Porosity Estimation of a Membrane Filled with Dextran Produced by Immobilized Dextransucrase

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Introduction

Dextransucrase (DSase) is an enzyme originating from *Leuconostoc mesenteroides*, a kind of *Streptococcus*. DSase produces dextran, a polymer of pyranose rings linked by α-(1,6) bonds and fructose from the substrate sucrose.[1] DSase which has been immobilized on a support will still react with sucrose resulting in a gradual decrease in the enzyme activity over time, indicating that the dextran product forms a complex with the active site of DSase. This in turn, results in an increase in the diffusional mass-transfer resistance of sucrose to the immobilized enzyme.[2,3] Dextran has a unique hydrophilic character because its hydroxyl groups located outside of the main polymer are soluble in water. Thus, when dextran is attached to the surface of a substance used for biomacromolecular separation, it inhibits the adhesion of biomacromolecules.

To date, we have performed dextran formation in porous membranes by utilizing the complex formation of DSase and dextran at the enzyme active site. To immobilize the DSase onto the surface of polymeric porous hollow-fiber membranes, polymer chains bearing suitable anion-exchange groups were appended to the pore surface by radiation-induced graft polymerization of glycidyl methacrylate, followed by subsequent conversion of the epoxy groups to amines. Permeation of a sucrose solution through a DSase-immobilized membrane generated a layer of dextran.[4] Next, to fill the membrane pore with dextran generated from DSase, the inorganic membrane, Shirasu Porous Glass membrane (SPG membrane), was used as a trunk membrane. The DSase solution was then permeated through SPG membrane to directly immobilize the DSase onto the pores. Subsequently, the sucrose solution was permeated again through the DSase-immobilized SPG membrane to form dextran. The pressure loss which occurred during the permeation of pure water through the dextran-produced membrane varied with the amount of dextran produced, which in turn, depended on the DSase.[5]

In this study, DSase was immobilizated onto SPG membranes in which the pore diameter was varied over the range of 500–2000 nm, and the sucrose reaction processes were investigated. The porosity change by generated dextran in the membrane was understood quantitatively using different-pore-diameter membranes. During the immobilization of DSase to the membrane in permeation mode, the enzyme immobilizing density is dependent on the space velocity of the DSase solution through the membrane, i.e., the residence time of DSase in the membrane. In addition, the dextran formation rate is dependent on the residence time of sucrose in the membrane. Thus, each diffusion process during the DSase immobilization and the sucrose reaction should be considered for controlling the membrane porosity for use in a separation process. The reaction process was summarized using the Peclet number, a dimensionless value that considers both the diffusion process of sucrose to immobilized DSase and the residence time of sucrose. The relationship between the pressure loss and the porosity of the membrane is discussed using the Kozeny-Carman equation to calculate the membrane porosity change.

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Table 1. Properties of SPG Membranes

	Pore Diameter (nm)	Number of Pore $\times 10^{12}$ (m^{-2})	Porosity ε	Pressure Loss* (kPa)
SPG_{500}	520	4.8	0.36	18.8
SPG_{800}	760	2.9	0.46	10.7
SPG_{1000}	950	1.4	0.35	7.5
SPG_{2000}	1780	0.56	0.49	3.4

*Pressure loss was determined at 1 m/h of flux at 298 K.

Experimental Section

Materials

Hydrophilized Shirasu Porous hollow-fiber Glass Membrane (SPG Technology Co., Ltd.) was used as the trunk inorganic material. This membrane had inner and outer diameters of 4 and 5 mm, respectively, with average pore diameters ranging from 500 to 2000 nm. SPG membranes with different pores are referred to as SPG_x membranes where x designates the pore diameter. Dextransucrase (EC 2.4.1.5) was purchased from Sigma (10 U/mg, D-9909 Lot No. 128H4026). Other reagents were of analytical grade or higher.

Determination of the number of pores of SPG membranes

The pore diameter and pore volume of SPG membranes were determined by BET methods using N_2 as a probe gas. The data were provided by SPG Technology Co., Ltd. The pore number was calculated as follows, with the assumption that the pore of SPG membranes is cylindrical:

$$r^2\pi Ln = \text{pore volume over SPG membranes} \quad (1)$$

where r, L, and n are the pore radius, membrane thickness, and the pore number of SPG membranes, respectively.

Immobilization of DSase and production of dextran in the membrane

The DSase solution was permeated through the SPG membrane pores at a rate of 20 mL/h at ambient temperature. The effluent penetrating the outside surface of the SPG membrane was continuously collected, and the activity of DSase in the effluent was determined to calculate the amount of DSase immobilized. The sucrose solution was permeated radially outward from the inner surface of the DSase-immobilized SPG membrane. The effluent was continuously collected and the fructose concentration produced by DSase was determined to estimate the amount of dextran produced. Water permeability, flux, and space velocity of solution are defined as follows:

$$\text{Flux}\,[\text{m/h}] = \text{permeation rate/inner area of the membrane} \quad (2)$$

$$\text{Space velocity}\,[\text{h}^{-1}] = \text{permeation rate of solution/membrane volume} \quad (3)$$

The detailed experimental methods for DSase immobilization and dextran production has been described in a previous article.[5]

Results and Discussion

Properties of SPG membranes

The properties of the SPG membranes with different pore sizes, ranging from 500 to 2000 nm, are summarized in Table 1. The number of pores decreased with an increase in pore diameter. However, the porosity of the SPG membranes was not constant because the structure of the membrane was not maintained if the porosity was increased. The pressure loss gradually decreased with an increasing pore diameter.

DSase immobilization to SPG membranes

The DSase solution was permeated through SPG membranes with various pore sizes, 500 to 2000 nm, as shown in Figure 1. The space velocity of the DSase solution was set to 190 h^{-1}. The amount of DSase immobilized to the SPG membranes was constant irrespective of the pore diameter of the membranes. By permeating the DSase solution, DSase was transported to the pore surface via convection flow, to rapidly immobilize the silanol group on the surface. DSase interacted with silanol group at the surface of the membrane via hydrophilic interaction. Immobilized DSase was not leaked by permeating buffer solution and pure water, indicating that DSase was strongly immobilized to the membrane.

The pore numbers of various SPG membranes are given in Table 1. The DSase was immobilized to the various pores of the membrane. If a unit of DSase is directly converted to a milligram of DSase, a 16 U DSase/g SPG membrane as shown in Figure 1 is the equivalent of a 1.6 mg DSase/g SPG membrane. Because the molecular weight of DSase is 184 kDa,[6] 8.69×10^{-9} mol DSase/g SPG membrane was immobilized to give a calculated value of 5.23×10^{15} molecules of DSase/g SPG membrane. For SPG_{500}, for instance, the number of DSase molecules immobilized per one cylindrical pore was calculated to be ~10.

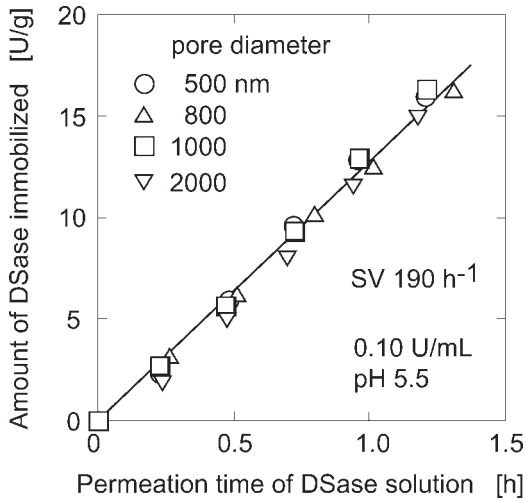

Figure 1. Amount of DSase immobilized to SPG membranes.

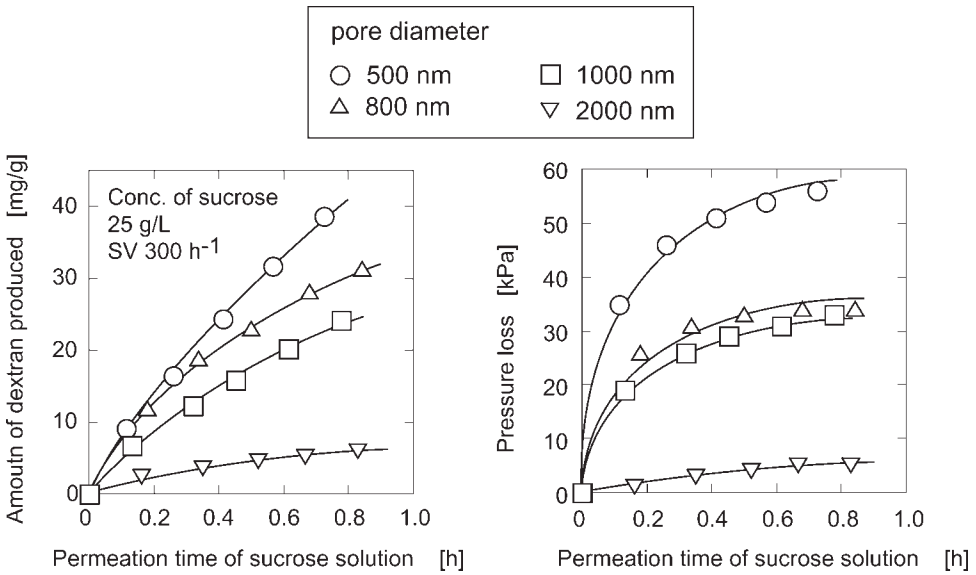

Figure 2. Permeation of sucrose solution through DSase-immobilized membranes to produce dextran: (a) amount of dextran produced and (b) pressure loss during the permeation.

Dextran formation to fill the pores of SPG membranes

Sucrose solution was permeated through DSase-immobilized membranes with different pores as shown in Figure 2. The space velocity of sucrose solution was set to 300 h^{-1}. With an increase in the permeation time of the sucrose, the amount of dextran produced was gradually increased. Together with the dextran production from DSase immobilized on the surface, the pressure loss of the membrane was also increased, indicating that the dextran which was produced filled the pores of the SPG membrane.

During substrate permeation through the membrane, the reaction process is classified into five steps: (1) substrate flows to the vicinity of the immobilized DSase via convection flow, (2) substrate diffuses to the DSase via a concentration gradient, (3) substrate reacts with DSase, (4) dextran is produced and fructose diffuses out again via a concentration gradient, and (5) fructose flows to the outside of the membrane. Steps 2 and 3 are the rate-determining steps for dextran formation because the diffusion coefficient of sucrose is larger than that of fructose and Steps 1 and 5 are negligible because of convection flow. The extent of DSase immobilization was the same for all membranes regardless of the pore size because approximately the same number of silanol groups existed on the surface of the pores. Thus a constant number of silanol groups generated a constant number of bound DSase molecules resulting in a constant intrinsic enzymatic reaction rate. Therefore, the difference in the reaction rate was due to the diffusion of sucrose to the immobilized enzyme, not the number of bound enzyme.

To analyze the diffusion step of sucrose to the immobilized DSase, the Peclet number (*Pe*), a dimensionless value was used and can be defined as follows[7]:

$$Pe[-] = [(\text{pore radius})^2/D]/(\text{residence time}) \quad (4)$$

where D is the diffusion coefficient of sucrose in water, 5.0×10^{-10}, and the pore radius is the diffusion length of sucrose flowing via convection.

The reaction rate coefficient of the DSase-immobilized membrane is calculated by a pseudo-first order equation. The relationship between the reaction rate coefficient and *Pe* is shown in Figure 3. With an increasing *Pe* number, that is, an increasing pore radius, the reaction rate coefficient decreased, indicating that the overall enzyme reaction is dependent on the pore radius.

Porosity change of the membrane determined by Kozeny-Carman equation

The membrane pore was filled with dextran produced by the immobilized DSase, resulting from the pressure loss change during permeation of the sucrose solution through the DSase-immobilized membrane. To evaluate the pore filling

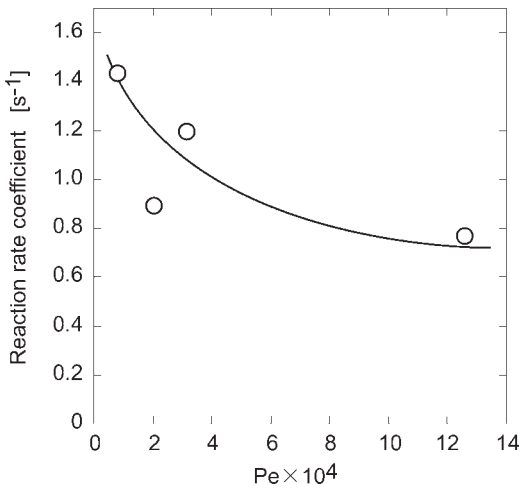

Figure 3. Reaction rate coefficient vs. Peclet number.

Table 2. Porosity Change of Dextran-Produced Membrane

	SPG_{500}	SPG_{1000}
Amount of DSase immobilized (U/g)	16.0	16.3
Amount of dextran produced (mg/g)	38.7	24.3
Pressure loss at a flux of 1 m/h (kPa)	301	188
Porosity	0.17	0.14

of the membrane by dextran, the porosity change of the membrane was calculated using the Kozeny-Carman equation.

The porosity of the SPG membrane and the membrane containing dextran was calculated using the following Kozeny-Carman equation[8]:

$$P_0 = [kS_0^2(1-\varepsilon_0)^2/\varepsilon_0^3]/(\mu L V_R) \quad (5)$$

$$P_D = [kS_D^2(1-\varepsilon_D)^2/\varepsilon_D^3]/(\mu L V_R) \quad (6)$$

where P, k, ε, μ, L, and V_R are the pressure loss, Kozeny constant, membrane porosity, viscosity, membrane thickness, and flow rate, respectively. The subscripts 0 and D designate the SPG membrane and the membrane containing dextran, respectively. Here, S_0 did not differ from S_D, because the specific surface areas were the same. By combining Eqs. 5 and 6, the permeation pressure loss ratio can be expressed by:

$$P_0/P_D = [(1-\varepsilon_0)^2\varepsilon_D^3]/[(1-\varepsilon_D)^2\varepsilon_0^3] \quad (7)$$

The pressure losses of SPG_{500} and SPG_{1000} in permeating pure water at 1 mL/h are summarized in Table 2 together with the data for the membranes containing dextran. Compared with the initial porosities as shown in Table 1, the membrane porosity is lowered by less than 50%. The DSase immobilization density on the pore surface and the amount of dextran produced are key factors in controlling the porosity of the membrane for use for specific molecules or colloidal particle separation.

Conclusions

Dextran was formed by the reaction of dextransucrase with sucrose. The enzyme was immobilized onto the inorganic Shirasu porous glass membrane with different pore diameters, and subsequently reacted with sucrose to produce dextran. The extent of DSase immobilization was constant irrespective of the membrane pore diameters in the permeation mode. In the dextran-production process, the diffusion of sucrose to the immobilized enzyme was the rate-determining step. The dextran production rate was summarized using the Peclet number, demonstrating that with an increase in Peclet number, the rate gradually decreased. Using the Kozeny-Carman equation, the estimated porosities of the pore diameters of 500 and 1000 nm were changed from 0.36 and 0.35 to 0.17 and 0.14, respectively. Controlling the dextran properties by the dextransucrase density on the pore surface and the sucrose reaction conditions with immobilized dextransucrase provides a specific porosity of the membrane.

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