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Reversible extension and shrinkage of solvent-responsive dextran chains produced by enzymatic reaction

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ABSTRACT

Dextransucrase produces dextran and fructose from sucrose as a substrate, and dextran forms a complex with dextransucrase at the active site. Dextran chains were formed on the surface of an inorganic porous membrane, Shirasu Porous Glass membrane, via the enzymatic reaction. Dextran-containing membrane was prepared by permeating dextransucrase solution and then sucrose solution. Pure water was passed through the dextran-containing membrane to measure the pressure loss, from which the membrane porosity was determined using the Kozeny–Carman equation. Production of dextran chains in the porous membrane reduced the membrane porosity by more than 50%. When water–methanol solutions were permeated through the dextran-containing membrane, the porosity of membrane increased with proportion of methanol, indicating shrinkage of dextran immobilized on the surface of membrane. When water and 50% (v/v) methanol solutions were alternately passed though the membrane, high and low pressures were reproducibly determined by permeation of water and methanol solution, respectively. Dextran chains produced by the enzymatic reaction thus showed reversible extension and shrinkage in water and methanol–water solution.

1. Introduction

Stimulus-responsive polymers whose structure and morphology are changed by temperature, pH, solvent, and added chemical reagents have been extensively studied. There have been numerous reports of surface modification by grafting functional stimulus-responsive polymers. Poly-(acrylic acid) brushes were formed on polystyrene-coated glass, giving a reversible coil-stretch conformation change in response to pH, ionic strength, and contact time [1]. Poly-(2-(dimethylamino)-ethyl methacrylate) brush was shrunken and extended in response to temperature and pH [2,3], and there are many other reports on stimulus-responsive synthetic polymers. Although pH-responsive biopolymers have been reported [4,5], there have been few reports on biopolymers showing response to other stimuli.

Dextran, composed of α-(1,6) glycoside bonded glucose units, is a polysaccharide produced by *Leuconostoc mesenteroides* and *Streptococcus* species. Because dextran has the characteristics of (1) flexible structure due to the free rotation of glycoside bonds, (2) high solubility in water due to multiple hydroxyl groups, and (3) high usability for biotechnology due to its biocompatibility and biodegradability, dextran is used as a functional polysaccharide. For instance, dextran and dextran derivatives are widely used as blood-expander, biosensor, and packed bed for chromatography (Sephadex).

We have reported surface modification of an inorganic membrane using dextran produced by the enzymatic reaction [6–10]. Dextransucrase (DSase) produces dextran and fructose from sucrose as a substrate, and dextran forms a complex with the active site of DSase [11–13]. Using this characteristic, DSase was immobilized on the surface of a matrix, and reacted with sucrose to produce dextran from the immobilized DSase, which acts not only as a biocatalyst for the production of dextran but also as a binder between the dextran and the matrix. Thus dextran produced by DSase immobilized on the surface is a terminal-immobilized polymer, similar to a polymer prepared by the "grafting-from" method, i.e. surface-initiated radical polymerization [14]. In this enzymatic method, dextran chains can be densely connected on the surface of the matrix due to their formation perpendicular to the surface, compared with the "graft-to" method [14] in which functional polymer is directly adsorbed on or coupled with the surface of the matrix. It is easy to control the amount and length of the dextran chains produced, by varying the concentration of substrate and the reaction time [15,16]. Unlike chemical methods such as radical-graft polymerization, amination, and epoxidation, this enzymatic reaction has the advantage of modifying the surface in mild conditions, thus avoiding degradation of the support. The enzymatic reaction technique has been used for control of the porosity of microfiltration membranes [6,7], rejection of colloidal particles [8,9], affinity

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adsorption of lectin [10], and prevention of protein adhesion [17].

Separation ability of colloids in membrane chromatography is reduced by fouling of pores, and anti-fouling of the membrane is important proposition to operate for long term. Non-specific interaction between a protein or a colloid-matrix is prevented by hydrophilization of the surface, which contributes to protection from fouling in the field of membrane chromatography [18]. Hydrophilization has been achieved by immobilization of hydrophilic polymers such as ethyl hydroxyethyl cellulose [19], poly-(hydroxyethyl methacrylate) (PHEMA) [20], and poly-(vinyl alcohol) (PVA) [21]. Hydrophilization using immobilization of dextran is also effective for prevention of non-specific interactions. Another problem is difficulty for size-exclusion membrane to collect the colloids and proteins after rejection. Using stimulus responsive polymer-containing membrane, the rejected colloids and proteins are eluted by changing volume of the polymer.

Soluble dextran has a random coil conformation due to hydrogen bonding via multiple hydroxyl groups. The Stokes radii of dextrans with molecular masses 10 and 2000 kDa are 2.7 and 27.2 nm, respectively [22]. The conformation of dextran is changed by surrounding media. For example, aggregation and precipitation of dextran are induced in methanolic media. Terminal-immobilized dextran chains have an extended structure due to hydrogen bonding with a number of water molecules. In methanolic media, dextran is dehydrated by methanol, causing the conformation of dextran to be constricted. Such solvent-responsive phenomena have been confirmed using polyacrylamide and poly (acrylic acid) grafted to PVDF [23]. Terminal-immobilized dextran chains play a role as a reagent for solvent-responsive materials.

In the present study, dextran chains were formed on the pore surfaces of an inorganic membrane by the enzymatic reaction, to confirm extension and shrinkage of dextran chains resulting from permeation of methanol–water solutions. While direct observation of the dextran is difficult because dextran cannot be isolated from the porous membrane, the quantitative change of pressure loss in permeating solutions can elucidate the behavior of the dextran chain. DSase was immobilized on the pore surfaces of the membrane in permeation mode, and sucrose was reacted with the immobilized DSase to produce the dextran chains. To prevent non-specific interaction of protein on membrane, the adsorption ability of bovine serum albumin (BSA) on dextran-containing membrane was compared with that on SPG membrane. Aqueous methanol solutions with varying relative proportions of water and methanol were allowed to permeate through the membranes, and the resulting extension and shrinkage of the dextran chains determined by evaluating the porosity change of the membrane using the Kozeny–Carman equation [24]. Latex-beads-containing solutions were permeated through the dextran-containing membrane, and then were eluted by permeation of 50% methanol–water solution to collect colloids rejected by the membrane.

2. Experimental

2.1. Materials and reagents

Shirasu porous glass (SPG) membrane, a porous hollow membrane matrix consisting of silica and alumina, was purchased from SPG Technology Co., Ltd. The SPG membrane (Lot No. PEN08B05 and PEN08A30) had inner and outer diameters of 4 and 5 mm, respectively, with effective length of 1.5 cm. The average pore diameter, porosity, and specific surface area were 1800–1900 nm, 49%, and 1.1 m^2/g, respectively. DSase from *Leuconostoc mesenteroides* (EC: 2.4.1.5, specific activity 185 U/mg, Lot No. 018K4014), albumin from bovine serum (Lot No. 075K7572), and latex beads (polystyrene, mean particle size 600 nm, Lot No. 076K1103) were purchased from Sigma Chemical Co. Sucrose was purchased from Wako Chemical Co. A syringe pump (S-1235, Atom Medical International, Inc) was used to pass DSase and sucrose solution through the SPG membrane.

2.2. Preparation of dextran-containing membrane

The immobilization of DSase on SPG membrane and the production of the dextran by reaction of DSase with sucrose were described in detail in a previous paper [6]. Briefly, DSase solution (0.2 U/mL) in 0.01 M acetate buffer at 5.5 was permeated through the SPG membrane for 30 min at 30 mL/h to immobilize the enzyme on the membrane pores. An activity of 1 U was defined as that required to produce 1 μmol of fructose in 1 min. The effluent was collected and the activity of DSase in the effluent was determined from the fructose concentration using the Somogyi–Nelson method [25,26]. The amount of DSase immobilized was calculated from the following equation.

$$\text{Amount of DSase immobilized on SPG membrane } (\text{U/g}) = \frac{C_0 - C}{W}\nu \quad (1)$$

where C_0 and C are the activities of DSase in feed solution and in effluent solution, respectively, ν is the volume of effluent solution, and W is the weight of the membrane.

Sucrose solution was permeated for 30 min at 30 mL/h to produce dextran chains from the immobilized DSase. The effluent was collected continuously and the concentration of fructose in the effluent was determined by the Somogyi–Nelson method. The density of dextran on the surface (Γ) was calculated from the following equation.

$$\Gamma\ (\text{mg/m}^2) = \int_0^{\nu} \frac{C_F}{WS} d\nu \quad (2)$$

where C_F and S are the concentration of fructose and specific surface area, respectively.

2.3. Estimation of porosity of dextran-containing membrane

To investigate the porosity of dextran-containing membrane, the pressure loss was determined during permeation of sucrose solution through the membrane. The membrane porosity was calculated from the flow rate and pressure loss by the Kozeny–Carman equation.

$$\frac{\Delta P}{V} = \frac{kS^2(1-\varepsilon)^2}{\varepsilon^3}\mu L \quad (3)$$

where ΔP, V, k, S, ε, μ, and L are pressure loss, permeation rate, Kozeny constant, specific surface area, porosity, viscosity of feed solution, and membrane thickness, respectively. Assuming that k, S, μ, and L before and after generation of dextran are constant, the ratio of the porosities of SPG membrane and the dextran-containing membrane is given by

$$\frac{\Delta P_1}{V_1} = \frac{(1-\varepsilon_1)^2\varepsilon_0^3}{(1-\varepsilon_0)^2{\varepsilon_1}^3}\frac{\Delta P_0}{V_0} \quad (4)$$

where subscripts 0 and 1 designate unmodified SPG membrane and the dextran-containing membrane, respectively.

2.4. Permeation of solutions with varying methanol content through dextran-containing membrane

Methanol–water solutions were permeated through SPG membrane and the dextran-containing membrane, and the pressure loss was determined to calculate the extension and shrinkage of the