FORMATION OF NANOTEMPLATES BY GENETICALLY ENGINEERED TRUNCATED S-LAYER PROTEINS WITH PRESERVED SELF-ASSEMBLY POTENTIAL.

P. Ryzhkov[1], K. Ostermann[1], A. Blüher[2], M. Mertig[2], G. Rödel[1]
[1]Technische Universität Dresden, Institut für Genetik, Zellescher Weg 20b, D-01217 Dresden, Germany
[2]Technische Universität Dresden, Institut für Werkstoffwissenschaft, Helmholtzstraße 7, D-01062 Dresden, Germany
pavel_m_ryzhkov@yahoo.com

Surface layer (S-layer) proteins are the outermost cell wall components, found in many types of bacteria and archaea. S-layers mostly consist of a singular type of protein, rarely of two or more different proteins [1]. These proteins form periodic ordered structures with oblique, square or hexagonal type symmetry. It is currently believed, that such structures are formed by non-covalent interactions in an entropy-driven self-assembly process [2]. The unit of the S-layer lattice is composed of 1 to 4 different proteins (p1 – p4 lattice types), thereby forming characteristic pores of 2 to 8 nm. The main factors that disrupt the formation of S-layers are changes in the pH value, changes of cultivation temperature, oxygen limitation, mechanical stress and application of different chemicals, interfering with hydrogen bonds formation (e.g. guanidine hydrochloride, urea). For a variety of S-layer proteins it was shown that the isolated protein monomers maintain the ability to form periodic self-assembly structures and recrystallize in solution, on lipid films (e.g. on the surface of liposomes [3]), or on liquid/air and liquid/solid phase interfaces. S-layer periodic structures are of high interest for different applications in nanotechnology. For example, they have been used as nanotemplates for the formation of metal clusters, for the immobilization of different reagents and as molecular sieves.

We will report on our investigations of the self-assembly potential of the *Sporosarcina* (*S.*) *ureae* S-layer protein SslA and genetically engineered derivatives. The native SslA S-layer exhibits a p4 square type symmetry and a lattice constant of 13.2 nm. Reassembled S-layer sheets have been shown to bind metal ions and to facilitate the formation of metal clusters [4]. Until recently, biotechnological work with the *S. ureae* S-layer protein was impeded by the fact that its amino acid and the corresponding DNA coding sequences were not known. We isolated and determined the DNA sequence of the *sslA* gene encoding the S-layer protein of *S. ureae* [5]. A Cys residue at the extreme carboxyl-terminal end – an unusaual feature for S-layer proteins – may explain the affinity of SslA lattices for heavy metal ions.

We constructed several truncated protein versions, expressed them heterologously in *E. coli* and analyzed their ability to self-assemble into supramolecular structures. Based on the results of multiple sequence alignments with the S-layer proteins SbsC from *Bacillus stearothermophilus* and SbpA from *Bacillus sphaericus*, for which an extensive truncation analysis was performed [6,7], it was estimated that the N- and C-terminal regions of SslA might be deleted without affecting its self-assembly properties. We constructed (i) an N-terminally truncated SslA with deletion of the first 341 amino acids, (ii) a C-terminally truncated SslA, which lacks the last 172 amino acids, and (iii) a SslA derivative with the above mentioned truncations at the both C- and N-terminal ends (see fig. 1). The respective SslA constructs were overexpressed in *E. coli*, monomerized with 5 M GuHCl after isolation, and dialysed against dH_2O for 24 to 48 hours to allow self-assembly. The native SslA was prepared from *S. ureae* cells and treated in the same way. Self-assembly products were precipitated onto the substrate surface and analysed by means of scanning (SEM) and transmission electron microscopy (TEM). Microscopy data revealed that all truncated protein versions formed nanosheets with typical dimensions in the range of 0.1-10 µm, that are similar to those formed by the native SslA protein (fig. 2). Based on the assembly studies with the native SslA protein on different surfaces (Si wafers, silanized Si wafers, glass, APTES-glass) self-assembly structures have a higher affinity to the surfaces with lower

hydrophobicity. This finding can be explained by a model, according to which the protein multilayer structures are first formed in solution at high protein concentrations, and thereafter attach with their exposed hydrophilic parts to the substrate surface.

In summary, our work shows that native as well as recombinant SslA monomers have the ability to form self-assembly structures upon crystallization in solution and on the substrate surfaces. Self-assembly into nanosheets of 0.1 to 10 µm is mediated by the central protein part and not impaired by C- or N-terminal deletions.

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Figures:

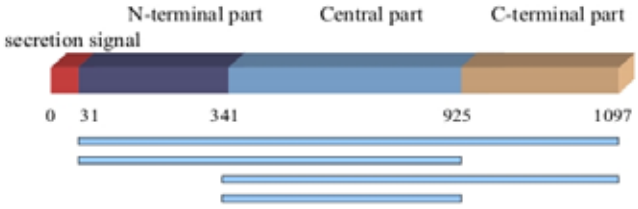

Fig.1 Schematic representation of SslA protein sequence: processed as well as truncated versions are indicated below

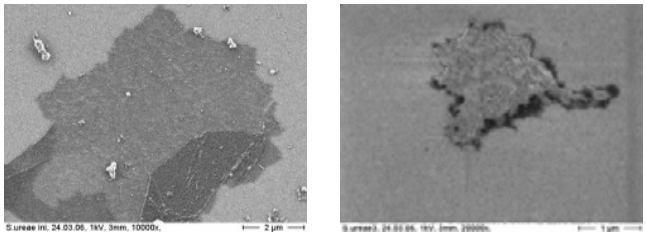

Fig.2 SEM images of self-assembly nanosheets: structures formed by the native protein (left) and the C-terminally truncated version (right) are shown as examples