Photocontrolled Adsorption Chromatography for Lysozyme Using Azoaromatic Polymer

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Synopsis

Photoresponsive polymeric adsorbents containing an azobenzene moiety were prepared, and adsorption behavior of lysozyme onto the adsorbents was studied. In the dark, the amount of lysozyme adsorbed increased with increasing hydrophobicity of the surface of the adsorbents. Therefore, it is suggested that hydrophobic interaction between lysozyme and the adsorbents plays an important role in their adsorption behavior. On irradiation with UV light, the amount of lysozyme adsorbed decreased. This result is due to increased polarity of the surface of the adsorbent induced by UV irradiation and the resultant reduction in hydrophobic interaction with lysozyme. When a column chromatography of lysozyme was carried out using the photoresponsive polymeric adsorbent as a packing material, lysozyme was eluted by photoirradiation in a system using water as the single solvent.

INTRODUCTION

Recently, polymeric adsorbents are used for purification or isolation of biosubstances and drugs. In general, when adsorbed materials are desorbed from the adsorbent, a gradient elution method is used by addition of organic solvents or surfactants into the eluent to change in the polarity of a moving phase. However, it is difficult to use the gradient elution method for desorption of the materials which are sensitive to the change in the chemical property of the environment surrounding them. In this process, if the polarity of a fixed phase can be controlled by external physical signals such as light, heat, etc., the desorption of the adsorbates from the adsorbent is possible in a system using water as the single solvent. In a previous article, we reported that the polarity of the surface of polymer film containing an azobenzene moiety was regulated reversibly by photoirradiation and the adsorption/desorption process of methyl orange on the polymeric adsorbents, which were prepared by the polymers containing an azobenzene moiety, was controlled by photoirradiation.[1] Furthermore, we have already reported on the photoinduced adsorption–desorption behaviors of cephalosporin antibiotics to the photoresponsive polymeric adsorbent.[2] In this paper, lysozyme which is one of the typical amphipathic proteins, is selected, and its adsorption–desorption behavior with the polymeric adsorbent containing an azobenzene moiety by photoirradiation will be studied with attention to the change in hydrophobicity of the surface. Moreover, the possibility of column chromatographic separation of lysozyme using the photoresponsive polymeric adsorbents as packing materials will be discussed.

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EXPERIMENTAL

Materials

Lysozyme was purchased from Seikagaku Kogyo Co., Ltd., in sixth recrystallized grade and used without further purification. 2-Hydroxyethyl methacrylate (HEMA)–ethylene glycol dimethacrylate copolymer microsphers (20–25 μ) was kindly supplied by Dr. T. Okano, Tokyo Women's Medical College. The styrene (St)–divinylbenzene copolymer beads, Amberlite XAD-2, was purchased from Rohm and Haas Co., Ltd., and washed sufficiently with methanol and water. *N,N*-dimethylformamide (DMF), ethylene glycol (EG), and water were purified in the usual way before use.

Preparation of Photoresponsive Polymeric Adsorbent

The polymers containing an azobenzene moiety in their side chain, poly(*p*-phenylazoacrylanilide) [poly(PAAn)] or PAAn—HEMA copolymer were synthesized by the homopolymerization or the copolymerization of corresponding monomers; this has been already reported.[1] HEMA microsphers or St beads were swollen with DMF solution of poly(PAAn) or PAAn—HEMA copolymers for 24 h. Then the polymeric adsorbents were filtered off and dried *in vacuo*.

Measurement of the Adsorption Amount

(a) Batch method: 0.2-g portions of photoresponsive polymeric adsorbent were transferred to test tubes to which aqueous solutions of lysozyme of specified concentration were added. The test tubes were shaken in a thermostated shaker bath. The concentration of free lysozyme was determined spectrophotomerically. Photoirradiation was carried out with a 500-W ultra high pressure mercury lamp and the wavelength was selected with a filter ($\lambda = 350 \pm 50$ nm). Following the attainment of equilibrium adsorption in the dark, light irradiation was carried out.

(b) Column method: The polymeric adsorbents were packed into a glass column (15 × 0.8 cm ID). A known concentration of an aqueous solution of lysozyme was passed through the column. After unbound lysozyme was rinsed with water, the flow of solution was made to stop, and photoirradiation to the column was carried out for 15 min. In order to obtain high irradiation efficiency, a reflective mirror was used. Then EG was added to change in the polarity of moving phase. The concentration of lysozyme of the eluted solution in the column was determined spectrophotometrically.

RESULTS AND DISCUSSION

Adsorption Behavior of Lysozyme onto the Photoresponsive Polymeric Adsorbent

Azoaromatic polymers show reversible cis–trans isomerization in their azobenzene moieties by photoirradiation, with a resultant change in their polarity.[1,3] In our previous article, we have reported that the values of the contact angle by water on the surface of poly(PAAn) film are 90° in the dark state and 77° in the

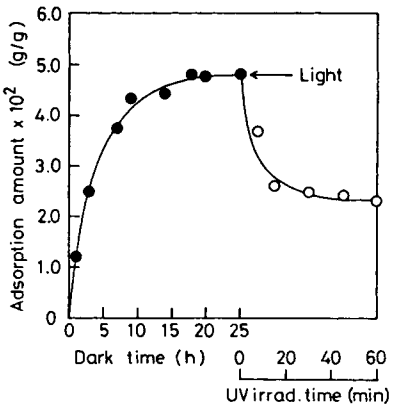

Fig. 1. Photoresponse in the amount of adsorption of lysozyme for HEMA microspher coated with poly(PAAn) at 25°C: (●) in the dark; (○) in light.

photoequilibrium state.[1] When this property is used for the polymeric adsorbents, the binding abilities of them are controlled by photoirradiation.

Adsorption behavior of lysozyme onto the photoresponsive polymeric adsorbents was analyzed by the batch method.

Figure 1 shows the change in the amount of lysozyme adsorbed onto the polymeric adsorbent, which was prepared by poly(PAAn), both in darkness and under light irradiation. It is clear that the amount of adsorption gradually increases in darkness and ultimately becomes saturated and that it begins to decrease with the irradiation of light and reaches an equilibrium after about 30 min. This time agrees with that required for the photoinduced polarity change in the adsorbent surface to become complete. From these results, it is suggested that lysozyme molecules adsorbed onto the polymer film through hydrophobic interaction are desorbed as a result of decrease hydrophobicity of the surface of the adsorbent.

Figure 2 shows the change in the amount of lysozyme absorbed on the polymeric adsorbents, which were prepared by PAAn—HEMA copolymers, plotted against the copolymer composition. The hydrophobicity of PAAn—HEMA

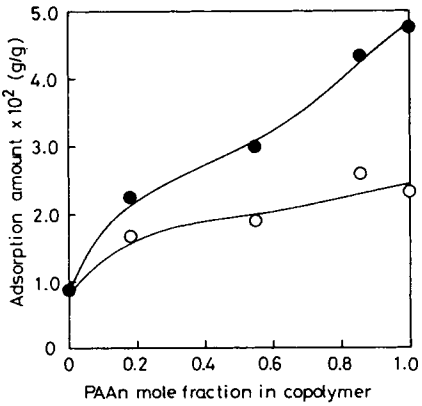

Fig. 2. Relationship between PAAn composition and the amount of adsorption of lysozyme for HEMA microspher coated with PAAn–HEMA copolymer at 25°C: (●) in the dark; (○) in the photostationary state.

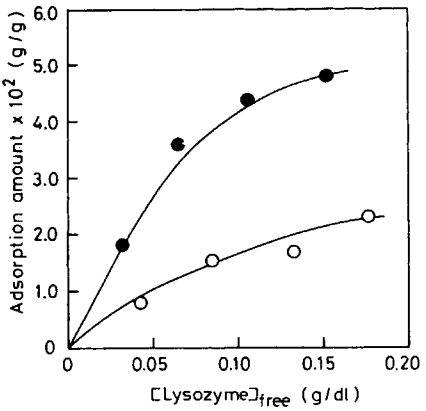

Fig. 3. Binding isotherms of lysozyme by HEMA microspher coated with poly(PAAn) at 25°C: (●) in the dark; (○) in the photostationary state.

copolymer increased with increasing hydrophobic PAAn composition. It is found that the amount of lysozyme adsorbed in darkness increases as the function of PAAn component increases and the surface of the adsorbent becomes more hydrophobic. It is suggested that the hydrophobicity of the polymer surface has influenced the adsorption of lysozyme onto the adsorbent. On irradiation with light, the amount of lysozyme adsorbed decreases for all copolymer compositions. The difference between the amounts of lysozyme adsorbed in darkness and after irradiation represents the amount of lysozyme desorbed onto the polymeric adsorbent by photoirradiation. Apparently, the value of the amount of lysozyme desorbed with increase of PAAn composition. In the PAAn—HEMA copolymers, the photoinduced polarity change on the surface was increased with increasing the PAAn composition. From this, there is a great contribution of the hydrophobic interaction toward the adsorption of lysozyme on the photoresponsive polymeric adsorbents.

Figure 3 shows the binding isotherms for lysozyme in the photoequilibrium state compared with it in the dark state. The amount of lysozyme adsorbed clearly decreases after photoirradiation. When the reciprocal of the adsorption amount was plotted against the reciprocal of the equilibrium concentration, a straight line isotherm was obtained. The intercept of this straight line gave the maximum amount of lysozyme adsorbed onto the polymeric adsorbent.[4] The value for the maximum amount of lysozyme adsorbed in the dark was 6.76×10^{-2} g/g and in the photoequilibrium state was 3.41×10^{-2} g/g. It was consequently found that 3.35×10^{-2} g lysozyme/adsorbent could be desorbed from the adsorbent by UV irradiation. These results lead to conclusion that the adsorption–desorption process can be controlled by light using the polymeric adsorbent containing azobenzene moiety in water solvent system.

Chromatography of Lysozyme on Column Packed with Polymeric Adsorbents

The following experiments were carried out to prove the applicability of the photoresponsive polymeric adsorbent to the column chromatography.

First, the adsorption chromatography of lysozyme was carried out using St beads as a packing material.

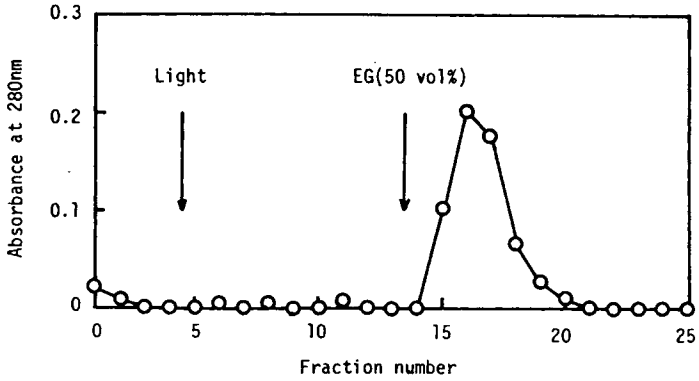

Fig. 4. Adsorption chromatography of lysozyme on column packed with St beads at 25°C. Elution: First step: flow of the solution was stopped and photoirradiation for 15 min; second step: EG (50 vol %) was added.

Figure 4 shows that the profile of the adsorption chromatography of lysozyme using St beads. After an aqueous solution of the lysozyme was passed through the column and lysozyme was adsorbed on the adsorbent, the photoirradiation to the column was carried out to elute the adsorbed lysozyme. However, the elution of lysozyme could not be found. Since the St beads were not coated with the photoresponsive polymer, they were insensitive to light. Then, when an EG (50 vol %) was added into the column, the absorbance at 280 nm was increased in the eluent, which corresponded with the elution of lysozyme. That is to say, lysozyme adsorbed on the St beads was desorbed by the change in the polarity of the moving phase. This suggests that lysozyme can also be eluted through a photoinduced polarity change in the fixed phase by using a photoresponsive polymeric adsorbent.

Figure 5 shows the results of such an experiment. It can be seen from the figure that a part of lysozyme can be eluted by irradiation of light and that further elution is effected by adding EG (33 vol %). The former corresponds to the polarity change in the fixed phase, and the latter to that of the moving phase. Comparison of Figures 4 and 5 reveals that the total elution amount of lysozyme is about 1.3 times larger under the conditions of Figure 5 than that in Figure 4. From these results, it is evident that a combination of the photoinduced polarity

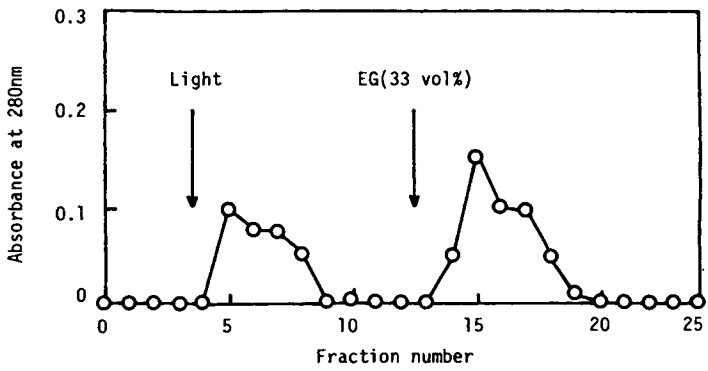

Fig. 5. Adsorption chromatography of lysozyme on column packed with St beads coated with poly(PAAn) at 25°C. Elution: First step was the same condition in Figure 4; second step: EG (33 vol %) was added.

change ensures a more efficient elution of lysozyme under milder conditions than with the gradient elution method alone. Therefore, it is expected that utilizing this type of photoresponsive polymeric adsorbent is a powerful method for separating the biosubstances which cannot be separated by the adsorption chromatography using the gradient elution method.

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