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Hydrogel microspheres III. Temperature-dependent adsorption of proteins on poly-N-isopropylacrylamide hydrogel microspheres

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Abstract: Precipitation polymerization of N-isopropylacrylamide (NIPAM) with methylenebisacrylamide (MBAAm) in water at 70 °C gave thermosensitive hydrogel microspheres. The adsorbability of proteins on the poly-NIPAM microspheres was found to depend on temperature. Below the lower critical solution temperature (LCST) of poly-NIPAM in an aqueous medium, that is, around 32 °C, the microspheres hold a large amount of water inside and their surface is hydrophilic enough to suppress the adsorption of proteins. On the contrary, above 32 °C, the microspheres deswell and their surface becomes hydrophobic and, consequently, susceptible to adsorption of a large amount of proteins. Proteins once adsorbed on the microspheres at a high temperature could be desorbed more or less by lowering the temperature to below 32 °C. The extent of desorption at low temperatures was found to depend on the incubation time for adsorption at high temperatures.

Key words: Hydrogel; thermosensitivity; protein; adsorption and desorption; hydrophilicity; microsphere

Introduction

Poly-N-isopropylacrylamide (poly-NIPAM) is attracting a great deal of attention because of its thermosensitivity [1–4]. Poly-NIPAM in aqueous medium has its lower critical temperature (LCST) at 32 °C and poly-NIPAM gel drastically changes its volume at the LCST. The phenomenon is caused by the reversible formation and cleavage of the hydrogen bond with temperature change. Extensive formation of hydrogen bonds between the amide group and surrounding water molecules below LCST brings about extensive swelling, and cleavage of the bond above the LCST results in deswelling. The above-mentioned response of poly-NIPAM to a temperature change is believed to change the surface of the polymer from a hydrophilic to a hydrophobic one. Therefore, it is expected that the adsorption of proteins on the poly-NIPAM microspheres changes with temperature because protein adsorption generally depends on the hydrophobicity of the surface of substrates.

In this study monodisperse hydrogel microspheres of poly-NIPAM were prepared by precipitation polymerization in water and used as an adsorbent of human gamma globulin (HGG) at 25 °C and 40 °C. The extent of desorption of proteins, having been adsorbed on microspheres at 40 °C, by lowering the temperature to 25 °C was measured as a function of adsorption time at 40 °C.

Experimental

Materials

N-isopropylacrylamide (NIPAM) was kindly presented by Kohjin Co. and purified by recrystallization from a mixture of 1 : 1 toluene and hexane. Methylenebisacrylamide (MBAAm) (Wako Pure Chemicals Co.) was distilled under a reduced pressure. Potassium persulfate (KPS) (Wako Pure Chemicals Co.) was recrystallized from water. HGG (Sigma) was stored in a refrigerator and used as-received.

Preparation of poly-NIPAM microspheres

5 g of monomers (NIPAM + MBAAm) were dissolved in 190 g water in a 200 ml round-bottomed flask equipped with a condenser, a nitrogen inlet, and a stirrer. Nitrogen was bubbled into the solution and the system was kept at 70 °C. 10 ml of an aqueous solution of 0.2 g KPS was added into the flask to initiate polymerization. The reaction was continued for 24 h under mild stirring. The resulting poly-NIPAM microspheres were centrifuged to separate them from the medium. They were redispersed in distilled water. This purification procedure was repeated three times.

The size of microspheres was measured by transmission electron microscopy and photon correlation spectroscopy (PCS) (Ohtsuka Electronics Co., LPA 3000/3100). The former gives the size of dry particles and the latter, a hydrodynamic size.

Adsorption

1 ml latex containing 0.1 g microspheres was mixed with 10 ml of an aqueous solution of 3.75 mg HGG. The mixture was incubated at each fixed temperature. The experiment was carried out at three pH values: 4, 7 and 10. An aliquot of the mixture was pulled out at a scheduled time. The sample was centrifuged and the amount of protein remaining in the supernatant was determined by a BIORAD protein assay or UV adsorption at 280 nm to calculate the adsorbed amount (Γ) of protein by Eqs. (1) and (2),

$$\Gamma\,(\text{g/g particle}) = \{3.75 \times 10^{-3} - C[11.0 - 0.1\,(1 + f\rho_w/\rho_p]\}/0.1 \quad (1)$$

$$f = (D/D_0)^3 - 1\ , \quad (2)$$

where C is the concentration of HGG in supernatant (g/g), f is the increment in volume of microsphere by swelling, ρ_w and ρ_p are the density of water and polymer, respectively, and D and D_0 are the hydrodynamic diameter and the diameter of unswollen (dried) microsphere, respectively.

Desorption was examined by lowering the temperature of the mixture from 40° to 25 °C after a certain time, and the amount of protein was determined in the same manner as above.

Results and discussion

Poly-NIPAM microspheres

A transmission electron micrograph of microspheres is shown in Fig. 1. The microspheres were monodisperse and of about 0.5 μm diameter in their dried state. The hydrodynamic diameter changed with temperature as shown in Fig. 2. A remarkable change was observed around 35 °C and no appreciable change took place around 25° or 40 °C. The hydrodynamic size of microspheres depended on the crosslinking density. The size of microspheres with denser crosslinkage had less temperature-dependence as expected, but the temperature range where the hydrodynamic size changed significantly was not affected by the crosslinking density.

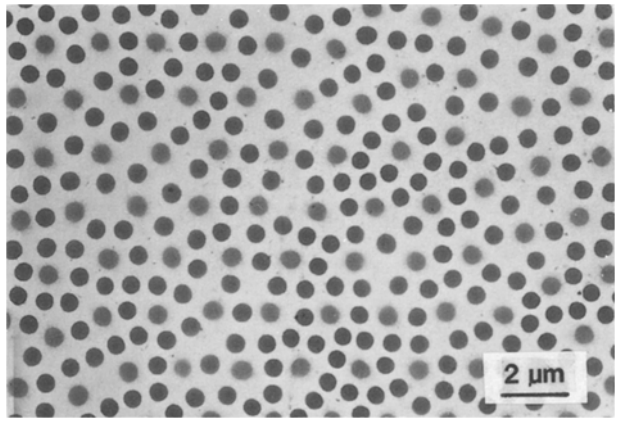

Fig. 1. Transmission electron micrograph of crosslinked poly-NIPAM microspheres. Polymerization: NIPAM 4.9 g, MBAAm 0.1 g, KPS 0.2 g, water 190 g at 70 °C for 24 h

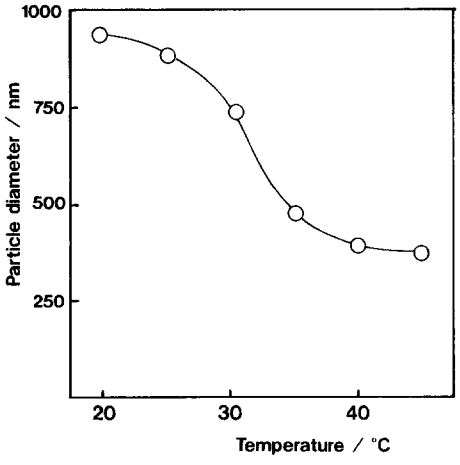

Fig. 2. Temperature dependence of the hydrodynamic size of poly-NIPAM microspheres

Adsorption of proteins on poly-NIPAM microspheres at 25 °C and 40 °C

Some results of the HGG adsorption onto the poly-NIPAM microspheres are shown in Fig. 3. These results indicate that a large amount of HGG is adsorbed at 40 °C, while a small amount is adsorbed at 25 °C. The temperature dependence of

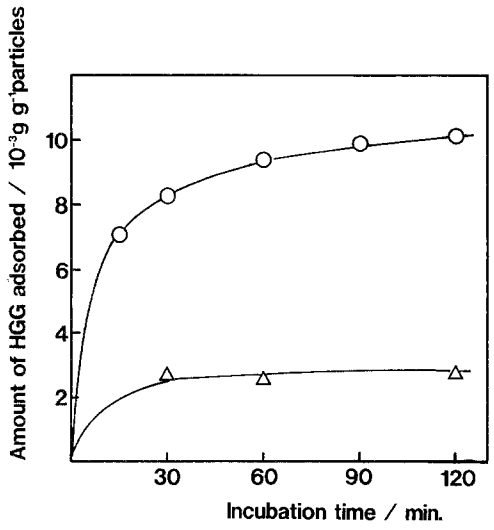

Fig. 3. Adsorption of HGG on crosslinked poly-NIPAM microspheres at different temperatures and pH 4. Microspheres: NIPAM/MBAAm = 0.45/0.05

adsorption was examined by measuring the adsorption of HGG at every five degrees interval between 25 °C and 40 °C. The saturated amount of adsorption did not increase linearly with temperature but changed remarkably around 35 °C.

Adsorption of HGG on nonthermosensitive styrene–acryloylpiperidine copolymer microspheres was also examined, but it showed no temperature dependence in the temperature range between 25 °C and 40 °C. In other words, only the thermosensitive microsphere system exhibited a characteristic temperature-dependent adsorption behavior around the LCST of poly-NIPAM. The amount of protein adsorption decreased with a rise in temperature in spite of the decreasing volume of microspheres. From these results it is concluded that temperature changes not only the swellability of the bulk of the polymer microspheres but also the hydrophilicity of their surface, and that the extent of adsorption of protein is decided by the temperature-dependent hydrophilicity of the surface.

The molecular area of HGG on the surface of shrunk poly-NIPAM microspheres at 40 °C was calculated from the maximum amount of HGG adsorbed at 40 °C and found to be about 2000 nm^2/molecule. This value is much larger than the expected value for the native HGG molecule. Such a discrepancy has been often observed for proteins adsorbed on some hydrophilic surfaces. So, it is reasonable to consider that the hydrophobicity of the surface of poly-NIPAM microspheres at 40 °C is not extremely high but moderate.

All of the above-mentioned results were obtained from the experiments at pH 4. Adsorption at different pH values gave different results. The equilibrium amount of HGG adsorbed at medium and high pH values was not large even at 40 °C as shown in Fig. 4. At these pH values, there is an electrostatic repulsive force between the HGG molecule and the poly-NIPAM microsphere because the latter has an anionic initiator residue ($-SO_4^-$ group) at the chain ends and HGG has a negative charge at pH values above its isoelectric point ($\sim$ 6.5). Therefore, the electrostatic force seems to be the most dominant factor determining the adsorption in our systems with a low ionic strength. The hydrophilicity of the microsphere surface would be the second important factor having a remarkable influence on the adsorption in a system where an electrostatic attractive force exists between the protein molecules and the thermosensitive microspheres. It would be worth mentioning that the pH change and HGG adsorption did not cause any appreciable change in the size of microspheres.

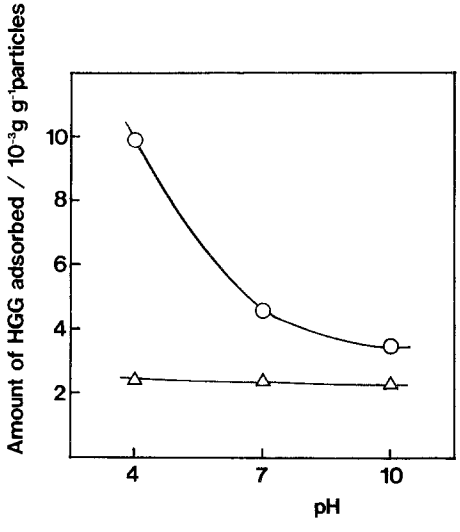

Fig. 4. Temperature and pH dependence of an equilibrium amount of HGG adsorbed on poly-NIPAM microspheres. ○: 40 °C, △: 25 °C

Desorption of proteins

The transition of the microsphere surface between the hydrophilic and the hydrophobic states should be reversible. This was confirmed by repetitive measurements of the hydrodynamic size of microspheres by changing the temperature alternately between 25 °C and 40 °C. Therefore, it is expected that the protein once adsorbed on the poly-NIPAM microspheres at 40 °C can be desorbed at 25 °C because of an increase in hydrophilicity of the microsphere surface. Desorption of HGG by lowering the temperature was examined and the result is shown in Fig. 5. When the incubation time for HGG adsorption on the poly-NIPAM microspheres at 40 °C was short, a large fraction of the adsorbing protein got desorbed by lowering the temperature to 25 °C. After a longer incubation time at 40 °C, the amount of protein desorbed decreased more drastically. Two possible causes can be pointed out for this irreversible desorption. One is the denaturation of protein in contact with poly-NIPAM at 40 °C. According to a preliminary investigation on the molecular conformation (amide I band by FTIR) of HGG, once adsorbed on microspheres and detached from them, there is little possibility for HGG molecules to undergo denaturation on adsorption and desorption, although more precise analysis is necessary. The other is partial anchoring or permeation of protein molecules into the polymer network. Cussler et al. [7] showed in their application for soy protein concentration that protein molecules hardly permeate the network of hydrogel beads. However, the possibility of permeation of HGG inside our microspheres is not necessarily excluded at present. In fact, the amount of desorption on cooling was low in the system of loosely crosslinked microspheres and this might be attributed to permeation of an appreciable amount of HGG in the microspheres.

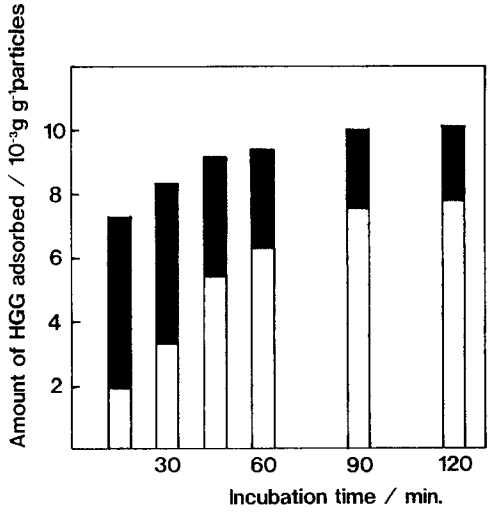

Fig. 5. Desorption of HGG, which was adsorbed at 40 °C, on lowering the temperature to 25 °C as a function of adsorption time at 40 °C. Total bar: amount of HGG adsorbed at 40 °C; solid part of bar: amount of HGG desorbed on lowering the temperature to 25°C

A large difference in the amount of adsorption of proteins between 25 °C and 40 °C suggests a possibility for some useful applications of the poly-NIPAM microspheres such as enzyme collector, protein scavenger, separator, etc. The temperature dependence would be observed also for the adsorption of some amphiphilic compounds other than proteins, in which polymer drugs and catalysts would be included.

Conclusions

Monodisperse poly-NIPAM microspheres of submicron size were prepared by precipitation polymerization. They had temperature-dependent adsorbability of proteins. Above the lower critical solution temperature of poly-NIPAM, the surface of microspheres becomes hydrophobic and able to adsorb a large amount of proteins. Decreasing the temperature of aqueous medium to lower than LCST resulted in the desorption of proteins from the microspheres. The extent of desorption at low temperatures was found to depend on the incubation time for the adsorption at high temperatures.

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