CHEMISTRY LETTERS, pp. 1263−1266, 1991.

Self-aggregates of Hydrophobic Polysaccharide Derivatives

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Hydrophobic polysaccharide derivatives bearing palmitoyl or cholesterol moieties form self aggregates in an aqueous solution. The critical concentrations to give the polymer aggregates depended on the degree of substitution of the hydrophobic moiety. Cholesterol - bearing pullulan showed a stronger binding for hydrophobic guest molecules and higher colloidal stability compared with the corresponding palmitoyl-bearing one.

Naturally occurring polysaccharides are one of the most abundant and diverse families of biopolymers, and are widely applied to various fields. [1] Recently, the hydrophobic polysaccharide derivatives have been prepared for a variety of applications as a carrier gel of hydrophobic chromatography, [2] polymer surfuctants, [3] material of immobilizing of enzyme, [4,5] and biological active polymer. [6,7] We also have developed liposomes or oil-in-water droplets as coated by cell-specific polysaccharides which bear a hydrophobic anchor and utilized them in biotechnology and/or medicine. [8-11] In spite of these applications only few studies of solution property of such the hydrophobic polysaccharide derivatives in aqueous media have been reported. [3, 12] We have found that palmitoyl- or cholesterol-substituted derivatives of naturally occurring polysaccharides such as pullulan, amylopectin, and dextran derivatives form self aggregates in an aqueous solution. In this communication, the solution characteristics of pullulan derivatives bearing long alkyl chain or cholesterol moieties are described.

Cholesterol-substituted polysaccharide derivatives were newly synthesized. To 1.22 g (7.0 mmol, as the glucose unit) of pullulan (Mw 50000, Hayashibara K. K.) in 100 ml of dry DMSO containing 8 ml of pyridine were added 0.412 g (0.74 mmol) of cholesteryl *N*-(6-isocyanatehexyl)carbamate which was synthesized by the reaction of cholesterol with hexyldiisocyanate. The reaction mixture was stirred at 100 °C for 8 h. After the solvent was removed in vacuo, 400 ml of ethanol were added and the suspension so obtained was stored overnight at 4 °C. The precipitates obtained was separated and purified by dialysis using Seamless Cellulose Tube (VISKASE SALES Corp.). The degree of substitution of cholesterol group was determined by ^{1}H-NMR and elemental analysis. In this case pullulan was

substituted to 5.5 cholesterol groups per 100 glucose units (degree of substitution: DS = 0.055) and coded as CHP-50-5.5. Other cholesterol derivatives which have different degree of substitution (DS = 0.018 and 0.041) were also synthesized by the same method. A palmitoyl pullulan derivative (OPP-50-5.4), which was substituted by 5.4 palmitoyl groups per 100 glucose units (DS = 0.054), was synthesized by the same method previously reported. [8] Structures of these pullulan derivatives are shown in Fig. 1.

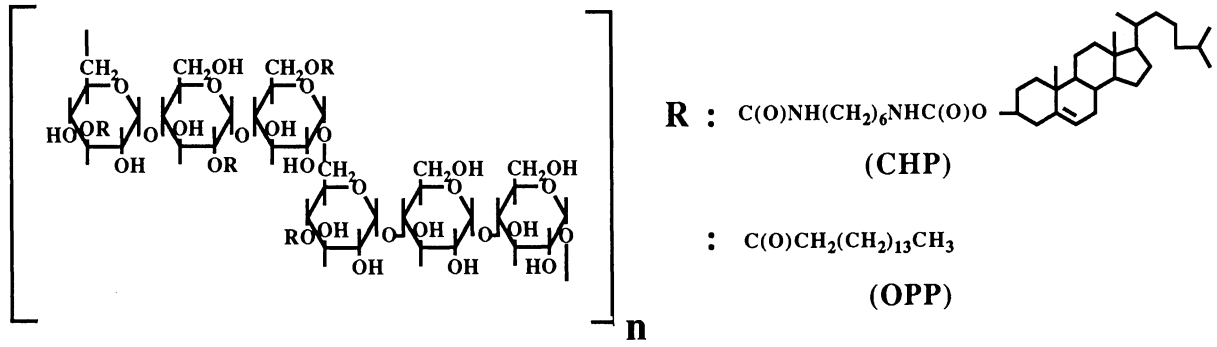

Fig. 1. Pullulan derivatives prepared.

First, the interaction between pullulan derivatives and a fluorescent probe, magnesium 1-anilino-8-naphthalenesulfonate (ANS), was investigated in an aqueous solution. ANS is a prove sensitive to a microscopic polarity. [13] The emission maximum of ANS is 515 nm at 25 °C in a neutral aqueous solution and the fluorescence was strongly quenched by hydration. When ANS (2.0×10^{-5} M) was added to an aqueous pullulan solution (5.0×10^{-5} M) at pH 7.0, the emission maximum did not change at all. However, in the presence of CHP (5.0×10^{-5} M) or OPP (5.0×10^{-5} M), the emission maximum shifted to 477nm, and the fluorescence intensity drastically increased. This means that the micropolarity around the probe corresponded to that of butanol, and the probe molecules were incorporated in the hydrophobic domain of the polysaccharide derivatives. The change of fluorescence intensity of ANS was investigated as a function of concentration of the pullulan derivatives at pH 7.0 and 25 °C. This plot gave a linear relationship with a critical point regarding the concentration of the polymer (Fig. 2). This phenomenon was very similar to that observed in various aqueous micellar systems. [14] CHP or OPP molecules certainly form polymer self-aggregates above this critical concentration. The critical concentrations of these polysaccharide derivatives are shown in Table 1. The higher substitution of the cholesterol moiety gives the lower critical concentration. The major driving force for the aggregation is ascribed to the hydrophobic interaction between hydrophobic moieties. The palmitoyl group was less effective for forming the self-aggregates.

Secondly, the binding constants between ANS (5.0×10^{-6}M) and the pullulan derivatives aggregates were estimated by Benesi-Hildebrand relationship.[12,15]

$$1/(I/I_0) = 1 / [\text{CHP or OPP}] \times 1 / K(I_\infty / I_0) + I_\infty / I_0$$

where K is the binding constant, I / I_0 is the relative fluorescence intensity at 477 nm (Ex : 380 nm) in the presence (I) and absence (I_0) of a given amount of the polymer, I_∞ is the postulated

fluorescence intensity if all ANS were incorporated to the polymer aggregates. The plot of $1 / (I/I_0)$ vs. 1 / [CHP or OPP] yields a straight line for each polysaccharide derivatives. The values obtained are also shown in Table 1. ANS was strongly incorporated to the polymer aggregates. The K-value increased with an increase in the substitution of cholesterol. Cholesterol residue offered a stronger binding site than palmitoyl residue.

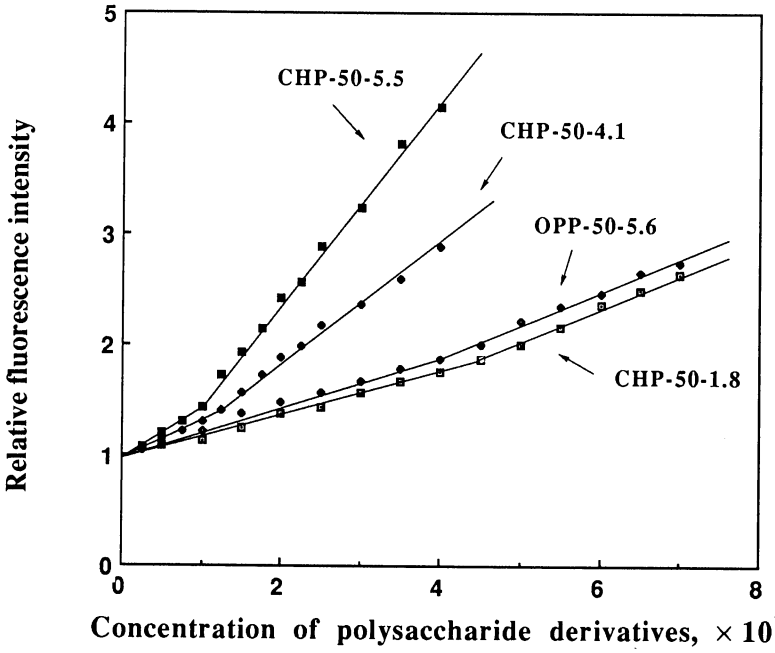

Fig. 2. Change of fluorescence intensity as a function of the concentration of polysaccharide derivatives at pH 7.0 and 25 °C. [ANS] = 2.0×10^{-5} M
Relative fluorescence intensity was plotted I/I_0 at 477 nm of emission spectra (Ex = 380 nm).

Table 1. Critical concentrations of pullulan derivatives and binding constants for ANS in an aqueous solution at 25 °C and pH 7.0

Polysaccharide derivatives	Critical concentration / $\times 10^7$ M	Binding constant / M^{-1}
CHP-50-1.8	4.5 ± 0.1	1.5×10^4
CHP-50-4.1	1.3 ± 0.1	2.4×10^4
CHP-50-5.5	1.0 ± 0.1	3.5×10^4
OPP-50-5.4	4.0 ± 0.1	0.9×10^4

Thirdly, in order to estimate the particle size of the polymer aggregates and to evaluate their colloidal stability, average hydrodynamic diameters (Dh) were measured by a dynamic light scattering method (DLS-700, Otsuka electronics.). The change of particle size was followed as a function of time. A 0.1 wt% (- 10^{-5} M) aqueous solution of the polymer aggregates was prepared under sonication at 20 W and 70 °C for 30 min. Hydrodynamic diameters determined were 195 nm for CHP-50-1.8, 164 nm for CHP-50-4.1, 135 nm for CHP-50-5.5, and

173 nm for OPP-50-5.4 respectively at 25 °C just after the sonication. Apparently, several pullulan derivative molecules intermolecularly aggregate because the diameter of parent pullulan without the hydrophobic residues was less than 20 nm. The OPP aggregate was rather unstable. The diameter of the aggregate increased up to 2300 nm after 3 h upon standing, and then precipitated after 24 h. On the other hand, CHP-50-5.5 aggregate was more stable and its size (Dh = 133 nm) did not change at all even after 24 h and no precipitates were observed. Hydrophobic moiety of the polysaccharides influenced the colloidal stability of the aggregates. Cholesterol moiety showed a higher stability compared with the corresponding palmitoyl moiety. Cholesterol-bearing amylopectin(Mw 112000, DS = 0.013) and dextran (Mw 19600, DS = 0.011) also formed self-aggregates in an aqueous solution (0.1 wt% for amylopectin derivative, Dh=226 nm and 1.0 wt% for dextran derivative , Dh = 116 nm). The particle size of polysaccharide aggregates also changed depending on the sonication time, the temperature and the ionic strength of the medium.

Our present polysaccharide aggregates have a potential capacity to encapsulate various substances mainly by hydrophobic interaction. Since it is possible to endow cell specificity to the aggregates by using cell-specific polysaccharide derivatives as reported previously, [9,10] the polysaccharide aggregates are expected to behave as a novel carrier for various lipophilic drugs, proteins, nucleic acids, and so on.

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(Received May 7, 1991)