Effect of Ethanol on Self-assembly of SbpA Surface Layer Protein

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Abstract

Bacterial cell surface layers (S-layers) are found on the cell envelope component of many bacteria and archaea. S-layers are made up of protein subunits which form a protective crystalline lattice at the outer cell wall. S-layers have attracted much interest in the scientific community due to their ability to self-assemble and produce supramolecular arrangements at the Nanoscale [1].

SbpA is a relatively well-studied S-layer protein and is a promising material for surface functionalization in biosensors and biofuel cells [2, 3]. In many of such applications it is exposed to solutions that have aggressive components with an effect on the protein's conformation. It is, therefore, important to verify the explicit effects of sample components that are potentially damaging to the protein layer, such as ethanol.

The aim of the present study was: (i) to test the effects of exposure to different amounts of ethanol of a readily recrystallised S-layer; and (ii) check the influence of ethanol at low concentrations added during the S-layer recrystallization phase. The latter is of interest as addition of ethanol can be used to tune the surface energy of the substrate, in this way acting as a surfactant.

SbpA recrystallisation has been carried out on silicon following the procedure reported by Vollenkle et al [2]. Readily assembled protein coatings on silicon were exposed to a range of concentrations of ethanol for 1 hour, after which the surface was imaged by the Atomic Force Microscopy (AFM) in MilliQ water. It has been found that SbpA structure showed gradual deterioration following the ethanol concentration; however, it sustained the distinct island nature of the films until the concentrations of ethanol up to 40% were reached (Figure 1). At concentrations exceeding this amount the film morphology changed dramatically (Figure 1), possibly due to unfolding of the self-assembly domain.

While the protein demonstrating unparalleled stability for ethanol exposure following the assembly, a different effect has been found when ethanol was added during the recrystallisation step. Here, additions of up to 5 % ethanol during the assembly stage did not interfere with the recrystallization process and in many cases resulted in better surface coverage and minimization of gaps between the islands (Figure 2). Exposure to higher ethanol content, in this case, quickly leads to the loss of the S-layer pattern and the crystallinity of the coating. These findings show promise in the use of SbpA as a substrate to create biosensors that can operate in the presence of ethanol.

References

[1] Sleytr, U., Messner, P., Pum, D., & Sara, M.. *Angewandte Chemie* **38** (1999) 1034-1054.
[2] Vollenkle C, Weigert S, Ilk N, Egelseer E, Weber V, et al. *Appl Environ Microbiol* **70** (2004) 1514-21.
[3] Ilk N, Vollenkle C, Egelseer EM, Breitwieser A, Sleytr UB, Sara M. *Appl Environ Microbiol* **68** (2002) 3251-60.

Figures

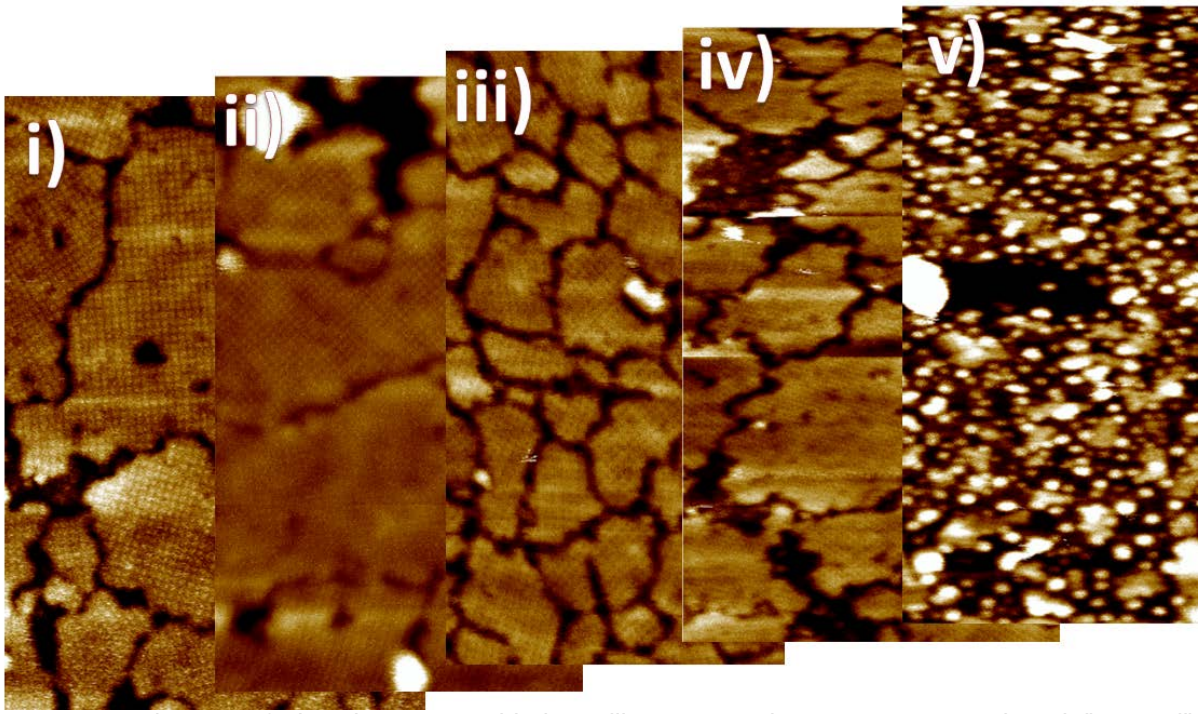

Figure 1. AFM images of SbpA layer assembled on silicon upon 1 hour exposure to ethanol: i) 10%, ii) 20%, iii) 30%, iv) 40%, v) 50%. Original images were acquired at 1x1 μm scan size.

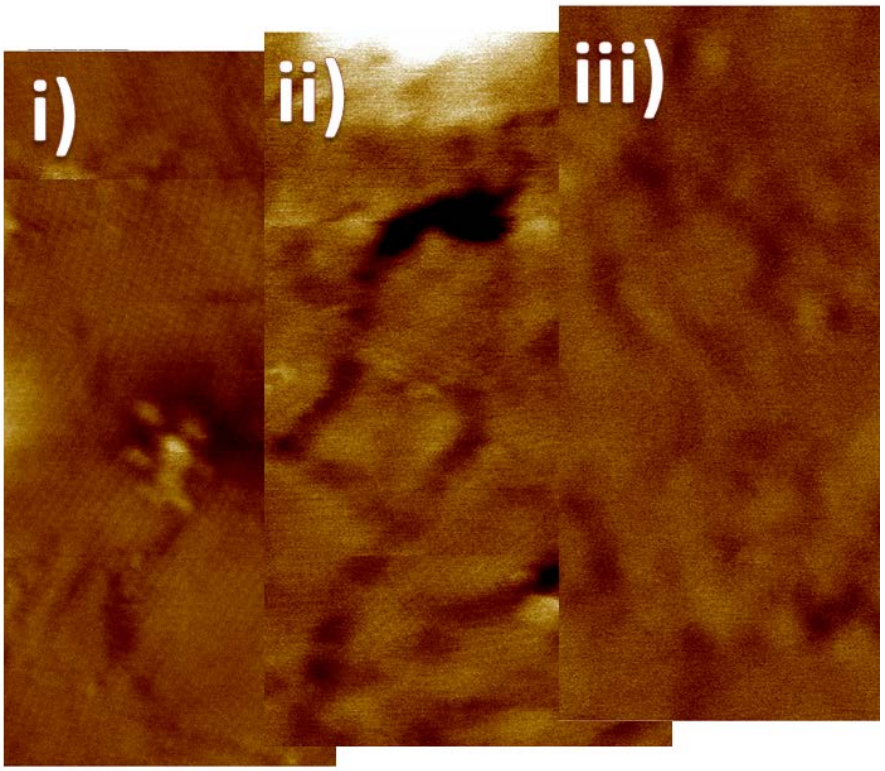

Figure 2. AFM images of SbpA layer assembled on silicon with addition of ethanol to recrystallisation solution: i) 2.5%, ii) 5%, iii) 7.5%. Original images were acquired at 1x1 μm scan size.