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Signal-responsive gating of nano-pores by self-assembled poly(acrylic acid)

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Poly (acrylic acid) was conjugated with cysteamine hydrochloride and the modified poly(acrylic acid) was self-assembled on a gold-coated porous polycarbonate membrane. Water permeation through the membrane was reversibly regulated by pH and ionic strength. The influence of chain length and the surface density of assembled polymer on water permeation was investigated. Permeation of ionic and non-ionic solutes such as deoxyribonucleotide (DNA) and polyethylene glycol (PEG) through the membrane, respectively, were also studied. The pore size was roughly estimated by permeation of these solutes.
Key words: Poly (acrylic acid), signal-responsive, self-assembly, porous membrane, intelligent material

1. INTRODUCTION

Intelligent materials have been the focus of increasing interest in various fields of biotechnology, medicine, robotics etc. Signal-responsive polymer gels are typical examples. They undergo reversible discontinuous volume changes in response to external stimuli such as changes in solvent composition, pH, temperature, electric field and light irradiation[1-5]. However, the mechanical fragility and slow kinetics of the swelling and deswelling processes have hindered their practical applications. To overcome these drawbacks, stimuli-responsive polymer-grafted nanoporous membranes have been designed by in situ graft polymerization from monomer[6,7]. Water permeation of the membrane in response to external stimuli and the permeation mechanism have been extensively studied. However, the effect of chain length and surface density of assembled polymers on the membrane permeation has not been clearly elucidated.

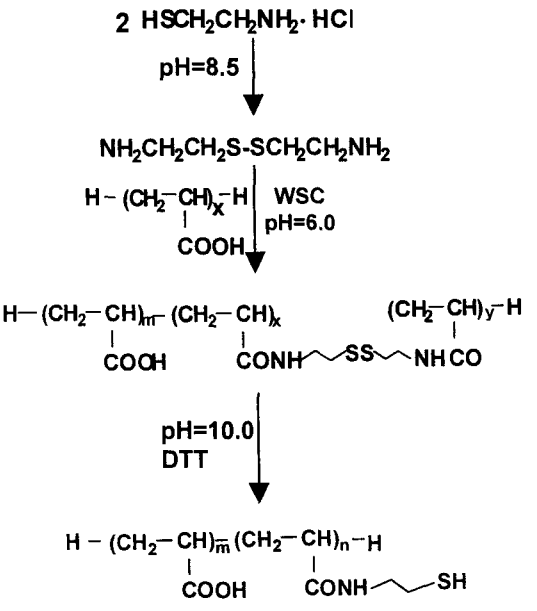
Therefore, We previously performed self-assembly of poly (glutamic acid) carrying thiol group on one end on a gold-coated porous membrane to develop highly intelligent signal-responsive materials[8]. By using the prepared polyelectrolyte, the length of graft polymer and the density could be easily adjusted.

In the present study, polyelectrolyte carrying multi-binding sites was prepared and its side-on type adsorption (self-assembly) was attempted. Comparing with the end-on type self-assembly[8] or graft-polymerization[6,7] which was previously performed, some differences in assembly behavior were found and the substance permeation was affected by the assembly state.

2. MATERIALS AND METHODS

2.1 Synthesis of modified PAA

Poly(acrylic acid) (PAA, Mw=30000, Aldrich) containing certain amount of thiol groups in the side chains was synthesized as shown in Scheme 1. The oxidation of cysteamine (Wako Pure Chemical Industry, Ltd., Osaka, Japan) was performed by incubation at pH 8.5 for 48 hours. The thiol content of the product was estimated from the absorbance at 412 nm of 3-carboxylato-4-nitro thiophenoylate (TNB) ions produced from the Ellman reaction of 5,5'-dithilbis (2-nitro-benzoic acid) (Nacalai Tesque, Kyoto, Japan) with the



Scheme 1. Preparation procedure of modified poly(acrylic acid)

thiol groups[9]. The oxidation continued until the conversion reached to 99.5%. The product was added to a solution containing 0.18 g of PAA in 20 ml Millipore-treated water, and the pH of the mixture adjusted to 6 with HCl. The mixture was kept at room temperature and continuously stirred. Subsequently, 240 mg of 1-ethyl-3-(3-dimethyl amino-propyl) carbodiimide was added in portions of about 24 mg at 10 min intervals. The reaction mixture was kept at pH 6 by adding HCl. After addition of the carbodiimide (water-soluble carbodiimide, WSC) the stirring was continued at 4℃ for 48h. The resulting PAA was hydrolyzed by adjusting the pH to 10 and then the disulfide dimers grafted onto the PAA were reduced to thiol group by 12 h treatment with (±)dithiothreitol (DTT, Wako Pure Chemical Industry, Ltd., Osaka, Japan). To remove excessive reactants, dialysis was conducted using a seamless cellulose tube (Amicon model, Millipore UFP1 LCC 24, cut off molecular weight of 5000). The dialyzed polymer was lyophilized and the obtained polymer was referred to PAA-SH. The content of cysteamine in PAA was determined by quantitative measurement of both amino and thiol groups, before and after DTT treatment respectively. The estimation of amino group is based on the reaction of amino group with 2,4,6-trinitrobenzene sulfonic acid (TNBS) and subsequent spectrophotometric determination of 2,4,6-trinitrophenyl (TNP) derivatives[10].

2.2 Self-Assembly

The synthesized PAA-SH was self-assembled on the surface of an Au-coated nanoporous membrane following the procedure shown in Figure 1. A porous polycarbonate (PC) membrane (Dupont nuclepore

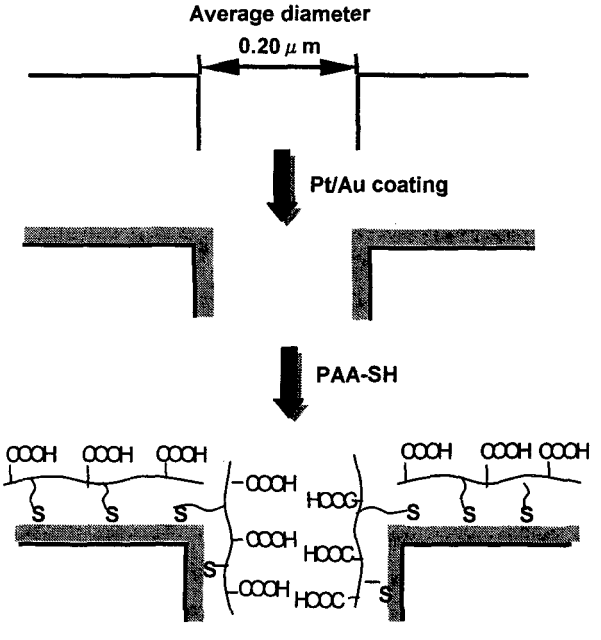


Fig. 1 Preparation scheme of signal-responsive gating pore by modified PAA on a nanoporous membrane

membrane; average pore diameter, 200nm) was coated with platinum and then with gold by an E-1010 ion sputter (Hitachi Co., Hitachi, Japan). The coated membrane was exposed to an aqueous solution of PAA-SH (pH=3.0~4.0) for 24 h. The surface-modified membrane was rinsed with Millipore-treated water until the pH of the washing liquid became neutral.

2.3 Surface Plasmon Resonance (SPR) Measurement

BIAcore SPR instrument was used to determine the amount of PAA-SH assembled on the cover glass (22 × 22mm, Matsunami Glass IND., LTD., Japan) onto which gold film (approximately 50 nm thick) was vapor deposited according to the same way as that for PC membrane. The fact that the PC membrane surface was fully covered with Au was confirmed by electron microscopy[8]. SPR was also utilized to estimate the concentration of polyethylene glycol (PEG) solutions. The calibration curves were obtained by measuring the SPR responses of known concentrations of PEG with different molecular weight.

2.4 Permeation Experiment

Solution permeation through the membranes assembled with the modified PAA was investigated using a centrifugal filter device (Microcon, YM-50, Millipore). The prepared membrane was mounted on a filter reservoir containing cellulose membrane, which attached to a vial for filtrate collection. The reservoir was filled with a certain amount of aqueous solution. The pH of the solution was adjusted using NaOH and HCl. The aqueous solution was allowed to flow under a constant centrifugal force for a prescribed time. The permeation rate was calculated by measuring the weight of solution permeating through the membrane.

3. RESULTS AND DISCUSSION

3.1 Chemical modification of PAA

The thiol content in PAA-SH increased with the increase of feed concentration, about 92~80% of feed cysteamine attached to the polymer.

3.2 Self-Assembly

The amount of the assembled PAA-SH was estimated from SPR spectrum. Different absorption behavior was observed (see Figure 2) for original PAA and PAA-SH. Significant binding of the PAA-SH to the Au surface occurred, whereas very little binding occurred for PAA.

The amount of the assembled PAA-SH was calculated from the change in the resonance signal before and after the gold surface was exposed to the PAA-SH solution (The monomer concentration of PAA was 1 M and carried 2 thiols per 100 carboxyls). It was reported that, in the case of protein adsorption on a polysaccharide-coated Au surface, 1000 resonance unit

of SPR response correspond to a surface concentration of about 1 ng/mm^2 independent of molecular size[11]. This calibration was also used for protein adsorption on bare Au surface[12]. Taking this into consideration, the amount of PAA-SH was roughly estimated to be 2.3×10^{-11} mol/cm^2.

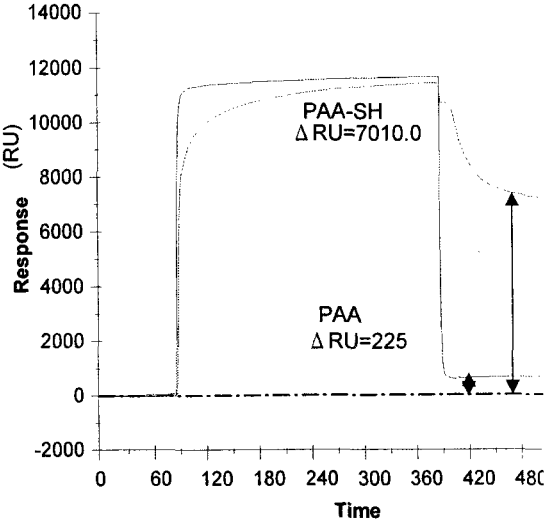


Fig. 2 SPR spectra showing the binding of PAA and PAA-SH to a gold-coated plate. Millipore treated water was used as both running and washing buffer.

In addition, the density of assembled PAA-SH was affected by the solution pH. Because at low pH PAA-SH has coiled structure and at high pH it has extended structure, the amount of adsorbed PAA-SH at low pH was higher than that at high pH.

3.3 Water permeation

The rate of water permeation through bare membrane was independent of pH, whereas permeation through the graft membrane depended upon pH. High permeation was observed at low pH and low permeation at neutral pH (Figure 3). It was considered that in the region of low pH, the PAA-SH chain was protonated and existed in tighten globular state to open the pores; while in the region of high pH, it was de-protonated to form an extended structure to cover the pores.

The water permeation through the membranes self-assembled with PAA-SH significantly depended on the chain length and density of graft chains. Assembly of PAA-SH containing higher content of thiol groups enhanced the water permeation and reduced the pH sensitivity. It was considered that the increase of thiol increased coupling points between the polymer and the Au surface, thus leading to lie-down state of assembled polymers. SPR spectra indicated that the amount of assembled PAA-SH with high thiol content was less than that with low thiol content, when the same concentration of PAA-SH was used.

The increase of surface density of assembled polymers reduced both the water permeation and the pH sensitivity. It was considered the increase of surface density reduced the polymer mobility on the surface.

3.4 Solute permeation

PEG and DNA with different molecular weights were used for permeation study. It was found that the permeation of low molecular weight PEG and DNA depended on pH, in the same way as H_2O. However, there was almost no difference in such solute permeation for different membranes and at different pH value when molecular weight exceeds 20000 for PEG (Figure 4) and 1kb for DNA (Figure 5). The molecular size was so large that it was difficult for them to permeate. It is known that PEG at low pH interacts with PAA. However, in the present study, the interaction occurred only at the surface so that no significant effect on the flow was observed.

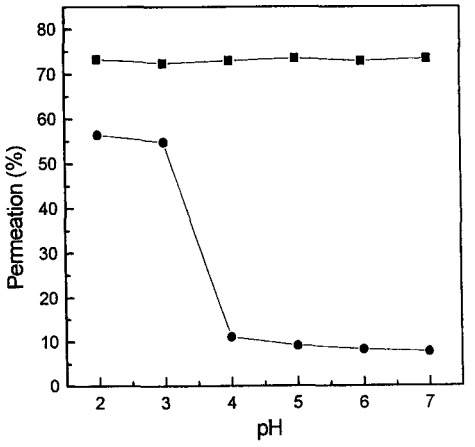


Fig. 3 pH dependence of water permeation for bare membrane (■) and membrane immobilized with PAA-SH (●). Thiol content was 2 thiols / 100 carboxyls and surface density was 1.8×10^{-11} mol/cm^2.

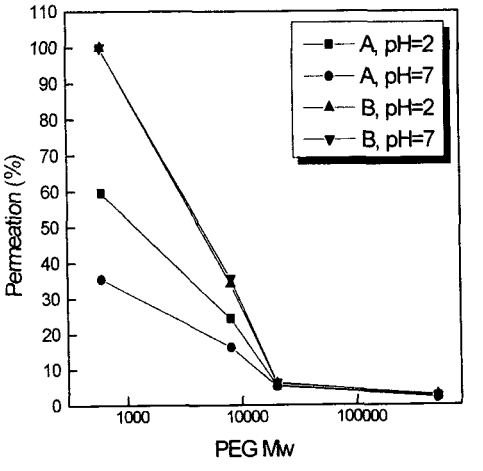


Fig. 4 PEG permeation through membranes assembled with PAA-SH containing 10 (A) and 30 (B) thiols per 100 carboxyls.

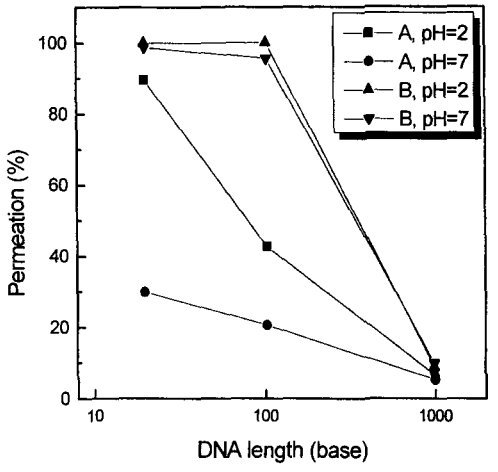


Fig. 5 DNA permeation through membranes assembled with PAA-SH containing 10 (A) and 30 (B) thiols per 100 carboxyls.

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