SHORT COMMUNICATIONS

Efficient Immobilization of Protein on Monodispersed Colloidal Silica Particles Modified by Copolymers of Maleic Anhydride and Styrene or Methyl Methacrylate

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Recent developments in colloid chemistry have made the preparation of many kinds of monodispersed inorganic colloids possible. We have been developing a new functional materials derived from monodispersed colloidal particles modified with polymer.^{1,2} One application is as a biomaterial carrier in biotechnology for cell labeling, immunoassay or affinity separation. In many cases, polymer latexes, such as poly(styrene) microspheres of micron size, have been employed as carrier particles.³ In latex immunoassay, polymer particles should have adequate stability and high density to attain high sensitivity and short test time. In this respect, applications of polymer particles as carriers are limited, because it is difficult to prepare polymer spheres with high density, in spite of controlling stability and surface hydrophobicity.⁴ A previous paper reported that monodispersed colloidal silica particles modified with poly(ethylene glycol) bind to bovine serum albumin (BSA) as a protein, and hybrid particles can be used in immunological agglutination tests.⁵ However, binding efficiency was low; amount of immobilized BSA was less than 20% of

dosed protein. This paper describes improvement in the immobilization of BSA on monodispersed colloidal silica modified with the copolymer of maleic anhydride and styrene or methyl methacrylate, and the applicability of hybrid particles to the immunological agglutination tests.

Poly(maleic anhydride-styrene) (P(MA-ST)) and poly(maleic anhydride-methyl methacrylate) (P(MA-MMA)) having a trimethoxysilyl terminal group were synthesized by the previous method.⁶ Reactions of the polymer couplers with ethanolic colloidal silica (23 wt%), consisting of 0.47 μm diameter particles, gave unimodal P(MA-ST)/SiO₂ and P(MA-MMA)/SiO₂ particles, composed of 26 mg g⁻¹ P(MA-ST) and 17 mg g⁻¹ P(MA-MMA), respectively, by the method described previously.⁶ The reaction of P(MA-ST)/SiO₂ or P(MA-MMA)/SiO₂ (0.1 g) with BSA (5 mg) was conducted in pH 5.6 phosphate buffer (0.03 M) solution (10 ml) at room temperature. The hybrid particles were stirred with 2-aminoethanol (0.6 g) in pH 7 buffer (10 ml) for 5 h. The amount of bound BSA was determined by spectrophotometric absorption of the

supernatant, separated by a centrifugation, at 280 nm. The anti-BSA antibody was purified by the method described previously.⁷ Agglutination tests were carried out by mixing 50 μl of 0.05 M phosphate buffer, containing the antibody at $10\text{ }\mu\text{g ml}^{-1}$ to $0.019\text{ }\mu\text{g ml}^{-1}$, with 50 μl of a BSA-immobilized particle suspension (0.1%) in the buffer in the well of a microplate.

Particles of P(MA-ST)/SiO₂ and P(MA-MMA)/SiO₂ were stable in neutral aqueous solution, but immersing them into pH 4.0 buffer solution brought about the partial elimination of bound P(MA-ST) or P(MA-MMA) on the silica. Hence, the reactions of composite particles with BSA were carried out

Figure 1. Immobilization of BSA on P(MA-ST)/SiO₂ (○) and P(MA-MMA)/SiO₂ (●) at pH 5.6.

Table I. Immunological agglutination tests of BSA-P(MA-ST)/SiO₂ and BSA-P(MA-MMA)/SiO₂^a

Particles	Immob. BSA mg g ⁻¹	Concentration/ $\mu\text{g ml}^{-1}$									
		10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.039	0.019
P(MA-ST)/ SiO ₂	5 Anti-BSA antibody	++	+	±	—	—	—	—	—	—	—
	5 γ -Globulin	—	—								
	13 Anti-BSA antibody	++	++	++	++	++	+	±	—	—	—
	13 γ -Globulin	—	—								
	27 Anti-BSA antibody	++	++	++	++	+	±	—	—	—	—
	27 γ -Globulin	—	—								
	32 Anti-BSA antibody	++	++	++	+	±	—	—	—	—	—
	32 γ -Globulin	—	—								
P(MA-MMA)/ SiO ₂	5 Anti-BSA antibody	++	++	++	++	+	±	—	—	—	—
	5 γ -Globulin	—	—								
	14 Anti-BSA antibody	++	++	++	++	++	++	+	±	—	—
	14 γ -Globulin	—	—								
	32 Anti-BSA antibody	++	++	++	++	++	+	±	—	—	—
	32 γ -Globulin	—	—								

^a Symbols ++, + and ± represent extent of agglutination and — designates negative.

in pH 5.6 phosphate buffer, though BSA has an isoelectric point at pH 4.7–4.9. The composite particles of P(MA-ST)/SiO₂ efficiently bound to most of dosed BSA at lower BSA concentration than 20 mg per unit gram of silica in the reaction solution. Increase in dose of BSA led to less immobilization efficiency; about 80% of the BSA was immobilized at BSA of 30 mg g⁻¹ (Figure 1). The amount of immobilized BSA attained a constant value of 40 mg g⁻¹, corresponding to the maximum amount for end-on packing. This was estimated by 14 nm major and 3.8 nm minor axis in an oval shape of BSA.⁸ It was also observed that P(MA-MMA)/SiO₂ bound to BSA at 20–30% less efficiency at 30 mg g⁻¹ dosed protein, compared with that on P(MA-ST)/SiO₂ (Figure 1). The high binding efficiency of P(MA-ST) is probably due to binding through hydrophobic interaction between P(MA-ST) and BSA. The present immobilization is not only very effective, but also useful, since there is no need for coupling reagents, such as *N,N'*-dicyclohexylcarbodiimide or glutaraldehyde.

The hybrid particles of BSA-P(MA-ST)/SiO₂ gave specific agglutination with the anti-BSA antibody, and did not non-specifically agglutinate with γ -globulin (Table I).⁹ The lowest agglutination concentration of the antibody, or sensitivity, was dependent on the amount of immobilized BSA on P(MA-ST)/SiO₂. Hybrid particles containing 13 mg g⁻¹ BSA exhibited the highest sensitivity. Probably, less immobilized BSA than 13 mg g⁻¹ on the composite undergoes degradation due to strong hydrophobic interaction between BSA and P(MA-ST), thus depress in sensitivity. At higher BSA immobilization, crowded BSA seems to lead to sterical hindrance of the recognition site in reaction with the anti-BSA antibody. BSA-P(MA-MMA)/SiO₂ containing 14 mg g⁻¹ BSA agglutinated with the antibody at higher concentration than 0.078

$\mu\text{g ml}^{-1}$, showing the highest sensitivity among the BSA-composites. P(MA-MMA)/SiO₂ gave higher sensitivity in the agglutination test than P(MA-ST)/SiO₂, in spite of lower immobilization efficiency. In the present immobilization, P(MA-ST) and P(MA-MMA) function not only as surface modifiers of silica, but also as linkers between silica and BSA. The copolymer of P(MA-ST) is more hydrophobic than P(MA-MMA). In general, deformation of the protein adsorbed or immobilized on a polymer surface increases with surface hydrophobicity.¹⁰ Therefore, the lower sensitivity of BSA-P(MA-ST)/SiO₂ may possibly be due to the extensive deformation of immobilized BSA, compared with that of protein on P(MA-MMA)/SiO₂.

The characterization of immobilized protein on P(MA-ST)/SiO₂ and P(MA-MMA)/SiO₂ particles and their search for applications are presently being made.

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