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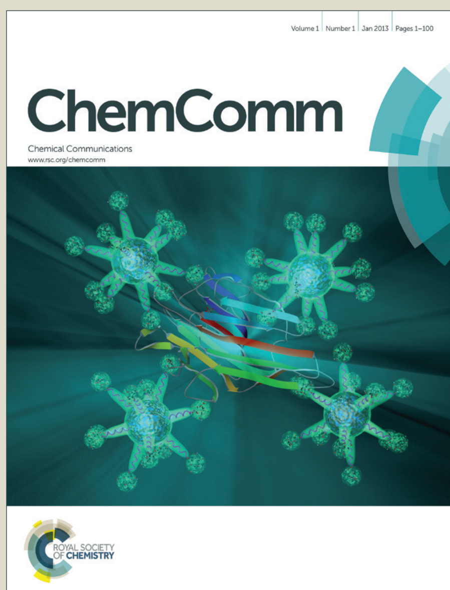

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ARTICLE TYPE

Dextransucrase-Catalyzed Elongation of Polysaccharide Brushes with Immobilized Mono-/Di-saccharides as Acceptors

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Quartz crystal microbalance (QCM) was used to monitor dextransucrase (DSase)-catalyzed polysaccharide elongation on the glucose-/maltose-ended self-assembly monolayer (SAM) surfaces. Kinetic parameters of the enzymatic elongation indicate that maltose is the promising substrate acceptor for DSase.

Polysaccharides are the core component of glycocalyx on the cell membrane surface, which are important in protein trafficking,[1] viral and bacterial infection,[2-3] and cell migration.[4] Polysaccharide brushes are therefore more favourable as biomimetic surfaces in biomedical science and technology.[5-7] However, it is extremely difficult to construct well-defined polysaccharide brushes because there are a series of challenges to perfectly control the regio- and stereo-chemistry of the saccharide chain via the ''traditional'' chemo-glycosylation reactions.[8-10] Alternatively, polysaccharide brushes can recently be constructed by enzyme-catalyzed elongation in vitro. This is a biomimetic process and it becomes increasingly promising due to its unique advantages such as green characteristics and regio-/stereo-selectivity. Enzymes from the glycosyltransferase family are the most used in this biomimetic process. They usually require activated substrates (nucleotides saccharide and saccharide-1-phosphates) as saccharide donors and oligosaccharides (maltoheptaose/iso-maltoheptaose and oligo-dextran) as saccharide acceptors.[11-16] However, the activated substrates are commonly unstable at ambient temperature and unimaginably expensive. The oligosaccharide acceptors are usually synthesized by a series of complicated reactions. Dextransucrase (DSase) is a kind of glycosyltransferase that can catalyze the transfer of D-glucose moiety from sucrose to a broad range of saccharide acceptors, including normal saccharides (mono-, di-, tri-, and oligo-saccharides) and unconventional ones (saccharide derivatives).[17-18] It is clear all these saccharides (as both donors and acceptors to this enzyme) are common and widely commercialized. Nevertheless, the Dsase-catalyzed D-glucose moiety transfer and saccharide elongation are usually carried out in solution and the saccharide acceptors have great effect on the enzymatic activity.[17-20] Nihira and co-workers studied the DSase-catalyzed saccharide elongation on gold surface immobilized with dextran as an acceptor.[11] They focused their attention mainly on monitoring the enzymatic process by quartz crystal microbalance (QCM). Despite its acknowledged importance, the effect of saccharide acceptors has rarely been manipulated experimentally on the model or practical surfaces. Furthermore, the structures and properties of the resulted polysaccharide brushes are not well understood also. Therefore, we use QCM to study the DSase–catalyzed saccharide elongation from the mono- and di-saccharide acceptors terminated self-assembly monolayer (SAM) on the gold surface. It is expected the resulted polysaccharide brushes will have defined structures than that using dextran as

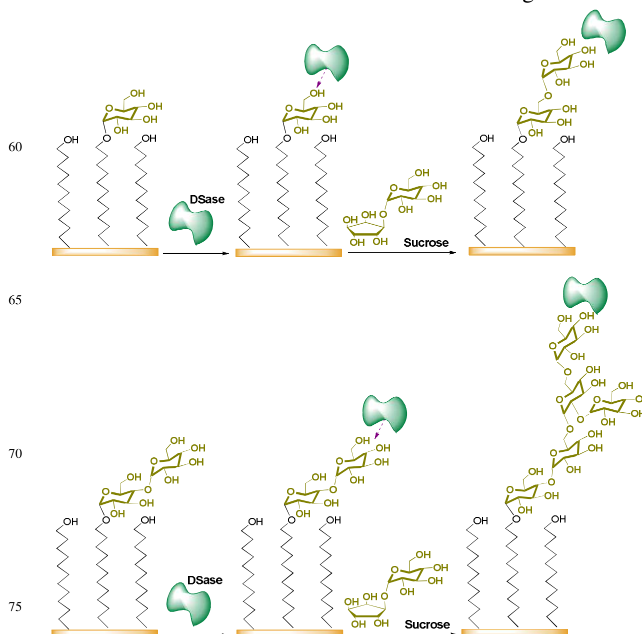

Fig. 1 Overall strategy for the DSase-catalyzed elongation of polysaccharide chains to form brushes on the gold surface assembled with a monolayer and then immobilized with different saccharide acceptors (glucose/maltose).

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acceptor. At the same time, we aim to give detailed results to demonstrate that the enzymatic activity depends on the nature of the immobilized saccharide acceptors.[21]

The overall strategy is schematically shown in Fig.1. Glucose and maltose are chemically attached to SAM fabricated on the gold surface by a typical procedure (ESI, Scheme S1).[21-23] The average density is 1.27 nm^{-2} and 1.30 nm^{-2} for the immobilized mono- and di-saccharide acceptors, respectively (ESI, Fig. S1). And the water contact angle is 36° ± 2° and 35° ± 2° for these saccharide-immobilized SAM surfaces (ESI, Fig. S2). The DSase–catalyzed saccharide chain elongation is then monitored in real time by QCM with sucrose as donor. DSase from *Leuconostoc mesenteroides* catalyzes the transfer of glucose moiety from sucrose to a saccharide acceptor and then to result dextran with 95% of α, 1–6 bonds and only 5% of α, 1–3 bonds.[24] Two steps in the enzymatic elongation process are monitored continuously from the time dependencies of QCM frequency changes, which include 1) recognition of DSase to the immobilized glucose/maltose acceptors; 2) enzymatic elongation of the complementary saccharide chains in the presence of sucrose (ESI, Fig. S3~S4).[25-26] Fig. 2 shows the QCM response curves for the recognition and then the binding of DSase to the glucose- and maltose-immobilized SAM surfaces, respectively. It can be seen that an immediate decrease appears in QCM frequency when the enzyme was injected over the surfaces. This frequency decrease is due to the specific binding of DSase to the saccharide acceptors, and it depends both on the enzyme concentration and the acceptor structure. For the glucose-immobilized SAM surface, the bound DSase increases from 0.15 to 1.16 $pmol\cdot cm^{-2}$ (Fig. 2(a)) with the enzyme concentration from 20 nM to 100 nM. At the same time, it increases from 1.54 to 7.73 $pmol\cdot cm^{-2}$ (Fig. 2(b)) on the maltose-immobilized SAM surface. We can calculate the rate constant of enzyme binding (k_{on}) and dissociation (k_{off}) from these curves. The dissociation constant (K_d) is then obtained from the ratio of k_{off} to k_{on} (ESI, Fig. S5~S6 and Eq. S1~S4). It is known that a low dissociation constant indicates a high specificity between the acceptor and the enzyme.[27-28] Tab. 1 summarizes the results. Compared with the glucose-immobilized surface (63.78 nM), K_d is obviously lower for DSase from the maltose-immobilized surface (35.09 nM). The values indicate that the affinity between DSase and maltose acceptor is greater than that between DSase and glucose acceptor.

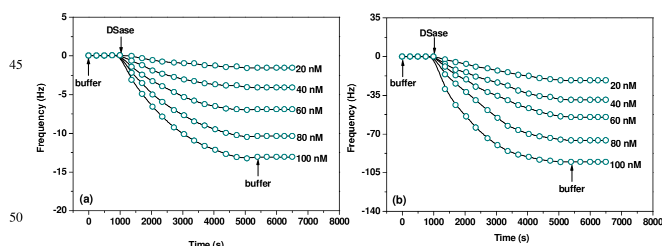

Fig. 2 Effect of enzyme concentration on DSase recognition/ binding onto the saccharide-immobilized surfaces. The curves were recorded by QCM for the surfaces with different saccharide acceptors: (a) glucose; (b) maltose.

Tab. 1 Kinetic parameters calculated from QCM curves presented in Fig. 2.

Acceptor	k_{on} ($10^{-3}M^{-1}S^{-1}$)	k_{off} ($10^{-4}S^{-1}$)	K_d (nM)	K_m (mM)	k_{cat} (S^{-1})
Glucose	2.54	1.62	63.78	18.30	0.0036
Maltose	3.99	1.40	35.09	8.09	0.021
Dextran[11]	89.00	16.00	17.98	3.40	3.50

The acceptor-bound DSase is expected to transfer glucose donor from sucrose and then to form dextran brushes on the surfaces. Fig. 3(a-b) shows the effect of sucrose concentration on the enzymatic elongation to the immobilized acceptors. The frequency decrease reflects a mass increase because sucrose accesses the active sites of the bound enzymes and results in the enzymatic attachment of glucose donor at the nonreducing end of the immobilized acceptors.[21] The amount of attached glucose increases with increasing sucrose concentration from 1 to 15 mM. It is from 12.56 to 62.78 ng cm^{-2} in the case of glucose as the acceptor and from 368.81 to 627.75 ng cm^{-2} in the case of maltose as the acceptor. These results indicate polysaccharide brushes are formed by the enzymatic elongation process. FT-IR/MR and XPS were used to analyze the chemical composition information of the polysaccharide brushes (ESI, Fig. S7~S9). It is obvious that XPS peak of saccharide acetal (O-C-O) enhances significantly due to the formation of polysaccharide brushes (ESI, Fig. S8~S9). ToF-SIMS spectra (ESI, Fig. S10) also show a series of specific secondary fragment ions from the polysaccharide brushes. Besides, the surface morphology changes measured by AFM further confirm the enzymatic elongation of polysaccharides on the mono-/di-saccharide immobilized surfaces (ESI, Fig. S11~S12). Fig. 3(c) presents the plots for the initial elongation rate (v_0) against sucrose concentration. It can be seen that v_0 increases with the increase of sucrose concentration, which is reasonable since more saccharide donors can access the active sites of DSases.[21] And v_0 is much higher for maltose acceptor than that for glucose one. Importantly, the DSase–catalyzed saccharide elongation can simply be described by the Michealis-Menten equation (ESI, Eq. S5~S7). Fig. 3(d) shows the reciprocal plots of v_0 against sucrose concentration. We can calculate the Michaelis constant (K_m) and the catalytic rate constant (k_{cat}) from slope and intercept of the plots. As can be seen from Table 1, the K_m and k_{cat} values are 18.30 mM and 0.0036 S^{-1} for the immobilized glucose acceptor, respectively. By contrast, the K_m and k_{cat} values are 8.09 mM and 0.021 S^{-1} for the immobilized maltose acceptor. The K_m value for maltose is higher than that obtained on the glucose immobilized surface and is almost consistent with the K_m value obtained on the dextran acceptor immobilized surface (3.40 mM[11]). It is worth to note the K_m value for the immobilized dextran acceptor is well consistent with that obtained in solution (K_m = 3.00 mM).[29] Previous work pointed out that the enzymatic activity of DSase would not be affected by the dextran acceptor immobilized surface. Our values indicate that the enzymatic activity of DSase has been largely affected by the glucose acceptor immobilized surface, thus the enzymatic

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elongation rate is much lower than the reported values. This result may be due to the use of short and stiff undecyl spacer and the low affinity between the enzyme and the glucose acceptor.[30-31] Meanwhile, it indicates that the enzymatic activity of DSase will not affected largely on the maltose acceptor immobilized surface. This is because the affinity between the enzyme and the maltose acceptor is high, and the decomposition rate of the enzyme-acceptor complex is so small (k_{off} = 0.00014 S^{-1}). DSase can quickly proceeds to elongation (k_{cat} = 0.021 S^{-1}) after binding to maltose (consequent formation of the enzyme-acceptor complex). These features were also observed for the enzyme-catalyzed elongation of saccharides in solution.[32-33]

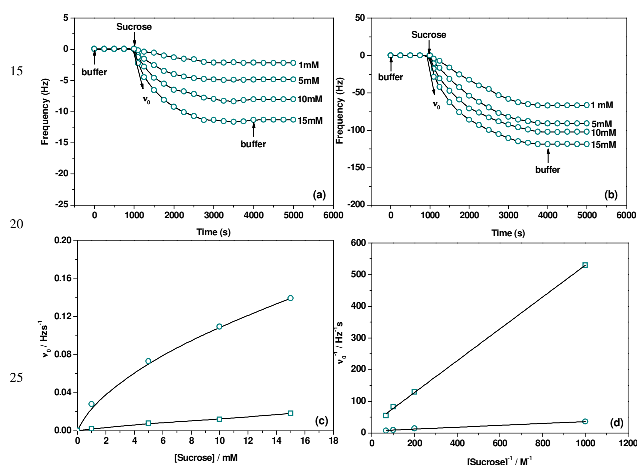

Fig. 3 (a-b) QCM curves for the enzymatic elongation on the glucose/maltose-immobilized surfaces, (c) Dependence of the initial elongation rate (ν_0) on sucrose concentration, (d) Reciprocal plots of ν_0 against sucrose concentration ((□: glucose as the acceptor; ○: maltose as the acceptor). 0.78 pmol·cm^{-2} of DSase was bound previously to the surface to induce the reaction).

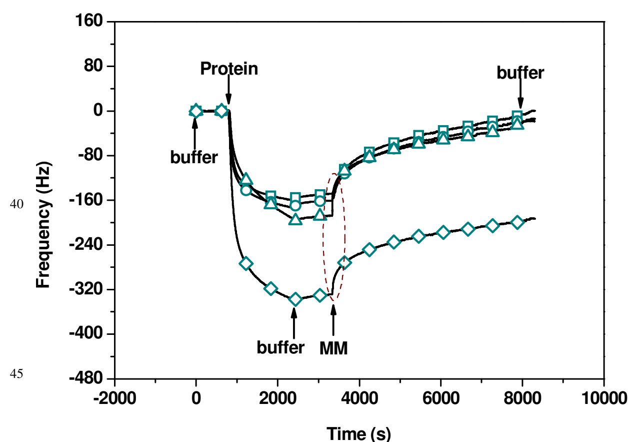

Fig. 4 QCM curves for the specific adsorption and the affinity elution of Con A on the fabricated surfaces. (□: the glucose acceptor immobilized surface; ○: the glucose acceptor immobilized surface after enzymatic elongation; Δ: the maltose acceptor immobilized surface; ◊: the maltose acceptor immobilized surface after enzymatic elongation.) (Con A: 20μg/mL, MM: 100 mM)

The synthesized polysaccharide (dextran) brushes are essentially linear polymers (α, 1–6 bonds) containing 5% of α, 1–3-linked branches with shorter side-chains.[34-35] Thus, more nonreducing residues would be available for lectins to bind. Con A is a well-known mannosyl/glucosyl–specific lectin.[36-37] An injection of Con A (20 μg/mL) results 162 Hz (7.97 pmol·cm^{-2}) and 165 Hz (8.12 pmol·cm^{-2}) binding response to the glucose and maltose acceptor immobilized surfaces, respectively. It increases to 200 Hz (9.80 pmol·cm^{-2}) and 360 Hz (17.72 pmol·cm^{-2}), 1.23-fold and 2.18-fold, for the surfaces after enzymatic elongation (Fig. 4). Obviously, there are more nonreducing glycosyl residues have been introduced to the maltose acceptor immobilized surface. It further suggests that the maltose acceptor is more efficient than the glucose ones in the DSase–catalyzed elongation of polysaccharide brushes. Besides, Fig. 4 also indicates methyl α-D-mannopyranoside (MM) can be used to dissociate almost all lectins from the studied surfaces with an exception of the maltose acceptor immobilized surface after enzymatic elongation. Only about 40% Con A can be dissociated from this surface. It may be attributed to multivalent interaction between Con A and the polysaccharide brushes.[38] These brushes show specific adsorption to Con A but high resistance to *ricinus communis agglutinin* 120 (RCA_{120}) and bovine serum albumin (BSA) (ESI, Fig. S13~S16). Fluorescence images of the surfaces also demonstrate the highly specific adsorption of Con A on the polysaccharide brushes (ESI, Fig. S17~S18). It means our glycocalyx-like polysaccharide brushes are effective for detecting the saccharide-protein interaction.

Conclusions

In conclusion, we have verified that DSase–catalyzed polysaccharide elongation can be directly carried out on the mono-/disaccharide acceptors (glucose and maltose) immobilized SAM surfaces. The saccharide acceptors have great effect on the enzymatic activity of DSase. Although the enzyme-catalyzed polysaccharide elongation is demonstrated on the model surface, it can easily be extended to other surfaces for practical applications. Polysaccharide brushes have great potential in biomedical technologies as biomaterials. Therefore, the described DSase-catalyzed polysaccharide elongation is also of significant promise in construction of glycocalyx-like biomimetic surfaces with specific saccharide-protein interaction.

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