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ORIGINAL CONTRIBUTION

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Adsorption behaviors of emulsifiers and biomolecules on temperature-sensitive polymer particles

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Abstract Adsorption and desorption behaviours of emulsifiers and biomolecules on the two kinds of temperature-sensitive composite polymer particles were compared. One (I) was produced by seeded emulsion copolymerization of dimethylaminoethyl methacrylate and ethylene glycol dimethacrylate with 0.17 μm-sized polystyrene seed particles. The other (II) was produced by seeded emulsion copolymerization of N-isopropylacrylamide (NIPAM) and N,N′-methylenebisacrylamide with 0.36 μm-sized styrene-NIPAM copolymer particles. The amount of adsorption at temperatures above each lower critical solution temperature (LCST) was found to be much higher for I than II. In both cases, at temperatures below the LCST, almost all lactalbumin hydrolysate molecules adsorbed above the LCST were desorbed but the desorptions of adsorbed egg albumin and lysozyme molecules were not so high. The adsorption and desorption were reversible for both particles and the efficiency was better for I than II. From these results, it is concluded that the adsorption/desorption of protein onto the temperature-sensitive polymer particles is controllable by changing the temperature below and above the LCST of the shell layer and the sensitivity is based on the surface property of the composite polymer particles.

Key words Temperature-sensitive – emulsifier – biomolecules – adsorption – surface

Introduction

In a previous article [1], composite polymer particles having temperature-sensitive property with lower critical solution temperature (LCST) around 35 °C were produced by seeded emulsion copolymerization of dimethylaminoethyl methacrylate (DM) and ethylene glycol dimethacrylate (EGDM) with 0.17 μm-sized polystyrene (PS) seed particles. This property is considered to be useful as a carrier for biomolecules in the biomedical field [2]. Makino et al. reported that composite particles prepared by seeded emulsion copolymerization of N-isopropylacrylamide (NIPAM) and N,N′-methylenebisacrylamide (MBAAm) with submicron-sized styrene (S)-NIPAM copolymer (P(S-NIPAM)) particles also had this temperature-sensitive property [3].

In this article, we carried out a comparative study on the effect of the temperature-sensitive property of the above two kinds of composite polymer particles on the adsorption and desorption behaviors of low molecular weight emulsifiers and some biomolecules under similar conditions.

Experimental

Materials

S was distilled under reduced pressure in a nitrogen atmosphere. DM and EGDM were of reagent grade and used as received. NIPAM (Kohjin Co., Japan) was purified by recrystallization. MBAAm (Wako Pure Chemicals Co.) was distilled under reduced pressure. Potassium persulfate (KPS) was recrystallized before use. 2,2′-azobis(2-amidinopropane) hydrochloride (AIBA), trimethyl stearyl ammonium chloride (TSAC), sodium dodecylbenzene sulfonate (DBS) and polyoxyethylene sorbitan monooleate (Tween 80) were also of reagent grade. Egg albumin (AL), lysozyme (LZ) and lactalbumin hydrolysate (LA), which were commercially supplied by Wako Pure Chemicals Co., were preserved in the refrigerator and used as received. Deionized water was distilled with a Pyrex distillator. Other chemicals used were of analytical grade.

Seeded emulsion copolymerization of DM and EGDM with PS seed particles

PS seed emulsion was prepared by emulsion polymerization of S in the presence of nonionic emulsifier with AIBA initiator under the conditions listed in Table 1. Seeded emulsion copolymerization of DM and EGDM was then carried out with the 0.17 μm-sized PS seed particles under the conditions presented in Table 1. Each conversion was about 98%. A portion of the coagulated composite particles was removed by centrifugation. The particles were washed repeatedly by serum replacement with deionized water to remove any traces of ionized salt and/or emulsifier. The solid content of the redispersed particles was adjusted in the range of 3 to 5 g/l. The conductance of the purified composite emulsion was under 10 μS/cm. Hereafter the produced particles are called as PS/P(DM-EGDM) composite particles.

Seeded emulsion copolymerization of NIPAM and MBAAm with P(S-NIPAM) seed particles

According to the recipes reported by Makino et al. [2], composite polymer particles consisting of poly-NIPAM as one component were produced as follows.

S-NIPAM copolymer (P(S-NIPAM)) particles were produced by emulsifier-free emulsion polymerization of S and NIPAM with KPS initiator under the conditions listed in Table 2. Seeded emulsion copolymerization of NIPAM and MBAAm was then carried out with 0.36 μm-sized P(S-NIPAM) seed particles under the conditions listed in Table 2. The produced composite particles were washed repeatedly by serum replacement with deionized water. The solid content of the redispersed particles was 26.1 g/l. Hereafter the produced particles are called as P(S-NIPAM)/P(NIPAM-MBAAm) composite particles.

The hydrodynamic diameters of particles at 40° and 25 °C were measured by dynamic light scattering.

Table 1 Preparation of polystyrene seed and PS/P(DM-EGDM) composite emulsions by emulsion polymerizations[a]

Ingredients		PS	PS/P(DM-EGDM)
PS emulsion[b]	(g)	–	66.22
Styrene	(g)	64	–
DM	(g)	–	8.33
EGDM	(g)	–	0.44
Tween 80	(g)	0.160	–
AIBA	(g)	0.256	2.00
Water	(g)	300	500
Polymn. time	(h)	24	8

[a] 60 °C, N_2, 100 rpm
[b] Polymer solid, 150 g/l
Abbreviations: PS, polystyrene; DM, dimethylaminoethyl methacrylate; EGDM; ethylene glycol dimethacrylate; Tween 80, polyoxyethylene sorbitan monooleate; AIBA, 2,2′-azobis(2-amidinopropane) hydrochloride.

Table 2 Preparation of P(S-NIPAM)/P(NIPAM-MBAAm) composite particles[a]

Polym. time (h)		Ingredients		
1st stage	24	Styrene	(g)	9.0
		NIPAM	(g)	1.0
		KPS	(g)	0.1
		Water	(g)	100
2nd stage	8	Seed emulsion[b]	(g)	55.0
		NIPAM	(g)	3.0
		MBAAm	(g)	0.2
		KPS	(g)	0.2
		Water	(g)	145

[a] 70 °C, N_2, 100 rpm
[b] emulsion solid, 90 g/l
Abbreviations: NIPAM, N-isopropylacrylamide; MBAAm, N,N′ methylene-bis-acrylamide; KPS, potassium persulfate.

Adsorption of emulsifiers

For each measurement, 10 ml of purified composite emulsion was mixed with 10 ml of emulsifier aqueous solution. The concentrations of the emulsifier in the mixtures were below the critical micelle concentration (CMC). The pH values of P(S-NIPAM)/P(NIPAM-MBAAm) and PS/P(DM-EGDM) composite emulsion were maintained at

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7 and 9, respectively. In order to examine the adsorption and desorption behaviours of the emulsifier onto them at 40° and 25 °C, the conductances of the mixtures were alternatively measured repeatedly at 40° and 25 °C. Before the measurement, the mixture was kept at the respective temperatures for 1 h.

The amount of emulsifier adsorbed was calculated by subtracting the emulsifier concentration in the medium from the initial concentration calculated under the assumption that the adsorbed emulsifier molecules do not contribute to the conductance. The emulsifier concentration in the medium was obtained from the measurement of conductance using each calibration curve, representing the relationship between the concentration and conductance, for the emulsifier aqueous solution at 40° and 25 °C.

Adsorption of biomolecules

For each measurement, 10 ml of purified emulsion was mixed with 10 ml of biomolecule aqueous solution. The pH value of the mixture was immediately adjusted at the isoelectric point of the biomolecule with a buffer solution. In order to examine the adsorption and desorption behaviors of each biomolecule onto the both composite particles at 40° and 25 °C, the amounts of adsorption at 40° and 25 °C was repeatedly measured alternatively after keeping at each temperature for 3 h, as follows. The mixture was centrifuged at 10000–15000 g, 40° or 25 °C for 10 min. Moreover, in order to completely remove the wafting particles, the supernatant was centrifuged twice at 25000 g. Biomolecule concentration in the medium was measured by ultraviolet spectrophotometry at 280 nm. The amount of biomolecule adsorbed was calculated by subtracting the biomolecule concentration in the medium from the initial concentration.

Results and discussion

Figure 1 shows the transmission electron micrographs of nonpurified P(S-NIPAM)/P(NIPAM-MBAAm) and PS/P(DM-EGDM) composite particles. In both cases, no by-produced particles was observed and the particles were monodispersed. The diameters and the coefficients of variation were, respectively, 0.4 μm and 5.8% for P(S-NIPAM) /P(NIPAM-MBAAm) and 0.18 μm and 2.4% for PS/P(DM-EGDM) composite particles. These suggest that in both cases seeded emulsion copolymerizations took place in the corresponding seed particles.

Figure 2 shows the amounts (mg/g) of anionic DBS emulsifier adsorbed onto the P(S-NIPAM)/P(NIPAM-MBAAm) composite particles at pH 7 and onto the PS/P(DM-EGDM) composite particles at pH 9. The measurements were carried out alternatively at 40° nd 25 °C. In both composite particles the amounts of adsorption were higher at 40° than those at 25 °C. The amount of adsorption at 40 °C was much higher for the PS/P(DM-EGDM) than that for the P(S-NIPAM)/P(NIPAM-MBAAm). In the case of PS/P(DM-EGDM) composite particles about 60% of the adsorbed emulsifier was

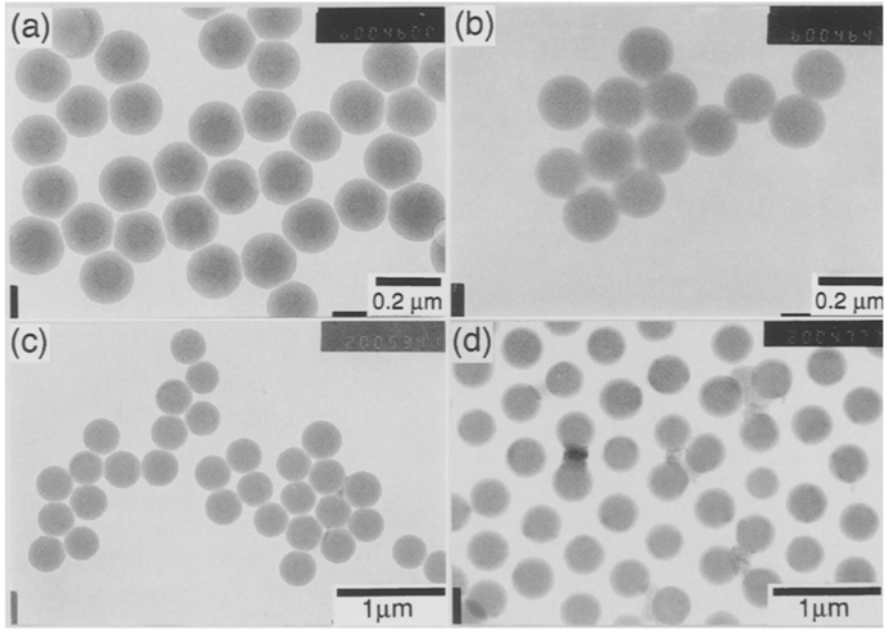

Fig. 1 Transmission electron micrographs of PS seed (a) and PS/P(DM-EGDM) composite (b), P(S-NIPAM) seed (c) and P(S-NIPAM)/P(NIPAM-MBAAm) composite (d) particles

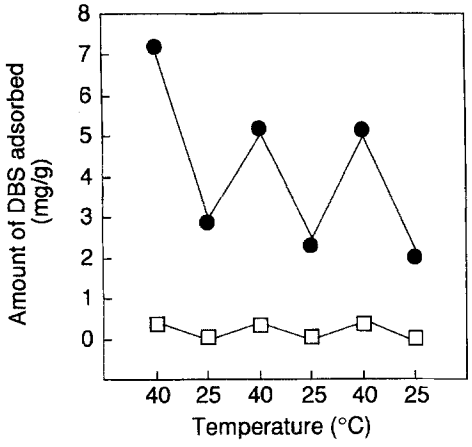

Fig. 2 Variations of the amount of sodium dodecylbenzenesulfonate adsorbed on P(S-NIPAM)/P(NIPAM-MBAAm) (□) at pH 7 and PS/P(DM-EGDM) (●) composite particles at pH 9 measured alternatively at 40° and 25 °C: emulsifier, 0.32 g/l

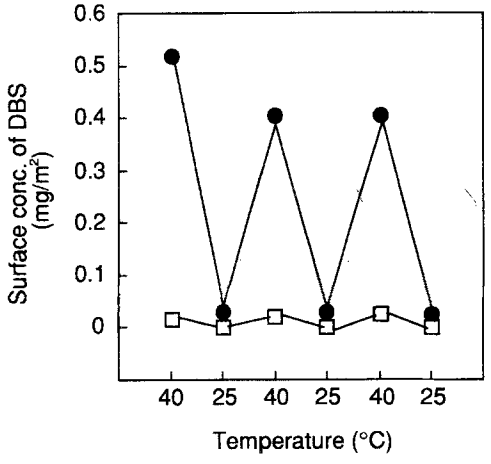

Fig. 3 Variations of surface concentration of sodium dodecylbenzenesulfonate adsorbed on P(S-NIPAM)/P(NIPAM-MBAAm) (□) at pH 7 and PS/P(DM-EGDM) (●) composite particles at pH 9 measured alternatively at 40° and 25 °C: emulsifier, 0.32 g/l; P(S-NIPAM)/P(NIPAM-MBAAm) system: hydrodynamic diameters at 25° and 40 °C were 695 and 200 nm; PS/P(DM-EGDM) system: hydrodynamic diameters at 25° and 40 °C were 1320 and 500 nm

desorbed, whereas in the case of P(S-NIPAM)/P(NIPAM-MBAAm) composite particles about 80% of the adsorbed emulsifier was desorbed by lowering the temperature to below the LCST. But the absolute amount of desorption was much higher for the PS/P(DM-EGDM) composite polymer particles. These behaviours were reversible between 40° and 25 °C.

Figure 3 shows the result replotted using the data shown in Fig. 2 as the variation of surface concentration (mg/m^2) of the anionic DBS emulsifier adsorbed. In both composite particles, the surface concentrations of the

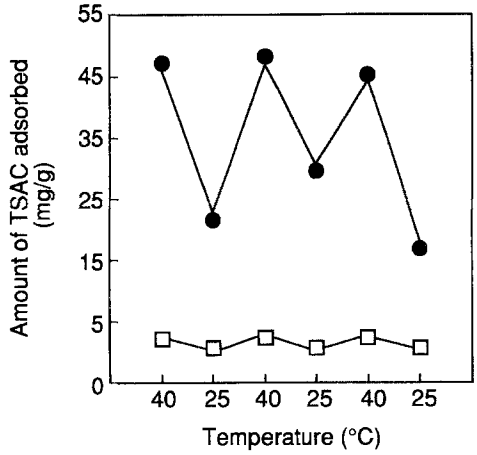

Fig. 4 Variations of the amount of trimethyl stearyl ammonium chloride adsorbed on P(S-NIPAM)/P(NIPAM-MBAAm) (□) at pH 7 and PS/P(DM-EGDM) (●) composite particles at pH 9 measured alternatively at 40° and 25 °C: emulsifier, 1.51 g/l

emulsifier at 40 °C were much higher than those at 25 °C and those at 25 °C were extremely low. In this way, there was apparently a great difference between the adsorption/desorption behaviors expressed as the amount of adsorption (mg/g) and the surface concentration (mg/m^2). This is because the total surface areas of both temperature-sensitive polymer particles at 25 °C were much higher than those at 40 °C because of the swelling and deswelling phenomena at temperatures below and above the LCST. Since the purpose of this article is to clarify the adsorption/desorption behaviors of emulsifiers and biomolecules onto the temperature-sensitive polymer particles, hereafter the results will be expressed as the amount of adsorption (mg/g).

Figure 4 shows the amounts of cationic TSAC emulsifiers adsorbed onto the P(S-NIPAM)/P(NIPAM-MBAAm) composite particles at pH 7 and onto the PS/P(DM-EGDM) composite particles at pH 9. The measurements were carried out alternatively at 40° and 25 °C. In both composite particles, the adsorbtion/desorption behaviors of TSAC emulsifier were observed to be similar as those observed for DBS emulsifier except that the amount of adsorption of TSAC at 40 °C was much higher than that of DBS. The difference in the amount of adsorption might be based on the difference in hydrophobicity between the cationic and anionic emulsifiers.

Figures 5, 6 and 7 show, respectively, the amounts of adsorption of LZ, AL, and LA onto both composite particles. All the measurements were carried out at the isoelectric points of the respective proteins under similar conditions. In all protein systems, the amounts of proteins adsorbed onto both composite particles at 40 °C were much higher than those at 25 °C. These behaviors were reversible

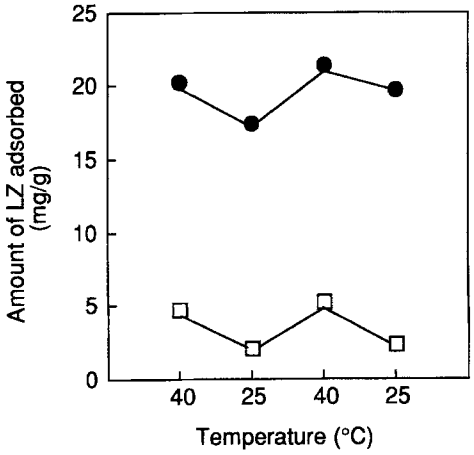

Fig. 5 Variations of the amount of lysozyme (LZ) adsorbed on P(S-NIPAM)/P (NIPAM-MBAAm) (□) and PS/P(DM-EGDM) (●) composite particles at pH 11.0 under the constant concentration of LZ against the total particle surface area, measured alternatively at 40 and 25 °C: PNIPAM system: particles, 26.10 g/l; LZ, 1.17 g/l; PS/P(DM-EGDM) system: particles, 3.47 g/l; LZ, 0.29 g/l

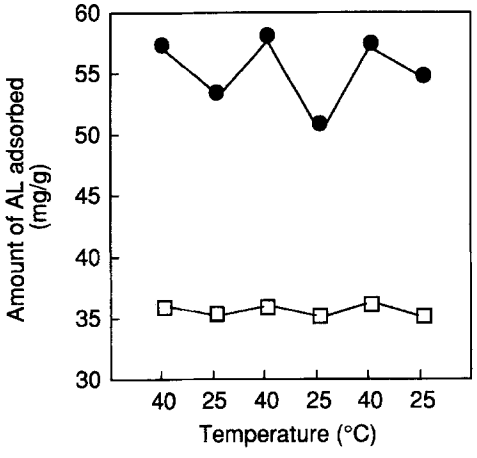

Fig. 6 Variations of the amount of albumin (AL) adsorbed on P(S-NIPAM)/P (NIPAM-MBAAm) (□) and PS/P(DM-EGDM) (●) composite particles at pH 4.9 under the constant concentration of AL against the total particle surface area, measured alternatively at 40 and 25 °C: P(S-NIPAM)/P(NIPAM-MBAAm) system: particles, 26.1 g/l; AL, 1.17 g/l; PS/P(DM-EGDM) system: particles, 5.07 g/l; AL, 0.37 g/l

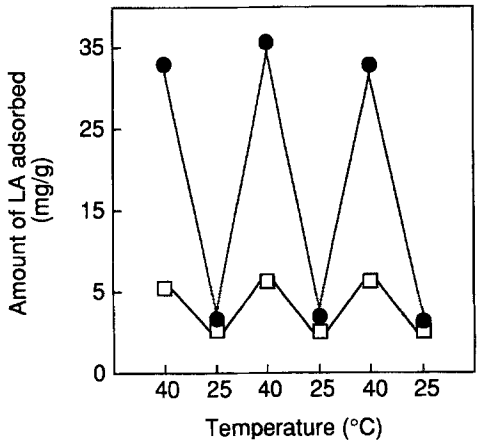

Fig. 7 Variations of the amount of lactalbumin hydrolysate (LA) adsorbed on P(S-NIPAM)/P(NIPAM-MBAAm) (□) and PS/P (DM-EGDM) (●) composite particles at pH 6.8 under the constant concentration of LA against the total particle surface area, measured alternatively at 40 and 25 °C: P(S-NIPAM) /P(NIPAM-MBAAm) system: particles 26.1 g/l; LA, 1.17 g/l; PS/P (DM-EGDM) system: particles, 3.47 g/l; LA, 0.29 g/l

between 40 ° and 25 °C except the case of LZ in which repeated measurements were only possible two times because of the low stability of LZ. In both composite particles, the amount of adsorption was decreased in the order of AL > LA > LZ. In the case of AL, only small amount of desorption was observed in the both composite particles. However, in the case of LA, almost LA molecules adsorbed above the LCST were desorbed and the amount of desorption was much higher for PS/P(DM-EGDM) than that for P(S-NIPAM)/P(NIPAM-MBAAm) composite particles. The adsorption behaviors of the proteins are usually influenced by the molecular characteristics particularly hydrophobicity and flexibility [4]. Since we have not enough information on those three kinds of proteins, we can only assume that the different adsorption behaviors are possibly based on the differences in molecular characteristics among the proteins.

From these results, it is concluded that the adsorption/desorption of protein onto the temperature-sensitive polymer particles is controllable by changing the temperature below and above the LCST of the shell layer and the sensitivity is based on the surface property of the composite polymer particles.

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