

Monitoring the formation of biosilica catalysed by histidine-tagged silicatein†

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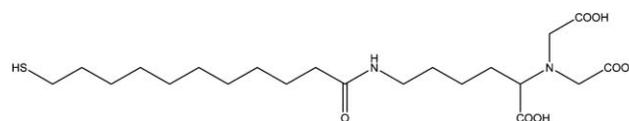
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Surface bound silicatein retains its biocatalytic activity, which was demonstrated by monitoring the immobilisation of silicatein using a histidine-tag chelating anchor and the subsequent biosilicification of SiO₂ on surfaces by surface plasmon resonance spectroscopy, atomic force microscopy and scanning electron microscopy.

Nanoscale control of polymerization of silicon and oxygen determines the structures and properties of a wide range of siloxane-based materials.¹ These materials typically require high temperatures, high pressures and the use of caustic chemicals.² In comparison, biological systems can synthesise siloxane-based materials at ambient temperatures and pressures and produce an amazing diversity of precisely nanostructured morphologies.³ Biosilicification in diatoms,⁴ sponges⁵ and grasses⁶ represents typical examples in this context. The study of biosilicification has led to the isolation of proteins that facilitate silica formation *in vivo*, in particular, silaffins in diatoms³ and silicateins in sponges^{5,7-9} have been studied and identified as constituents of biosilica (silica formed by proteins) showing an accelerated silica formation from monosilicic acid solution *in vitro*.⁸ To verify the specificity and significance of the above proteins in biosilicification, a variety of synthetic polymers including polypeptides and di-block co-polypeptides have been investigated for their role in silicification in solution.^{8,10,11} The process of silica formation in marine sponges is thought to be mediated by a family of catalytically active structure-directing enzymes, the so-called silicateins. It has been shown that silicateins facilitate the formation of SiO₂ in solution at neutral pH using tetraethoxysilane (TEOS) as a monomer,^{5,7,9} whereas no polymerization of TEOS was observed at neutral pH in the absence of silicatein.¹² To understand the operation of silicatein and the formation of the resulting silica structures at the molecular level by means of surface methods, we present the immobilization of silicatein which was isolated from marine sponge *Suberites domuncula*.

In order to be able to immobilise silicatein onto surfaces we synthesised a chelator alkanethiol based on the idea that silicatein expresses a short affinity sequence of histidines (histidine-tag) that is capable of binding metal ion complexes such as nitrilotriacetic acid (NTA). It's known that NTA, when complexed with Ni²⁺, selectively binds proteins whose sequence is terminated with a sequence of histidines.¹³ The structure of the NTA-terminated alkanethiol, whose synthesis was adopted from similar structures reported recently,¹³ is shown in Scheme 1.

The self-assembled monolayer formation of the chelating alkanethiol NTA and the immobilization of the protein to the gold (111) surface were monitored using the surface sensitive optical technique of surface plasmon resonance (SPR). The



Scheme 1 Nitrilotriacetic acid (NTA) terminated alkanethiol.

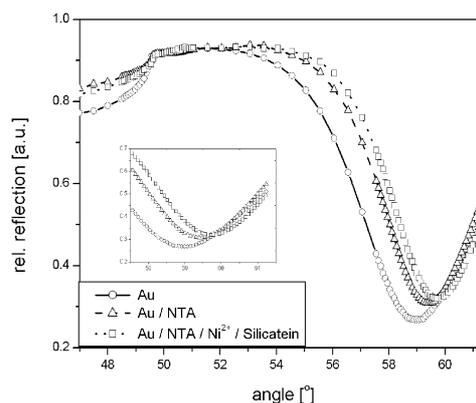


Fig. 1 SPR curves of gold (circles), NTA alkanethiol on gold (triangles) and immobilized silicatein thereon (squares).

plasmon spectra were recorded against ethanol on bare gold slides, after self-assembly of chelating alkanethiol NTA and after immobilization of silicatein on the gold surfaces, both Ni²⁺ coordinated as well as without coordination of Ni²⁺. Fig. 1 displays the corresponding SP spectra of some experiments and a closer view of the corresponding minima reflectivity angle. The shifts of the plasmon curves were calculated, corresponding to angular changes of 0.420° (monolayer of NTA alkanethiol on bare gold) and 0.720° (silicatein on a Ni²⁺ coordinated surface). In a separate experiment immobilization of silicatein without Ni²⁺ could not be detected, supporting the chelating efficiency of histidine-tags in the presence of Ni²⁺. These shifts in the surface

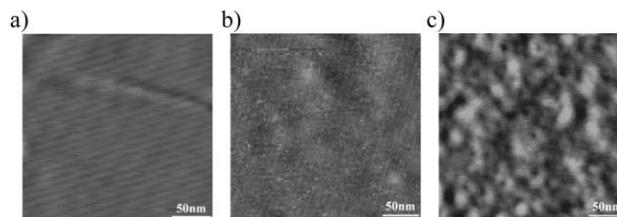


Fig. 2 SFM height pictures of (a) the gold (111) surface, (b) NTA alkanethiol on gold and (c) immobilized silicatein (same scale for all images).

† Electronic supplementary information (ESI) available: experimental details and SEM picture of SiO₂ polymerized on NTA functionalized surfaces. See <http://www.rsc.org/suppdata/cc/b4/b410283e/>

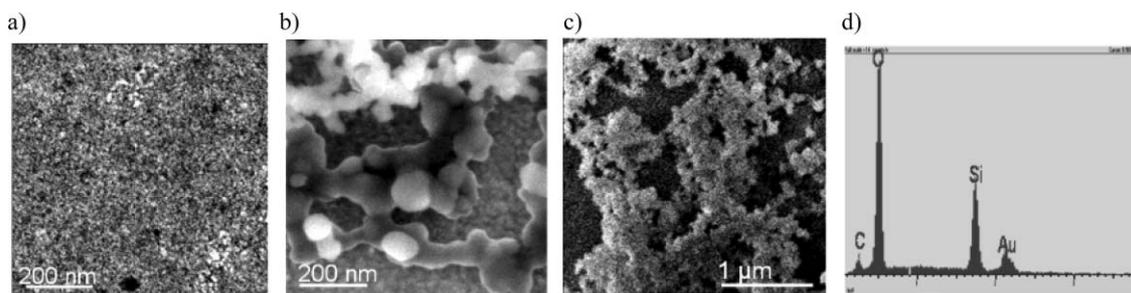


Fig. 3 SEM images of silicization onto surfaces functionalised with NTA alkanethiol without Ni^{2+} (a) and NTA alkanethiol with Ni^{2+} chelating silicatein (b, c). No observable formation of SiO_2 occurred on NTA alkanethiol modified surfaces (a). In contrast, Ni^{2+} chelated silicatein immobilization onto NTA alkanethiol surfaces induced the formation of SiO_2 (b,c), as evidenced by the EDX-spectrum (d).

plasmon resonance angle were converted by complex Fresnel calculations to an average thickness of monolayer of NTA alkanethiol and silicatein layer assuming a refractive index of $n = 1.5$ for both layers. NTA alkanethiol resulted in a 1.6 nm thick film, while silicatein immobilization increased the thickness by 2.8 nm, which matches the theoretical diameter of 3.0 nm for a protein with a molecular weight of 24 000 Da. SPR control experiments showed that silica is not released into the solution.

In order to confirm that the silicatein was immobilized on the surfaces functionalized with NTA alkanethiol self-assembled monolayers (SAMs), direct evidence by scanning force microscopy (SFM) was provided. Figs. 2a–c show the SFM height images obtained in the intermediate contact mode of the Au (111) crystal surface, the NTA monolayer surface, and the surface of immobilized silicatein film, respectively. The height and roughness analysis of the topologies of these surfaces supported strong evidence that the modification of Au(111) by NTA alkanethiol SAMs, and subsequently the immobilization of silicatein on the surfaces of NTA alkanethiol SAMs had both taken place. The roughness of the Au (111) surface was 0.590 nm, which is rather flat, but the roughness of the NTA SAMs surface and the silicatein film surface were 1.415 and 2.384, separately, which were rougher compared with the Au (111) plane. The increase of the roughness illustrates that the uncoated gold surface is atomically flat, but the NTA-coated gold samples and the silicatein films are only molecularly flat. We believe that the SFM image in Fig. 2c arises from the surface of an immobilized silicatein film chemisorbed on the NTA monolayers.

In order to confirm the activity of silicatein through the ability to precipitate silica from TEOS on the surface, we performed two sets of experiments: one, using a gold surface modified with NTA or NTA with Ni^{2+} and a second, with gold surfaces functionalised with immobilized silicatein. Under these similar sets of conditions for both experiments, we could find structured silica only on the silicatein immobilized surfaces, using SEM characterisation, as shown in Figs. 3b and 3c. Fig. 3a shows the results after having performed the precipitation of silica on the surface modified by NTA thioalkane only. Clearly, no formation of silica was observed. Furthermore, the surface became black when exposed to the electron beam in the scanning electron microscope which indicated the presence of organic species, such as NTA alkanethiol, that decomposed upon exposure to the electron beam. On surfaces functionalised with silicatein, nanospheres of silica had been precipitated (Figs. 3b and 3c). Structures of interconnected nanospheres with a diameter of about 70–300 nm were observed. Fig. 3d shows the energy dispersive X-ray (EDX) analysis spectrum of the material synthesised on the surface, which gives evidence that it consists of silica. Analyzing the EDX spectrum of the surface without immobilized silicatein during the silicization process showed that no silica was formed here.

The formation of interconnected silica spheres in the vicinity of surface bound protein indicates that the active site of the immobilized protein is oriented towards the solution and that the reaction is diffusion controlled. This is corroborated by the silicification of TEOS with silicatein used as an additive in solution, which resulted in no precipitation of SiO_2 on NTA functionalised surfaces, as can be seen in the ESL.† It is obvious from the SEM pictures shown in Figs. 3b and 3c that the coverage of the surface with silica is heterogeneous. About 70% of the surface was covered with silica structures. Whether this is due to the limited reaction time of 8 hours or an imperfect homogeneous immobilisation of the silicatein is the subject of ongoing investigations.

To conclude, we present a novel NTA terminated alkanethiol which can be successfully self-assembled onto gold surfaces. These functionalised surfaces could immobilise histidine-tagged silicatein using the efficient chelating properties with Ni^{2+} . The results presented here demonstrate that silicatein, previously shown active for catalysing and structurally directing the polycondensation of silicon alkoxides in solution when immobilised onto surfaces, is also capable of catalysing and templating the polycondensation of monomeric silicon alkoxides to form silica structures on surfaces.

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