then investigated by HPSEC. One mM ADC, which is excess to the cholesteryl group (84 μM) of CHP, was added to the CHP-β–CD complex solution. The peak attributed to the CHP self-aggregate was observed again (Figure 2d). The particle size of the CHP self-aggregate re-formed was measured by dynamic light scattering. The hydrodynamic radius (Rh = 20 nm) of the nanoparticle re-formed after the addition of ADC a little increased compared with the ordinary self-aggregate (Rh = 16 nm). The re-formed self-aggregate was also monodisperse even without ultrasonication. The binding constant (3.2×10^4 M^{-1}) of ADC[10] with β–CD is larger than that of cholesterol (1.6×10^4 M^{-1}).[11] Therefore, ADC competitively complexed with β–CD instead of the cholesteryl group of CHP. The free CHP self-aggregated again in water (Figure 3).

In conclusion, we showed the control of the self-aggregation and dis-aggregation of the hydrophobized polysaccharide by a host-guest interaction with β–CD. The complexation of soluble proteins with the CHP self-aggregate is also controlled by this method. Biosimulation of various biological functions such as chaperone system is in progress in our laboratory.

The authors are grateful to Professor Hitoshi Yamaoka and Dr. Hideki Matsuoka, Kyoto University, for their kind help in DLS measurements. This work was supported by Grant-in-Aid for Scientific Research in Priority Areas (No. 09232235 and 08455440) from the Ministry of Education, Science, Culture and Sports, Japan, and Terumo Science and Technology Foundation.

Reference

1 M. Mayhew, A. C. R. da Silva, J. Martin, H. E-bromage, P. Tempst, and U. Hartl, *Nature*, **379**, 420 (1996).
2 K. Akiyoshi, S. Deguchi, N. Moriguchi, S. Yamaguchi, and J. Sunamoto, *Macromolecules*, **26**, 3062 (1993).
3 K. Akiyoshi, S. Deguchi, H. Tajima, T. Nishikawa, and J. Sunamoto, *Macromolecules* **30**, 857(1997).
4 K. Akiyoshi and J. Sunamoto, *Supramolecular Science*, **3**, 157 (1996).
5 T. Nishikawa, K. Akiyoshi, and J. Sunamoto, *Macromolecules*, **27**, 7654 (1994).
6 T. Nishikawa, K. Akiyoshi, and J. Sunamoto, *J. Am. Chem. Soc.*, **118**, 6110 (1996).
7 R. Breslow and B. J. Zhang. *J. Am. Chem. Soc.*, **118**, 8495 (1996)
8 G. G. Gustavo , C. Aurora. S. M. Luis, and T. Gloria, *Langmuir*, **13**, 2235 (1997)
9 A. Harada, H. Adachi, and M. Kamachi, *Polymer Preprints, Jpn*, **45**, 2472 (1996)
10 W. Meik and W. Gerhard, *Macromol. Rapid. Commun.* **17**, 731 (1996).
11 K. Shiotani, K. Uehata, T. Irie, K. Uekama, D. O.Thompson, and V. J. Stella, *Pharm. Res.*, **12**, 78 (1995).