Carbohydrate Polymers 74 (2008) 627–631

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Dextran formation on hydroxyapatite by immobilized dextransucrase to control protein adsorption

Hidetaka Kawakita *, Akihito Gyotoku, Hirokazu Seto, Keisuke Ohto, Hiroyuki Harada, Katsutoshi Inoue

*Department of Applied Chemistry, Faculty of Science and Engineering, Saga University, 1-Honjo, Saga 840-8502, Japan*

ARTICLE INFO

*Article history:*
Received 24 January 2008
Received in revised form 7 April 2008
Accepted 10 April 2008
Available online 16 April 2008

*Keywords:*
Dextran
Dextransucrase
Hydroxyapatite
Protein
Adsorption
Enzyme immobilization
Size-exclusion effect

ABSTRACT

Dextran was formed on the surface of hydroxyapatite by the enzymatic reaction of dextransucrase to control protein adsorption. The hydroxyapatite substrate was immersed in a dextransucrase solution (0.3 U/mL, pH 5.5) to immobilize dextransucrase to the hydroxyapatite surface at 6.0 U/mL. The dextransucrase-immobilized hydroxyapatite was then reacted with a sucrose substrate to generate dextran from the active sites of the immobilized dextransucrase. The amount of dextran produced changed with reaction time and substrate concentration, generating dextran at a maximum of 50 mg/g. When the proteins, bovine serum albumin and γ-globulin, were adsorbed to the surfaces of both hydroxyapatite and dextran-generated hydroxyapatite, the amount of protein adsorbed decreased with increasing amount of dextran produced, indicating that the protein did not reach the adsorption sites of hydroxyapatite as a result of steric exclusion by dextran. The protein adsorption performance revealed that the generated dextran on HAP can size-exclude proteins.

© 2008 Elsevier Ltd. All rights reserved.

## 1. Introduction

Dextran is a polysaccharide with an α-(1,6) pyranose ring linkage, and has the following unique characteristics; (1) flexibility of the random structure, (2) high solubility due to the hydroxyl group, and (3) high stability. When using dextran for molecular immobilization, its flexible structure is useful in maintaining the structure and activity of the molecule during immobilization. The hydrophilicity of the dextran attached to the surface inhibits unexpected molecular adhesion. Dextran has been used as a blood volume expander (Terg et al., 1996) and as a gel for gel permeation chromatography (Porsch & Sundelof, 1994).

Dextransucrase (DSase) produces dextran and fructose using sucrose as a substrate. The product dextran forms a complex with DSase at the active site (Mooser & Iwaoka, 1989; Robyt, Kimble, & Walseth, 1974; Tanriseven & Robyt, 1992). The mechanism for synthesis of a sequence of α-(1,6)-linked glucose residues in dextran involves two nucleophiles at the active site which attack sucrose and displace fructose to give two β-glucosyl intermediates (Monchois, Willemot, & Monsan, 1999; Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). Progression of the enzymatic reaction allows dextran to increase its molecular mass; the overall activity of the immobilized dextransucrase gradually decreases, because dextran with an increasing molecular mass similar to that of DSase restricts the diffusion of sucrose (Kaboli & Reilly, 1980; Monsan, Paul, Auriol, & Lopez, 1987). We previously prepared dextran on a pore surface by using immobilized DSase to fill the membrane pore, where the dextran changed the membrane porosity (Seto, Kawakita, Ohto, Harada, & Inoue, 2007). Using the Kozeny–Carman equation based on pressure loss and the permeation rate of pure water, the membrane porosity was dramatically and continuously altered by changing the amount of dextran produced.

Polymer adsorption at solid–liquid interfaces is a widely studied phenomenon connected with important processes including colloidal stabilization, flocculation, adhesion, and coating (Bolivar et al., 2006; Hoorfar, Kurz, Policova, Hair, & Neumann, 2006; Xu, Persson, Lofas, & Knoll, 2006). The reduction of protein adsorption by coating the material with the polymer is of significant importance for many biological and biotechnological applications. Especially in preparing the biomaterials, the adsorption regulation was necessary because the binding protein inhibits the unique characteristics of the material. The protein combines with the material surface via ionic-exchange interaction through the carboxylic and amino groups, or through the hydrophobic interaction via the aromatic groups in the amino acid residues. The regulation of protein adsorption to the substrate surface should be founded based on the protein's size resulting from the three dimensional structure.

There are many surface modification methods that rely on dextran's own characteristics (Chern, Lee, & Tsai, 1999; Fournier, Leonard, Le Coq-Leonard, & Dellacherie, 1995; Miksa, Irish, Chen, Composto, & Eckmann, 2006). Mainly, dextran is first modified

* Corresponding author. Tel.: +81 952 28 8670; fax: +81 952 28 8670.
*E-mail address:* kawakita@cc.saga-u.ac.jp (H. Kawakita).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.04.013

with certain functional groups and then used to surface modify a material substrate via hydrophobic and ionic interactions. It is difficult to regulate the dextran on the surface at high density because of the excluded volume of the functional dextran. It is possible that the functional dextran immobilized on the surface captures molecules through the functional groups. The proposal of the novel modification method using dextran will provide us with a sophisticated biomaterial.

In this study, hydroxyapatite (HAP) was used as a test material for surface modification. HAP has been widely studied as a biomaterial because it has a high biocompatibility, which ensures its wide use as a medium for cell-immobilization and growth (Kandori, Tsuyama, Tanaka, & Ishikawa, 2007; Matsumoto et al., 2004; Nancollas et al., 2006; Woo, Seo, Zhang, & Ma, 2007). DSase was immobilized on the surface of HAP, and then sucrose was reacted with the immobilized dextransucrase to polymerize the dextran from the active sites of DSase. By changing the amount of dextran produced, it was possible to determine the adsorption behavior of different proteins for further verification of the protein adsorption control afforded by the dextran generated from the immobilized dextransucrase (see Fig. 1).

## 2. Materials and methods

### 2.1. Materials

Hydroxyapatite (HAP) was obtained from Sigma–Aldrich Chemical Ltd. The diameter of HAP was sieved to give molecules below around 150 μm. Dextransucrase (DSase) from *Leuconostoc mesenteroides* (D-9909 Lot No. 128H4026, 10 U-DSase/mg-DSase) was purchased from Sigma Chemical Co. and used without further purification. Bovine serum albumin (BSA, $M_w$ 67 kDa) and γ-globulin from human serum ($M_w$ 170 kDa) were obtained from Wako Chemical Ltd. Other reagents were of analytical grade or higher.

### 2.2. Preparation of dextran from immobilized dextransucrase on HAP

HAP was immersed in 10 mM acetate buffer (pH 5.5) for 1 h to equilibrate the surface of the HAP. 50 mg of HAP was mixed with 1 U/mL of DSase solution (1 mL) dissolved in 10 mM acetate buffer (pH 5.5) for a prescribed time to immobilized DSase on HAP. After immobilizing DSase, DSase-immobilized HAP was washed with acetate buffer. An activity of 1 U was defined as the amount of enzyme required to produce 1 μmol of fructose in 1 min. The amount of DSase immobilized was evaluated from the activity of DSase.

The as-prepared DSase-immobilized HAP was reacted with a sucrose solution (10 mM acetate buffer, pH 5.5, 20 mL) for 24 h at 303 K to produce dextran from the active sites of the immobilized DSase. The concentration of fructose, a by-product of the enzymatic reaction, was determined by evaluating the amount of dextran produced using the Somogyi–Nelson method (Nelson, 1944; Somogyi, 1952). The surface of the modified HAP was observed by scanning electron microscopy (KEYENCE VE-9800).

### 2.3. Protein adsorption to the dextran-containing HAP

BSA and γ-globulin were dissolved in 10 mM acetate buffer at pH 5.5. Individual protein solution (5 mL) was mixed with the HAP test material and with the dextran-produced HAP for 4 h at 303 K. The solution was filtered to remove the HAP, and subsequently the concentration of BSA in the remaining solution was determined via the Bradford method (Bradford, 1976). Protein adsorption inhibition percentage was defined as follows

$$\begin{aligned}\text{Protein adsorption inhibition percentage [\%]} &= 100[(\text{the amount of protein adsorbed to HAP}) \\ &\quad - (\text{the amount of protein adsorbed to modified HAP})] \\ &\quad /(\text{the amount of protein adsorbed to HAP}) \end{aligned} \quad (1)$$

## 3. Results and discussion

### 3.1. Dextran formation by DSase reaction on HAP

To form dextran by the DSase reaction on HAP, DSase was immobilized on HAP via immersion. The isoelectric point of DSase is 4.1, forming an ionic interaction with HAP at pH 5.5. As shown in Fig. 2, DSase was immobilized quickly, for 5 min, on HAP. This immobilization was not at equilibrium yet, meaning there was still the space on the surface for DSase immobilization.

DSase-immobilized HAP was reacted with a sucrose substrate to generate varying amounts of dextran by changing the reaction time and initial concentration of the sucrose, as shown in Fig. 3. The amount of dextran produced increased, and then leveled off, suggesting that the sucrose diffusion to DSase was limited due to the coverage of dextran generated from the immobilized DSase on HAP. The molecular weight of the dextran increased because the amount of DSase immobilized on HAP was set at a constant. From Fig. 3b it can be predicted that increasing the initial concentration of sucrose caused a gradual generation of dextran from the immobilized DSase. The surfaces of HAP and dextran-generated HAP were observed by SEM shown in Fig. 4. The amount of DSase immobilized and the amount of dextran generated were set at 6.0 U/g and 32 mg/g, respectively. The surface of dextran-gener-

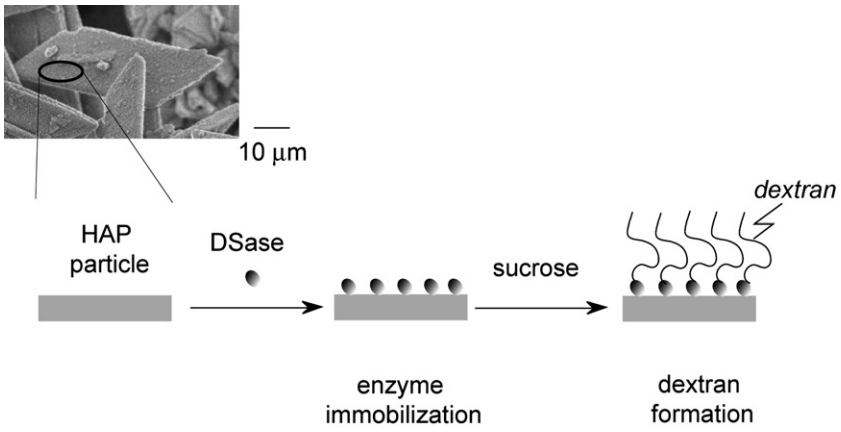


**Fig. 1.** HAP modification scheme with dextran generated by immobilized dextransucrase.

Download English Version:

https://daneshyari.com/en/article/1386435

Download Persian Version:

https://daneshyari.com/article/1386435

Daneshyari.com