Synthesis of Polymer-Coated Silica Particles with Specific Recognition Sites for Glucose Oxidase by the Molecular Imprinting Technique

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A polymer layer around silica beads, composed of acrylamide, acrylic acid, and crosslinkers, was able to preferentially recognize glucose oxidase (GOD) from a mixture of proteins. Matching between the ζ-potential of GOD and that of its imprinted polymer is suggested to be important in enhancing the recognition effect.

The method of molecular imprinting is a useful technique for preparing host compounds for molecular recognition.[1,2] There have been only a few papers concerning molecular imprinting of proteins such as ribonuclease A[3] and urease.[4] We have reported previously that acrylic acid–acrylamide copolymer particles, in a thin layer around silica beads, exhibited specific binding characteristics for the protein glucose oxidase (GOD, EC 1.1.3.4).[5]

In this paper, we report the use of acrylamide (AAm) and negatively-charged acrylic acid (AAc) or positively-charged *N,N*-dimethylaminopropyl-acrylamide (DMAPAAm) to prepare the polymer particles. Different combinations of these monomers were compared to find which preparation of the polymer particles gave the most specific recognition sites for GOD.

The method of preparing the polymer particles is described in more detail in our previous paper.[5] Acrylamide (AAm) was mixed, in varying quantities, with either AAc or DMAPAAm to bring the total amount of the monomer mixtures to 0.0150 mol. Two kinds of crosslinkers, 130 mg *N,N'*-methylene-bisacrylamide and 120 mg *N,N'*-(1,2-dihydroxyethylene)-bisacrylamide were mixed with the monomer mixtures in 12 mM phosphate buffer solution. Silica beads with amino groups on the surface (LiChroprep NH2, Merck; particle size 0.025-0.040 mm) were allowed to react with acryloyl chloride, so that vinyl groups were introduced to the surface of the beads. The surface-activated silica beads with vinyl groups and GOD were added to the phosphate buffer solution which was adjusted with phosphoric acid to pH 4.2 or pH 2.8. Polymerization was initiated by adding ammonium persulfate and *N,N,N',N'*-tetramethyl-ethylenediamine in an atmosphere of nitrogen gas. The polymerization reaction was carried out under centrifugation. Polymer pellet obtained was composed of two parts: non-silica bead and silica bead-containing parts. The polymer particles were prepared by pressing the polymer pellets containing silica beads through a sieve (0.053 mm mesh size). To obtain the polymer particles for the rebinding experiments the template molecules (GOD) had to be removed by washing the imprinted polymer particles with 240 mM phosphate buffer solution (pH 4.2 or 2.8).

A competitive rebinding experiment of both GOD and glucose 6-phosphate dehydrogenase (G6PD, EC1.1.1.49) mixtures was carried out under the same pH conditions as those in which the polymer particles were prepared. After incubation, the polymer particles were centrifuged and the supernatant was used to detect remaining enzyme activity[6] of GOD and G6PD.[7]

The amount of GOD adsorbed onto the GOD-imprinted polymer particles, Q(GOD), was calculated by subtracting the GOD activity of the supernatant from that of the standard solution.[8] Amounts of GOD adsorbed onto the GOD-imprinted particles depended upon the monomer (AAc or DMAPAAm) compositions in the preparation of the polymer particles. On the other hand, adsorption amounts of G6PD onto the GOD-imprinted particles did not depend on the monomer compositions, and against expectation, almost all G6PD was adsorbed onto the particles. This suggested that some non-specific binding of GOD and G6PD to the GOD-imprinted particles also occurred. The experimental conditions used for this competitive rebinding test were very severe because G6PD, which has a lower molecular weight than GOD, can disturb the adsorption of GOD onto the GOD-imprinted sites.

In order to elucidate the effect of the specific binding of GOD to the GOD-imprinted sites, the competitive adsorption of both GOD and G6PD mixtures onto human serum albumin (HSA)-imprinted reference polymer particles was measured. The HSA-imprinted particles were synthesized using the same method and the same monomer compositions as for the GOD-imprinted particles. By subtracting the amount of GOD adsorbed onto the HSA-imprinted reference particles, Q(REF(HSA)), from that adsorbed onto the GOD-imprinted particles, Q(GOD), the amount of GOD specifically bound was calculated. The amount specifically bound was highest in the GOD-imprinted particles prepared with about 0.050 mL of AAc monomer at pH 4.2, while no GOD was found to be specifically bound to particles prepared at pH 2.8 (Figure 1). The result of particles prepared at pH 4.2 clearly showed the effects of imprinting. A weak ionic

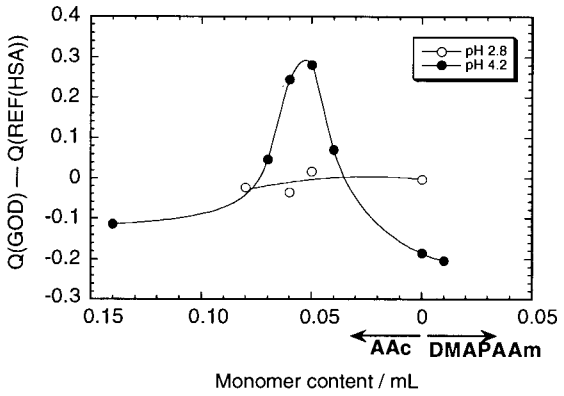

Figure 1. Specific binding of GOD onto GOD-imprinted particles vs. the monomer (AAc or DMAPAAm) content used in the preparation of the particles at pH 2.8 or 4.2. A monomer content of zero means that AAm is the only monomer contained in the polymer particles.

interaction between GOD and the particles is necessary for GOD to specifically bind with the GOD-imprinted sites. In particles prepared at pH 2.8, such ionic interactions cannot be expected because the carboxyl groups of the particles are not dissociated at that pH. The particles showing the highest amount of specific binding had a zeta-potential (ζ -potential) of about -5 mV at pH 4.2. All the particles prepared at pH 2.8 had a ζ -potential of nearly zero at pH 2.8, though the particles contained AAc. The GOD itself had a ζ -potential of about +10 mV and +20 mV in phosphate buffer solutions of pH 4.2 and 2.8, respectively. We noticed that at pH 4.2, the absolute value of the ζ -potential of the particle is very close to that of GOD, but the sign is opposite.

In conclusion, both a weak electrostatic interaction and a matching shape between the template molecule and the imprinted polymer particle are expected to enhance the recognition effect. In the molecular design of imprinted polymers, we suggest that a match between the surface potential (ζ -potential) of the template molecule and that of its imprinted polymer is important. These results are applicable to synthesizing imprinted polymers for other proteins.

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References and Notes

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7 GOD- and G6PD-adsorbed particles and the GOD and G6PD, which were removed by washing the particles with 240 mM phosphate buffer solution, had the enzyme activities.

8 $Q(GOD) = ([GOD]_0 - [GOD]) / [GOD]_0$, where $[GOD]_0$, [GOD]: GOD concentration in the protein solution before and after contact with the GOD-imprinted particles.