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Detection of Transglucosidase-Catalyzed Polysaccharide Synthesis on a Surface in Real Time Using Surface Plasmon Resonance Spectroscopy

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Biological systems that involve enzyme catalysis at insoluble surfaces require analyses beyond classical solution state enzymology. Such strategically important systems include the cell wall and the highly organized starch granule. Enzyme-catalyzed glycosyl transfer onto surfaces has been monitored using nonreal-time methods, such as mass spectrometry,[1] lectin affinity,[2] radiolabeling,[3] or fluorescent affinity-based systems[4] (see Supporting Information for other surface enzyme systems). Direct monitoring by SPR spectroscopy is not feasible with a limited number of glycosyl unit transfers due to sensitivity limits. This method has however been used to monitor nucleotide polymerization and bacterial cell wall peptidoglycan synthesis.[5] Although it has also been used to detect surface polysaccharide synthesis,[6] the measurements have not been truly real time and quantitative. This was because it was the enzyme that was immobilized and the product was typically only transiently bound to the surface. To our knowledge, the only example of the real-time analysis of enzymatic polysaccharide extension of a surface has been with a quartz crystal microbalance.[7] We now describe a real-time method for monitoring polysaccharide synthesis on a surface using SPR spectroscopy and a model system.

Alternansucrase from *Leuconostoc mesenteroides* NRRL B-1355 (E.C. 2.4.1.140) catalyzes the transfer of glucose from sucrose to an acceptor to give alternan, a soluble glucan polymer with predominantly alternating α-1,6 and α-1,3 glycosidic links. Carbohydrate acceptors such as glucose are extended at their nonreducing ends by this enzyme.[8] We have used NMR spectroscopy with [1-$^{13}C^{Glc}$]sucrose to establish that carboxymethylated α-1,6 dextran is an acceptor for this enzyme (Figure S1). When the enzyme was injected together with sucrose over a carboxymethyl α-1,6 dextran chip surface, there was an immediate increase in SPR response units (RU) due to a bulk refractive index change associated with sucrose (Figure 1). During the coinjection, there was a time-dependent increase in RU. At the end of each coinjection, there was an immediate bulk refractive index change as the solution returned to buffer only. This was followed by a slow dissociation of material from the surface that enzyme-only control injections showed was enzyme (Figure 1). An injection of NaCl solution removed the remainder of the enzyme (consistent with electrostatics being a component of the enzyme-surface interaction),[9] and the subsequent baselines remained stable over many hours.

It was possible to estimate how much enzyme was bound at the end of a coinjection by taking the maximum response at that time point and subtracting the bulk sucrose response together with the response of the irreversibly bound material at the end of that experiment (Figure S2). This showed that the enzyme concentrations used were well below the enzyme's apparent K_D for the surface in the presence of sucrose (estimated to be ~150 μg mL^{-1}, assuming a simple binding equilibrium). Furthermore, coinjections of different durations showed that enzyme binding had reached equilibrium (see Supporting Information).

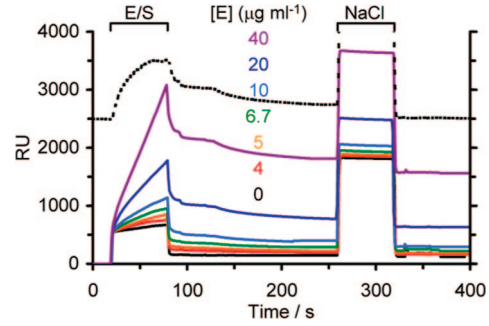

Figure 1. SPR spectroscopic detection of alternansucrase activity. Solid lines are coinjections of enzyme (E; concentrations are defined in the chart) and sucrose (S; 11.7 mM) on a carboxymethyl dextran chip. The broken line (displaced for clarity) is an enzyme-only injection (50 μg mL^{-1}). Each injection was followed by one of NaCl (150 mM).

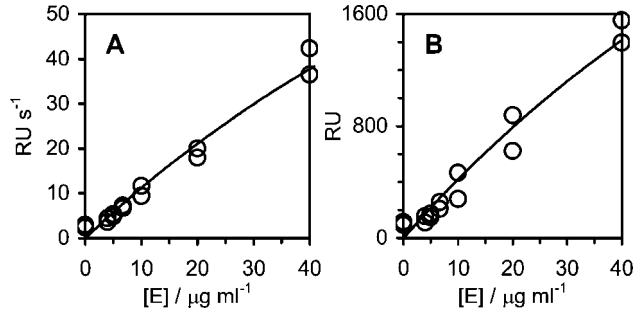

Figure 2. Enzyme activity determined by SPR spectroscopy as a function of its concentration, [E]. (A) The gradient at the end of each coinjection ($r^2 = 0.973$ with a best fit line assuming a K_d of 150 μg mL^{-1}) and (B) the increase in baseline after each coinjection−wash cycle ($r^2 = 0.961$).

The gradient at the end of each coinjection was dependent on enzyme concentration (Figure 2A) in a manner that was consistent with the amount of enzyme bound to the surface. This showed that it was possible to directly determine enzyme activity by SPR spectroscopy in real time. The increase in baseline after each coinjection and wash cycle exhibited a similar dependency (Figure 2B). Therefore, end-point measurements could also be made. The salt washes were very effective, but small background rates (~5% of the maximum) associated with sucrose-only injections indicated a low level of persistently bound enzyme. The detection limit of enzyme activity was of the same order of magnitude as that obtained using a standard solution state assay based on fructose release (20 μL of ~0.17 U mL^{-1}).[10]

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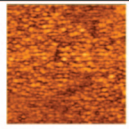
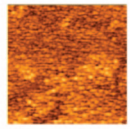
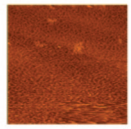
Table 1. AFM Images (20 × 20 μm^2) of a Chip Surface Before and After Exposure to Alternansucrase and Sucrose

solution[a]	AFM image	peak to trough height (nm)	rigidity (pN nm^{-1})[b]
buffer only		90	35 ± 3
sucrose		66	22 ± 2
enzyme		36	48 ± 3
enzyme & sucrose		415	14 ± 2

[a] 20 mM sodium succinate/HCl, pH 5.4, and either sucrose (292 mM) or enzyme (1.6 U mL^{-1}), both or neither 21 °C. [b] Estimated by measuring the modal slope values of cantilever deflection−distance curves.

The K_m of the enzyme for sucrose was determined using both the solution state assay and the SPR spectroscopy method (Figure S3). The K_m in solution was 3.3 ± 0.6 mM. The corresponding values on a surface were 3.8 ± 1.4 mM from end-point measurements and 5.8 ± 1.8 mM from gradient measurements, both statistically indistinguishable from that in solution.

It was possible to estimate the turnover number of the enzyme on the surface. This was derived from the rate of polymer synthesis in RU s^{-1} divided by the amount of enzyme bound in RU at the end of each coinjection. Such values needed correction for the different responses associated with dextran and protein on such a chip surface (refractive index increments *dn/dc* are 0.15 and 0.18 mL g^{-1}, respectively[11]) together with their molecular masses (162 and 225 000 Da for glucoside units and enzyme, respectively). An apparent k_{cat} value of 80 ± 12 s^{-1} at the surface was ∼48% of that in solution (167 ± 5 s^{-1}). A lower intrinsic turnover rate on the surface is unlikely to be due to substrate specificity because, after initial carboxymethyl dextran extension, the acceptor substrate would have been hydrated alternan. A more likely explanation is that the enzyme required a rate-limiting transition from initial electrostatic to subsequent catalytically productive binding modes.

Alternan is an essentially linear polymer, but it contains some α-1,6-linked branches, accounting for 10% of all linkages, with side-chain lengths of only two or three residues.[8] Thus, more nonreducing ends would be available for a lectin to bind to a chip after modification. An injection of concanavalin A gave a 5.6-fold greater binding response after surface modification, and this was carbohydrate specific because it was inhibitable by methyl α-mannoside. These obervations are consistent with an alternan-modified surface.

The presence of new material on the chip surface where coinjections had taken place was visible to the naked eye (Figure S4) due to the altered dispersion of light. Atomic force microscopy also revealed the presence of additional material on the surface (Table 1). The unmodified carboxymethyl dextran hydrogel surface had a peak-to-trough height of 90 nm, consistent with previous measurements.[11] Analysis of the gradients obtained from the cantilever deflection versus distance data, as the AFM cantilever was driven into the sample, produced a measure of the rigidity of the surface (Table 1). The presence of sucrose led to a modest lowering of the peak-to-trough height and a slight softening of the surface, perhaps due to an exchange of water with glucose within the hydrogel. The presence of enzyme gave a more significant decrease in peak-to-trough height and a slight hardening of the surface, perhaps due to the enzyme acting as a counterion and screening repulsive charges on the polymer. A similar effect has been observed with NaCl.[12] The presence of both enzyme and sucrose gave a 4.6-fold increase in peak-to-trough height and a significant softening of the surface. These results indicated the formation of significant quantities of amorphous polymer on the chip surface.

In summary, we have developed a method that will have wide utility in surface polysaccharide enzyme studies including screens. The preparation of appropriate substrate surfaces could take advantage of recent developments in carbohydrate arrays.[13]

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Supporting Information Available: Further citations, detailed methods and additional results, analyses, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Consistent with electrostatic binding were the pI of the enzyme, the rate of synthesis being dependent on the degree of dextran carboxymethylation, and the greater inhibition of the enzyme by NaCl on a surface compared with that in solution (see Supporting Information).
(10) Amylosucrase forms an insoluble α-1,4 linked glucan from sucrose (Rolland-Sabaté, A.; Colonna, P.; Potocki-Véronèse, G.; Monsan, P.; Planchot, V. J. *Cereal Sci.* **2004**, *40*, 17−30). We have shown that α-1,6 dextrans are not acceptors with this enzyme and surface extension cannot be detected despite enzyme binding to the surface. Therefore, substrate specificity was maintained on the surface and noncovalent interactions between glucan produced in bulk solution and the surface did not occur.
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