CHEMISTRY LETTERS, pp. 1727-1730, 1992.

Self-aggregates of Hydrophobized Polysaccharide as a Host for Macromolecular Guests

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Nano-particles, which are self-aggregates of hydrophobized pullulan partly substituted cholesterol moieties (CHP), formed a complex with various globular and soluble proteins such as hemoglobin, peroxidase, myoglobin, and cytochrome c. The nano-particle also showed an excellent colloidal stability and almost no dissociation of the protein from the complex.

Creation of a new function by an architecture as built with artificial supramolecular assemblies is of considerable interest, especially, in modern materials science.[1] Supramolecular chemistry is defined as the chemistry of non-covalent molecular assembly.[1a] Based on this concept, host-guest complex systems[1a,2] or self-organizations[1b,1c,3] have extensively been investigated. Much attention has been paid in order to control molecular recognition and reactivity through interaction of the relatively low molecular weight molecules with various hosts.[3b] For the self-organization or the host-guest complex between different macromolecules and the control of their function upon the complex formation, however, not so many have been investigated.[4] We have recently reported that hydrophobized polysaccharide derivatives, which are partly substituted by hydrophobic moiety, form a sort of self-aggregates in water.[5] For example, cholesterol-bearing pullulan (CHP, Fig. 1) forms a stable and monodispersive nano-particle and complexes with various small hydrophobic molecules. It provides even a chiral environment.[5b] In this communication, we would like to describe the CHP self-aggregates as a host which is able to complex with another macromolecules such as various globular and soluble proteins.

CHP was synthesized by the same method as that previously reported.[5a] CHP-55-1.6 which bears 1.6 cholesterol groups per 100 glucose units of pullulan (Mw 5.5×10^4, Mw/Mn = 1.6) was used in this study. Monodispersive particles of CHP self-aggregates were prepared by swelling the polymer in water for 12 h at 60.0 °C followed by ultrasonication twice at 40 W and room temperature for 5 min using a probe type sonifier (TOMY, UR-200P). The hydrodynamic diameter of the aggregate

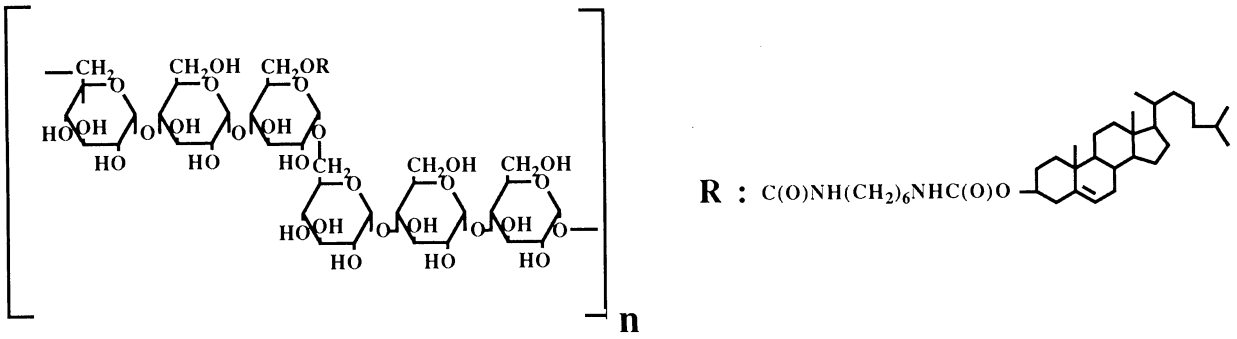

Fig. 1. Structure of hydrophobized pullulan (CHP).

was approx. 27 nm by dynamic light scattering method (DLS) on a Photal DLS-700 (Otsuka Electronics Co., Osaka).

First of all, complexation between myoglobin (from horse skeletal muscle, Sigma, Type I) and the CHP self-aggregate was investigated by means of gel chromatography. An appropriate amount of the enzyme (final concentration 10^{-6} - 10^{-4} M) was coincubated at 25.0 °C in 2.0 ml of 0.5 wt% aqueous polysaccharide solution (9.5×10^{-5} M) containing 5 mM MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.2) and 200 mM NaCl. The resulting mixture was submitted to a Sepharose 4B column (ϕ 1.8 × 30.0 cm, flow rate 0.3 ml/min, at 25 °C). The elution of the protein, which was followed by UV and fluorescamine methods,[6] gave two peaks. The former peak overlapped with the elution of CHP self-aggregate which was detected by refractive index and phenol/sulfonic acid methods,[7] while the latter was the elution of free enzyme. The CHP-protein complex was isolated by gel chromatography. Figure 2 shows a time course of the complexation between the CHP self-aggregates and myoglobin at various concentrations. The complexation reached an equilibrium after 10 h at 25 °C. Maximum binding amount of the protein to the aggregate depended on the initial concentration of protein adopted, and a saturation phenomenon of the complexation was observed (Fig. 3). Considering that the aggregation number of the self-aggregate is approximately 14, as determined by static light scattering (SLS) method, it is reasonable to assume that an one-to-two "host-guest" complex is formed between the two macromolecules, the CHP self-aggregate and myoglobin(Fig. 3). The retention time of the complex by size exclusion column chromatography (TSKgel 3000SW$_{XL}$, ϕ 0.78 × 30.0 cm, flow rate 0.5 ml/min at 25 °C, detected at 407 nm) was almost the same with that of the CHP self-aggregate. This means that guest proteins were incorporated inside the host CHP self-aggregate and the surface of the protein is effectively covered by CHP molecules. Because the retention time should be altered if the protein is simply adsorbed on the surface of the CHP self-aggregate. The complex showed an excellent colloidal stability without any precipitation and no dissociation of the protein from the complex was observed at

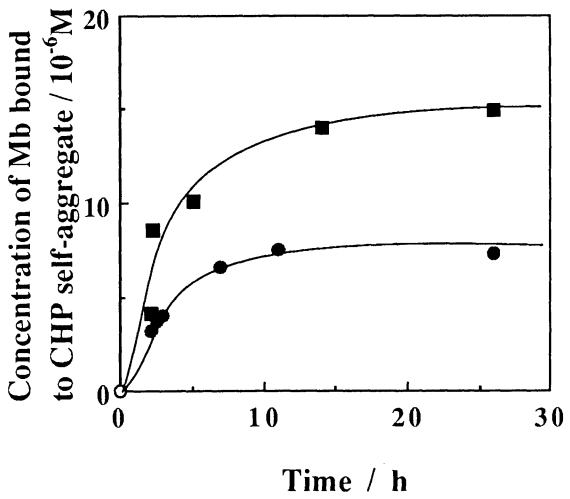

Fig. 2. Complexation between CHP self-aggregate and myoglobin as a function of time at pH 7.2 (5.0 mM MES buffer containing 200 mM NaCl): [CHP-55-1.6] =9.5 × 10^{-5} M, [Mb] = 5.9 × 10^{-5} (-●-), 3.0 × 10^{-4} M (-■-).

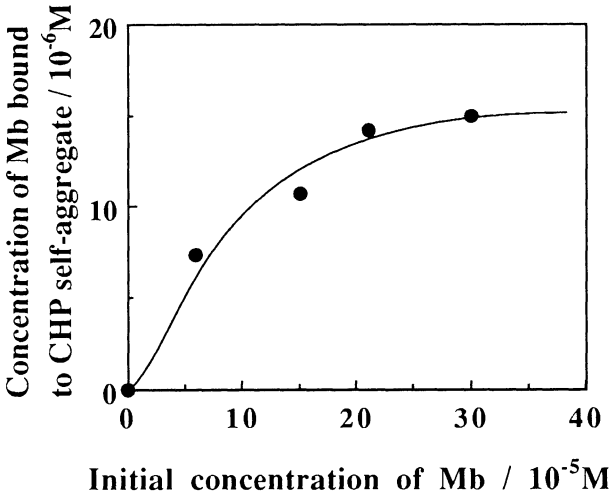

Fig. 3. Amount of myoglobin (Mb) bound to CHP self-aggregate (9.5 × 10^{-5} M) as a function of the initial concentration of Mb added. Coincubation was carried out at 25 °C for 24 h in 5.0 mM MES buffer and containing 200 mM NaCl (pH 7.2).

all even after 1 week at pH 7.2 and 25 °C. The complexation was not much influenced by the pH of the medium under the region studied. This was true at both the initial stage after 1 h and final stage reached at equilibrium after 24 h (Table 1). Amount of the protein bound to the CHP self-aggregate was not altered at all even if the NaCl concentration was increased from 20 mM to 200 mM. These results show that electrostatic interaction is not so important for the complexation between the nonionic CHP self-aggregate and the protein. In addition, of course, the formation of CHP self aggregate and the size of the aggregate also were not influenced by the changes of ionic strength and pH.

Table 1. Effect of pH and salt on the amount of Mb bound to CHP self-aggregate [a)]

Incubation time / h	Bound Mb at various pH / 10^{-6} M			
	pH 5.5	pH 6.2	pH 7.2	pH 7.2[b)]
1	4.7 ± 0.5	3.3 ± 0.5	4.0 ± 0.5	3.9 ± 0.5
24	8.9 ± 0.5	8.1 ± 0.5	7.4 ± 0.5	7.4 ± 0.5

a) In 5.0 mM MES buffer containing 200 mM NaCl, [CHP-55-1.6] = 9.5 × 10^{-6} M, [Mb] = 5.9 × 10^{-5} M. Coincubation was carried out at 25 °C.

b) In 5.0 mM MES buffer containing 20 mM NaCl.

In order to investigate the effect of the molecular weight or the size of globular proteins on the complexation, various proteins having different molecular weights were complexed at constant molar ratio. Various proteins (5.9×10^{-5} M), hemoglobin from bovine (Sigma), peroxidase (HRP) from horseradish (Sigma, TypeI), and cytochrome c (Cyt c) from horse heart (Sigma, Type III) were coincubated with the CHP self-aggregate (8×10^{-6} M) for 1 h at pH 7.2 and 25 °C. The sequence of extent of the binding was the following; Hb (Mw = 64500, 5.7×10^{-6} M) < HRP (Mw = 40000, 7.7×10^{-6} M) > Mb (Mw = 17000, 5.0×10^{-6} M) > Cyt c (Mw = 12500, 3.0×10^{-6} M). The binding of the proteins to the aggregate was affected by molecular weight of proteins. The CHP self-aggregate is considered to consist of hydrophobic domain of cholesterol groups stacked and hydrophilic region of polysaccharide skeleton that offers many hydrogen bonding. Such a structure of microdomain of CHP self-aggregate plays an important role for the complexation with soluble proteins which have both hydrophobic and hydrophilic patches on their surface.

In conclusion, the present macromolecular complex is a new host-guest complex or a sort of supramolecular assembly between the two different macromolecules. The change in the function of enzymes and/or the third-order structural change of proteins will be further expected through such the complexation. The characterizations of these macromolecular complexes are under investigation.

References

1) a) J-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, **27**, 89 (1988); b) H. Ringsdorf, B, Schlarb, and J. Venzmer, *ibid.*, **27**, 113 (1988); c) M. Ahlers, W. Muller, A. Reichert, H. Ringsdorf, and J. Venzmer, *ibid.*, **29**, 1269 (1990).

2) D. J. Cram, *Angew. Chem., Int. Ed. Engl.*, **27**, 1009 (1988); J. Rebek, Jr. *ibid.*, **29**, 245 (1990).

3) a) T. Kunitake, *J. Macromol. Sci., Chem.*, **A-13**, 587 (1979); b) J. H. Fendler, "Membrane Mimetic Chemistry," John Wiley & Sons, New York (1982).

4) N. Wangner, K. Dose, H. Koch, and H. Ringsdorf, *FEBS Lett.*, **132**, 313 (1981); I. Hamachi, S. Noda, and T. Kunitake, *J. Am. Chem. Soc.*, **113**, 9625 (1991); K. Naka, A. Ohki, and S. Maeda, *Chem. Lett.*, **1991**, 1303.

5) a) K. Akiyoshi, S. Yamaguchi, and J. Sunamoto, *Chem. Lett.*, **1991**, 1263; b) K. Akiyoshi and J. Sunamoto, "Organized Solutions," ed by S. E, Friberg and B. Lindman; Marcel Dekker, Inc, New York (1991), Chap. 20.

6) P. Bohlen, S. Stein, W. Dairman, and S. Undenfrien, *Arch. Biochem. Biophys.*, **155**, 213 (1973).

7) M. Dubois, K. A. Gilles, J. K. Hamiliton, P. A. Rekers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(Received July 17, 1992)