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Giant dextran particles formed by dextransucrase immobilized on a tube surface in a laminar flow

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ABSTRACT

Complexation of dextran with the active sites of dextransucrase was used to produce dextran on a tube in a laminar flow. A dextransucrase solution was passed through a Teflon tube at flow rates of 10 and 50 mL/h to immobilize the enzyme on the inner surface of the tube, and then sucrose solution was passed through the enzyme-immobilized tube to form giant dextran particles. The Reynolds number during the procedure was 4 or 20. The distribution of dextran along the flow direction in the tube was determined colorimetrically and showed that the dextran formed was distributed such that the amounts of dextran were higher near the ends than in the middle section of the tube. Direct observation of the dextran after colorization with phloxine showed that giant dextran particles, up to approximately 20 μm in size, were formed. The distribution of dextran produced by the enzymatic reaction in a laminar flow was determined quantitatively.

1. Introduction

Dextran is a polysaccharide that has α-(1,6) glycoside linkages between glucose units. Because of its hydrophilicity and flexibility, dextran has been used for the separation of biological materials on dextran-coated surfaces [1,2]. When dextran is directly attached to a surface, because it has only hydroxy functional groups, it may detach from the surface during use. To prevent detachment, dextran has been chemically modified to enhance attachment to surfaces. For example, a silicate group was introduced chemically into dextran, and the modified dextran was attached to an amino-group-modified surface [3]. However, during the chemical reaction, the molecular weight of dextran decreased and aldehyde groups were introduced into the polymer [4], resulting in degradation of the properties of the polysaccharide.

Dextransucrase (DSase) catalyzes the reaction of sucrose to form dextran and fructose, and the dextran that is produced forms a complex with the active sites of DSase [5]. The mechanism for the synthesis of the sequence of α-(1,6)-linked glucose residues in dextran involves two nucleophiles at the active site; these attack sucrose and displace fructose to give two β-glucosyl intermediates. During the reaction, the active site interacts weakly with α-(1,6)-linked glucose. This enzymatic characteristic is useful for modification of the surfaces of materials. DSase is immobilized on the surface, and then the sucrose reaction is catalyzed with the immobilized DSase, forming dextran on the surface. Dextran is a very large polysaccharide; in solution, the molecular weight of dextran can be more than 200 kDa [6]. Okahata and co-workers evaluated the transferase reaction of DSase occurring on the surface of a quartz-crystal microbalance at the molecular level [7]. Kawakita and co-workers modified membrane surfaces with dextran produced by the DSase reaction [8–10]. Dextran was attached to the membrane surface through DSase. This method of dextran production through DSase is a powerful technique; it does not change the structure of dextran during the modification, and thus provides direct access to the intrinsic properties of dextran.

The flow mode was used for the modification of porous membranes, using the DSase reaction. The membrane was coupled to a flow system and DSase and sucrose solutions were flowed through the membrane, immobilizing the DSase and producing dextran. The enzyme and sucrose were transported via convection and diffusion to the membrane surface, resulting in enzyme immobilization and dextran production at the surface. Assuming that the pores of the membrane can be represented as a bundle of tubes with circular cross-sections, the Reynolds numbers for the enzyme and sucrose solutions are low in a laminar flow regime.

There have been many investigations of fluid flow in reaction and separation systems. Recently, micro-electro-mechanical systems (MEMS) prepared by a lithographic technique have provided smart flow for separation of cells [11], immobilized microfluidic enzyme reactors [12], and syntheses [13]. Changes in the flow affect separation and production, and also change the overall residence time in the system, as well as changing local concentration distributions. When dextran is formed on a surface by an enzymatic

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reaction in MEMS, the dextran distribution on the surface should be considered.

In the present study, the distribution of dextran produced by a DSase reaction with sucrose in a laminar flow on the inner surface of a tube was determined (Fig. 1). A DSase solution was passed through a Teflon tube to immobilize the enzyme via hydrophobic interactions, and a sucrose solution was passed through the DSase-immobilized Teflon tube in a laminar flow. Verification of polymer-surface modification with dextran using DSase reactions will achieve the next breakthrough in the modification of such reactions for application to MEMS. To vary the distribution of the immobilized enzyme, the flow rate of the enzyme solution through the tube was set at 10 or 50 mL/h. The enzymatic reaction on the tube forms giant particles of dextran. The distribution of dextran formed in the flow direction was determined using a colorimetric method. Giant dextran particles with dimensions greater than 1 μm were observed by optical microscopy, which was used to determine the number and size distributions of dextran particles on the inner surface of the tube. Near the tube ends, the boundary layer of the fluid is fused, giving non-uniform production of dextran in the direction of flow within the tube.

In previous technologies, directly modifying the surface of a polymeric material using a polysaccharide was difficult. However, the immobilization of DSase and the generation of dextran from DSase enable modification of the material surface. This study included the modification of the inner surface of a Teflon tube under laminar flow with a polysaccharide produced using a DSase reaction, and determining the distribution of the generated dextran along the flow direction. MEMS flow systems are widely used in the medical, analytical, and chemical engineering fields. The purpose of this study is not to describe direct applications of dextran-modified surfaces, but to increase the scientific understanding of dextran formation in a laminar flow, based on chemical engineering.

2. Materials and methods

2.1. Materials

A Teflon tube (F-8011-01C) was obtained from Flon Industry, Tokyo, Japan. The inner diameter of the tube was 1 mm, and the length was 1.0 m. DSase from *Leuconostoc mesenteroides* (EC: 2.4.1.5, specific activity 185 U/mg, Lot no. 018K4014) was purchased from the Sigma Chemical Co. (St Louis, MO, USA). Sucrose was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The dextran (200 kDa) used for blank experiments was purchased from the Sigma Chemical Co. A syringe pump (S-1235, Atom Medical International Inc., Tokyo, Japan) was used to pass DSase and sucrose solutions through the Teflon tube.

2.2. Enzyme immobilization and production of dextran on the tube surface

A DSase solution (20 mL; 0.2 U/mL) in 0.01 M acetate buffer at pH 5.5 was passed through the Teflon tube at flow rates of 10 and 50 mL/h at ambient temperature to immobilize the enzyme on the inner surface of the tube. An enzyme activity of 1 U was defined as that required to produce 1 μmol of fructose in 1 min. The effluent was collected and the DSase activity of the effluent was determined from the fructose concentration using the Somogyi–Nelson method [14,15]. The amount of DSase immobilized was calculated from the equation:

$$\text{amount of DSase immobilized (U/g)} = \int_0^v \frac{(C_0 - C)}{W} dv \quad (1)$$

where C_0 and C are the activities of DSase in the feed solution and effluent, respectively, v is the volume of effluent solution, and W is the weight of the tube. The DSase-immobilized tubes prepared at enzyme solution flow rates of 10 and 50 mL/h are referred to hereafter as DSase(10) and DSase(50) tubes, respectively.

Sucrose solutions (20 mL) with flow rates of 10 and 50 mL/h were used to produce dextran from the immobilized DSase. The effluent was collected continuously and the fructose concentration in the effluent was determined using the Somogyi–Nelson method [14,15]. The dextran density on the surface was calculated using the equation:

$$\text{amount of dextran produced (mg/cm}^2) = \int_0^v \frac{C_F}{WS} dv \quad (2)$$

where C_F and S are the concentration of fructose and the surface area of the tube, respectively. The error bars obtained with respect to the amount of immobilized DSase and the amount of dextran were based on the R^2 value of the calibration curve for the Somogyi–Nelson method.

The Reynolds number (Re) is defined as follows:

$$\text{Re} = \frac{\rho u D}{\mu} \quad (3)$$

where μ, ρ, u, and D are the solution viscosity, solution density, solution flow velocity, and tube diameter, respectively.

2.3. Quantitative observation of dextran produced on the tube

The tube with dextran on the surface was cut into 10 cm segments. Each tube segment was immersed in concentrated sulfuric acid for 24 h to dissolve the dextran, and the dissolved dextran was quantitatively determined by the sulfuric acid–phenol method [16]. The error bars for the amount of dextran on the inner surface of tube were based on the R^2 value of the calibration curve for the sulfuric acid–phenol method.

A phloxine solution (1.0 g/L) with a flow rate of 10 mL/h was passed through each dextran-coated-tube segment to colorize the dextran. After washing the tube segments with water in permeation mode, the segments were dried in a vacuum. The surface of each tube segment was observed using an optical microscope (VH-S5, Keyence, Osaka, Japan) with a VH-Z500W lens (Keyence). The sizes of giant dextran particles larger than 1.0 μm on the inner surfaces of the tubes were determined quantitatively. The particle sizes were determined from images of the observed dextran particles using the KEYENCE_VH-M_174 software. The number of dextran samples observed was about 100. Observations were performed for every tube segment to determine the distribution of dextran along the flow direction of the tube. Error bars were added based on the numbers and size distributions between the highest and lowest values observed in the samples.

3. Results and discussion

3.1. Dextran production on tube surface in a laminar flow

Because DSase forms a complex with dextran at the active sites during the enzymatic reaction, dextran was easily attached to the inner surface of the tube. A DSase solution was flowed through the Teflon tube to immobilize DSase on the surface, and then a sucrose solution flow was used to produce dextran from the immobilized DSase. The immobilization of DSase took place via transport of DSase to the vicinity of the surface, diffusion of DSase to the surface, and immobilization of DSase on the surface. The surface of the Teflon tube is hydrophobic, and the hydrophobic group in DSase is considered to have interacted with the tube material via hydrophobic interactions. During the steps outlined above, a change in the