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Relevance of glycosylation of S-layer proteins for cell surface properties

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ABSTRACT

Elucidating the building principles and intrinsic features modulating certain water-associated processes (*e.g.*, surface roughness in the nanometer scale, surface hydration and accompanied antifouling property, *etc.*) of surface structures from (micro)organisms is nowadays a highly challenging task in fields like microbiology, biomimetic engineering and (bio)material sciences. Here, we show for the first time the recrystallization of the wild-type S-layer glycoprotein wtSgsE from *Geobacillus stearothermophilus* NRS 2004/3a and its recombinantly produced non-glycosylated form, rSgsE, on gold sensor surfaces. Whereas the proteinaceous lattice of the S-layer proteins is forming a rigid layer on the sensor surface, the glycan chains are developing an overall soft, highly dissipative film. Interestingly, to the wtSgsE lattice almost twice the amount of water is bound and/or coupled in comparison with the non-glycosylated rSgsE with the preferred region being the extending glycan residues. The present results are discussed in terms of the effect of the glycan residues on the recrystallization, the adjoining hydration layer, and the nanoscale roughness and fluidic behavior. The latter features may turn out to be one of the most general ones among bacterial and archaeal S-layer lattices.

1. Introduction

One of the most fascinating cell envelope structures in prokaryotic organisms is two dimensional arrays of protein or glycoprotein subunits, termed S-layers [1]. The widespread occurrence as one of the most common envelope surface structures in archaea and bacteria and the high physiological expense of S-layers raise the question, which selection advantage has S-layer carrying organisms in their natural and frequently highly competitive habitats. Moreover, the exploitation of S-layers as part of more complex supramolecular structures, particularly as patterning element for nanobiotechnological applications and in synthetic biology is a rapidly developing field [1–3].

Bacterial S-layers are highly porous protein mesh works with a unit cell size in the range of 3–30 nm, a thickness of 5–10 nm, and an estimated porosity of approximately 70%. In many S-layer lattices two or more distinct classes of pores in the range of approximately 2–8 nm have been identified. The planar assemblies of identical protein or glycoprotein subunits can be aligned in lattices with oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetry [1–7]. So far no general biological function has been found and many of the functions assigned to S-layers, for instance, isoporous and/or protective coating of the cell, surface recognition and cell adhesion to substrates, receptor–substrate interactions, templated fine-grain mineralization, as well as mediation of pathogenicity-related phenomena still remain hypothetical [1,8,9].

In addition to unique physicochemical surface properties, the repetitive topographical characteristics of S-layers should be considered as relevant feature affecting hydrodynamic surface properties of cells. It is tempting to speculate that the defined roughness of S-layer surfaces determines the flow resistance of cells in natural environments in a similar way. Studies on friction flows of liquids at nanopatterned interfaces have shown that the slippage of fluids at channel boundaries is greatly increased by using surfaces that are patterned on the nanometer scale [10].

Another important feature of S-layer proteins is that many of them comprise of glycans, the carbohydrate moieties of glycoproteins exposed on the cell surface [11–13]. The cell envelope may exclusively comprise of S-layer glycoproteins or, as in the case of *Geobacillus stearothermophilus* NRS 2004/3a which was investigated in the present study, of an intermixture of S-layer proteins and glycoproteins with different glycosylation patterns [14,15]. From a general point of view, S-layer glycoproteins have been implicated in a multitude of cellular processes, including immune response, intracellular targeting, intercellular recognition, and protein folding and stability [5,16]. Not only the presence of

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carbohydrate moieties but also the density of glycan display, glycan heterogeneity and molecular scale precision of interaction are most relevant aspects [12,17,18]. Only little is known about structure–function relationships of S-layer glycoproteins. However, one may speculate that surface-exposed glycans may improve the flagella-driven mobility of glycoprotein-carrying microorganisms in complex, natural habitats (*e.g.*, soil, mud, sediments, glycocalyx, biofilms, body fluids) [19].

In general, two major classes of antifouling materials, namely polyhydrophilic and polyzwitterionic materials have been figured out [20]. It is hypothesized that the antifouling ability of materials is tightly correlated with a hydration layer near the surface [21], because a tightly bound water layer forms a physical and energetic barrier to prevent protein adsorption on the surface. Water molecules residing on and/or penetrating into antifouling materials can be classified into two types of "surface-bound" waters formed by (1) hydrogen bonding for hydrophilic materials and by (2) even more strongly ionic solvation for zwitterionic materials [20,22]. Furthermore, spatially controlled binding, *i.e.*, patterning, of bioactive molecules on designated regions of solid surfaces is of great importance for the design of antifouling, bioactive surfaces for, *e.g.*, diagnostics and sensors [23]. In this context it is interesting to note that an excellent antifouling property of S-layers can also be deduced from the perfectly clean surfaces of bacterial cells seen in TEM micrographs of freeze-etched preparations [1,24–26]. Even when cells were harvested from complex environments or growth media containing macromolecular components the S-layer lattices were never masked by adsorbed molecules like, *e.g.*, proteins. Most recently the unique antifouling and cytophobic properties of S-layers were successfully exploited for the coating of microfluidic channels in lab-on-a-chip devices [27,28].

Moreover, native S-layer proteins from several *Bacillus* strains are comprised of carboxyl groups, which are neutralized by an equal number of amino groups and thus, leading to a charge neutral outer surface [29–31]. In addition to these polyzwitterionic characteristics it is self-evident that crystalline S-layer lattices are highly polyhydrophilic because water molecules may penetrate and lock to form a hydrogen–bond network in the pores and bonds to groups on the protein surface [32]. Moreover, hydration of S-layer glycoproteins may even be increased by water-exposed, highly hydrophilic glycan residues. Thus, S-layer lattices in general may be considered as highly hydrated, ultrathin biological antifouling materials.

Considering the combination of antifouling properties (including a hydration layer adjoining the surface), increased slippage and higher capillary permeability, the presence of S-layers may facilitate flagella-driven cell locomotion in natural habitats [19]. Interestingly, a repeated change between relocation and adhesion was described for archaea [33]. This relocate-and-seek behavior would enable the cells to seek for and remain in a favorable surrounding and would, therefore, be of great advantage for them. These features may justify the energy expense of S-layer protein synthesis and may turn out to be one of the most general ones among bacterial and archaeal S-layers [1].

In the present study, we have exploited for the first time, the relevance of glycosylation at its "native" condition, where a mixture of (glycosylated) S-layer proteins completely covered the surface of the ubiquitous organism *G. stearothermophilus* NRS 2004/3a, which is not specialized for specific habitats. The recrystallization characteristics and surface properties (i.e., surface hydration, nanoscale fluidic behavior) of this so-called wild-type SgsE (wtSgsE) glycoprotein (schematically depicted in Fig. 1A) are compared to the recombinantly produced protein SgsE (rSgsE), which is N-terminally truncated by 130 amino acids and is lacking the covalently linked carbohydrate moiety (Fig. 1B) [34]. This truncated form has been chosen because (1) the broadest knowledge has accumulated for this non-glycosylated counterpart of wtSgsE, (2) identical S-layer lattice formation compared to wild-type (oblique symmetry, a = 11.6 nm, b = 9.4 nm, and $\gamma \approx 78°$ [35]), and (3) highest yield of recombinant protein production [34]. Herein we show that wtSgsE and rSgsE reveal very similar and laterally homogeneous morphology as determined by atomic force microscopy (AFM). Surface plasmon resonance (SPR) spectroscopy and quartz crystal microbalance with dissipation monitoring (QCM-D) are used to elucidate qualitative differences in the adsorption and self-assembly process, the final mass deposited per unit area, and the coupled and bound water within and on the lattice formed by the glycoprotein wtSgsE and the protein rSgsE, respectively.

2. Experimental

Unless otherwise stated, all solvents and reagents were purchased from Sigma–Aldrich (Vienna). Buffer solutions were prepared with Milli-Q water (resistivity: $\geqslant$18.2 MΩ cm^{-1}).

2.1. Bacterial strain and growth conditions

G. stearothermophilus NRS 2004/3a was obtained from the N. R. Smith Collection, US Department of Agriculture (Peoria, IL) and was grown in a fermenter on modified S-VIII medium at 55 °C as previously described [35]. Cells were separated from culture broth by continuous centrifugation (Sepatech 17 RS centrifuge; Heraeus, Vienna, Austria) at 16,000×g and 4 °C. The biomass was stored at −20 °C.

2.2. Preparation of the wild-type S-layer glycoprotein samples

Wild-type S-layer glycoprotein was isolated from cell wall preparations by extraction with 5 M guanidine hydrochloride (GHCl; Fluka, Buchs, Switzerland) according to a previously described procedure [35]. Purification of the wild-type protein wtSgsE was performed and monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE; see Supplementary data, Fig. S1) as previously described [36].

2.3. Preparation of the recombinant protein

All experiments were carried out with a 130 amino acid N-terminal truncation (rSgsE$_{131-903}$) [34] of the S-layer protein SgsE of *G. stearothermophilus* NRS 2004/3a. In the following, rSgsE$_{131-903}$ is called rSgsE for simplification.

Escherichia coli DH5a (Invitrogen, Lofer, Austria) was used for cloning; overexpression of proteins was accomplished in *E. coli* BL21 Star (DE3). Both *E. coli* strains were grown at 37 °C in Luria–Bertani broth (LB broth) supplemented with kanamycin (50 mg mL^{-1}). Overexpression and purification of the recombinant protein were performed and monitored by SDS–PAGE (data not shown) as previously described [34]. Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad, Vienna, Austria) using BSA as standard. The molecular weight of rSgsE was calculated to be 82,800 Da [37].

2.4. Atomic force microscopy (AFM)

A Digital Instruments Nanoscope IIIa (Santa Barbara, CA, USA) was used with a J-scanner (maximal scan size, 130 mm). Standard 200 mm long oxide-sharpened silicon nitride cantilevers (NanoProbes, Digital Instruments) with a nominal spring constant of 0.06 N m^{-1} were used for imaging. The S-layer proteins on the QCM-D quartz crystals were imaged by AFM at a scan rate between 4 and 5 Hz. The applied force was kept to a minimum during