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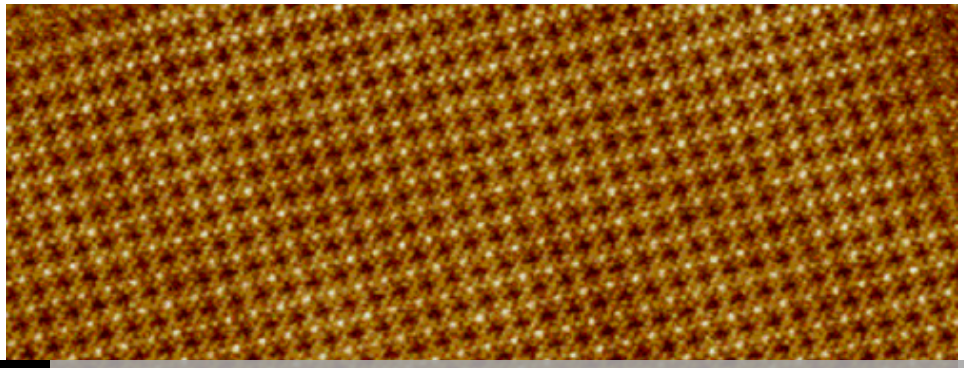
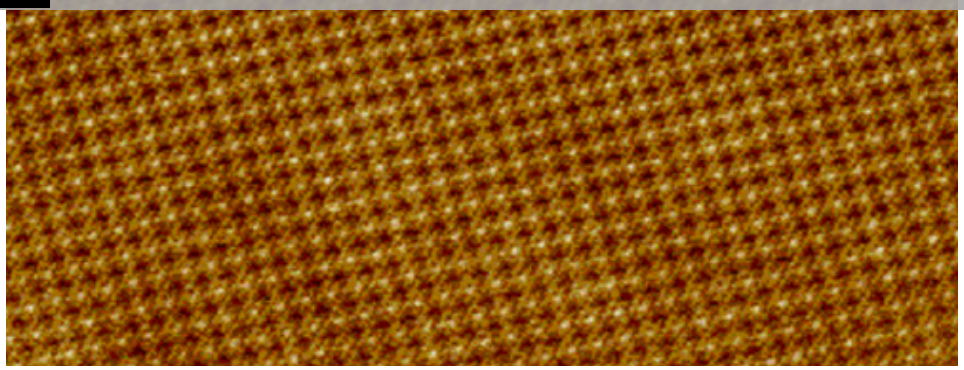


Pre-master Thesis

Isolation and Analysis of S-layer Proteins From Different Bacterial and Archea Species



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Title: Isolation and Analysis of S-layer Proteins From Different Bacterial and Archea Species

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Part I

Introduction

CHAPTER 1

INTRODUCTION

1.1 Crystalline Bacterial Cell Surface Layers

1.1.1 Occurrence and Structure of Surface Layers

Crystalline surface layers (s-layers) have been identified in a number of different bacteria and archaea species. The s-layers are composed of identical single proteins or glycoprotein species (s-layer proteins)with a M_w between 40 and 200kDa. They have shown the ability to self-assemble into monomolecular lattices when isolated from the cell. The lattice type can have the symmetry of oblique (p1,p2), square (p4), or hexagonal (p3, p6) as shown on Figure 1.1. The unit cell dimensions is in the range of 3-30 nm. One morphological unit consist of one, two, three, four, or six identical proteins depending of the lattice type. S-layers are generally 5 to 25 nm thick. They are a highly porous protein meshwork and as s-layers are composed of identical subunits they also exhibit pores of identical size and morphology. The size of the pores is in range of 2-8 nm. [U.B. Sleytr et al., 2007] [D. Pum et al., 2004] [M. Sará and U.B. Sleytr, 2000]

1.1.2 Reassembly of Surface Layer Proteins

Isolated s-layer proteins have shown to reassemble into crystalline arrays in suspensions and at interfaces, such as solid supports and the air-water interface. The typical isolation and purification techniques of s-layer proteins involves mechanical disruption of the cells and centrifugation steps to separate the cell-wall fragments. A high concentration of hydrogen-bond-breaking agents, such as guanidine hydrochloride are used to completely solubilize the s-layers into their constituent subunits. The recrystallization of s-layer proteins occur upon dialysis of the disintegrating agent.

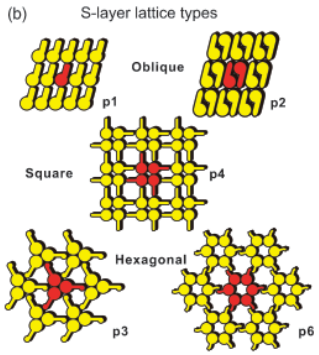


Figure 1.1: The five different lattice types formed by s-layer proteins

At solid interfaces the crystal growth of the s-layer proteins is initiated simultaneously at several randomly nucleation points. The growth proceeds in-plane until the crystalline domains meet. This leads to closed, coherent mosaic of individual micrometer large s-layer domains. [D. Pum et al., 2004] [E.S. Györvary et al., 2003]

1.2 Project Description

One of the main challenges in the development of biosensors is the minimization and integration of biosensor platforms/arrays. The design of the biosensor array needs to be in concurrently with the smaller reaction volumes, the larger numbers of detection sites, and the integration of various functionalities. The reduction of costs and resources is another important aspect in the development of biosensor array. [A. Agah et al., 2005]

Molecular and biological self-assembly systems have the potential to deal with the challenges in size and design of biosensor arrays. One of these systems are the s-layers. These are composed of s-layer proteins with the ability to self-assemble into monomolecular lattices when isolated from the cell. [U.B. Sleytr et al., 2007] [D. Pum et al., 2004]

The aim of this project is first and foremost to isolate the s-layer proteins from different bacteria and archaea species and then study the self-assembly properties of s-layer proteins. Secondly will the aim be to construct a functional biosensor based on the binding of enzyme to the s-layer array.

The species that will be investigated are; *Haloferax volcanii*, *Haloferax mediterranei*, *Halobacterium salinarum*, *Deinococcus radiodurans* and *Sulfolobus solfataricus*. AFM will be used to study the self-assembly of the s-layer proteins on a mica surface and a silicon surface. SPR will investigate the binding between enzyme and the s-layer. Traditional methods for protein studying will also be used to examine the s-layer proteins. This include methods as; gel electrophoresis (SDS-PAGE) and protein concentration determination methods (ultraviolet absorbance and/or Bradford protein assay).

Part II

Materials and Methods

CHAPTER 2

MATERIALS AND METHODS

2.1 Bacterial Strains and Growth Conditions

2.1.1 *Haloferax volcanii*, *Haloferax mediterranei*, *Halobacterium salinarum*, and *Deinococcus radiodurans*

A preculture of 50 mL was incubated at 250 rpm and 37°C for approximately a week. The preculture was then added to a cell culture to give a volume of 250 mL. This was incubated at 250 rpm and 37°C for approximately a week.

2.1.2 *Sulfolobus Solfataricus*

A preculture of 50 mL was incubated at 250 rpm and 70°C overnight. The preculture was then added to a cell culture to give a volume of 250 mL. This was incubated at 250 rpm and 70°C overnight.

2.2 Isolation of S-layer Proteins From Cell Wall

2.2.1 *Haloferax volcanii* and *Haloferax mediterranei*

The cell culture was centrifuged at 7,000g for 30 minutes. The pellet was resuspended in 200 mL modified medium. 60 mL of 0.5M EDTA was added and the solutions was incubated at 250 rpm for 30 minutes. The solution was centrifuged three times; 15 minutes at 3,000g, 5 minutes at 7,000g, and 10 minutes at 15,000g. [M. Sumper et al., 1990] The supernatant was kept in a -80°freezer overnight before being freezedried. The result from the freezedrying was kept at -4°C. 2g of the produckt from the freezedrying was dissolved in 5 mL of 5M guanidine hydrochloride

overnight at 60°C. The solution was then centrifuged at 20,000 g for 30 minutes. Centricon centrifugal filter devices was used to desalt the solution.

2.2.2 *Sulfolobus solfataricus* and *Halobacterium salinarum*

The cell culture was centrifuged at 10,000g for 30 minutes. The pellet was resuspended in 150 mL of HEPES buffer. The solution was centrifuged at 10,000g for 30 minutes. The pellet was resuspended in 5 mL of 5M guanidine hydrochloride overnight at 60°C. The solution was then centrifuged at 20,000 g for 30 minutes. The supernatant was dialyzed against 10 mM $CaCl_2$ for a minimum of 24 hours. The final solutions was kept at -4°C. [U.B. Sleytr et al., 1986]

2.2.3 *Deinococcus Radiodurans*

Almost the same as with *Sulfolobus solfataricus* and *Halobacterium salinarum*. The dialyze was replaced with the Centricon centrifugal filter devices.

2.3 Analysis of S-layer Protein Assembly

2.3.1 SDS-PAGE

A 7.5% SDS-PAGE was used to analyze the isolated s-layer proteins. 10 μL sample was mixed with 10 μL sample buffer and then boiled for 5 minutes. The samples were loaded onto the gel and it was run at 130 V for 90 minutes. 5 μL of low range molecular standard was used. The stacking gel was cut of and the separation gel was stained using Comassie briliant blue.

2.3.2 Atomic Force Microscopy

10 mL of solution with s-layer proteins was added to a freshly cleaved mica surface or silicon surface. After 10 minutes the surface was dried and analyzed in the AFM.

Part III

Results

CHAPTER 3

RESULTS

3.1 SDS-PAGE

Figure 3.1 shows the SDS-PAGE gel with the isolated s-layer proteins from *H. volcanii*, *H. mediterranei*, *D. radiodurans*, *S. solfataricus*, and *H. salinarum*.

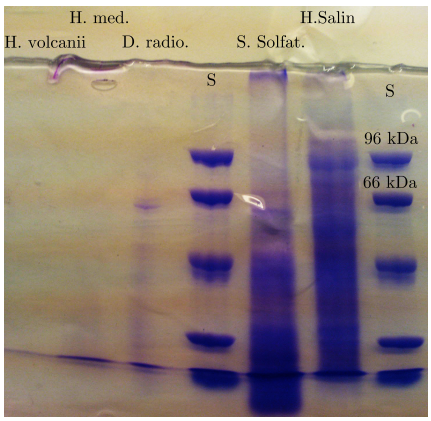


Figure 3.1: A SDS-PAGE gel with the isolated s-layer proteins from H. volcanii, H. mediterranei, D. radiodurans, S. solfataricus, *and* H. salinarum.

The lanes with *H. volcanii* and *H. mediterranei* does not show any bands indicating that no proteins have been isolated.

The lane with *D. radiodurans* show several weak bands indicating a low concentration of the isolated proteins. Studies have shown that the size of the s-layer protein is around 120 kDa. None of the visible bands are seen within this size range. The most distinct band lies just below 66 kDa.

The lanes with *S. solfataricus* and *H. salinarum* show several clear bands indicating a high concentration of isolated proteins. No previous studies of *S. solfataricus* mention the size of the s-layer protein. It is therefore not possible to compare the results from the SDS-PAGE. However, several bands are located between 40 kDa and 200 kDa which is size range of s-layer proteins in general. Studies of *H. salinarum* have shown a size of the s-layer protein around 100 kDa. From the *H. salinarum* lane a distinct band is located around this size.

3.2 AFM

Figure 3.2 shows AFM images and profiles of s-layer proteins isolated from *S. solfataricus*.

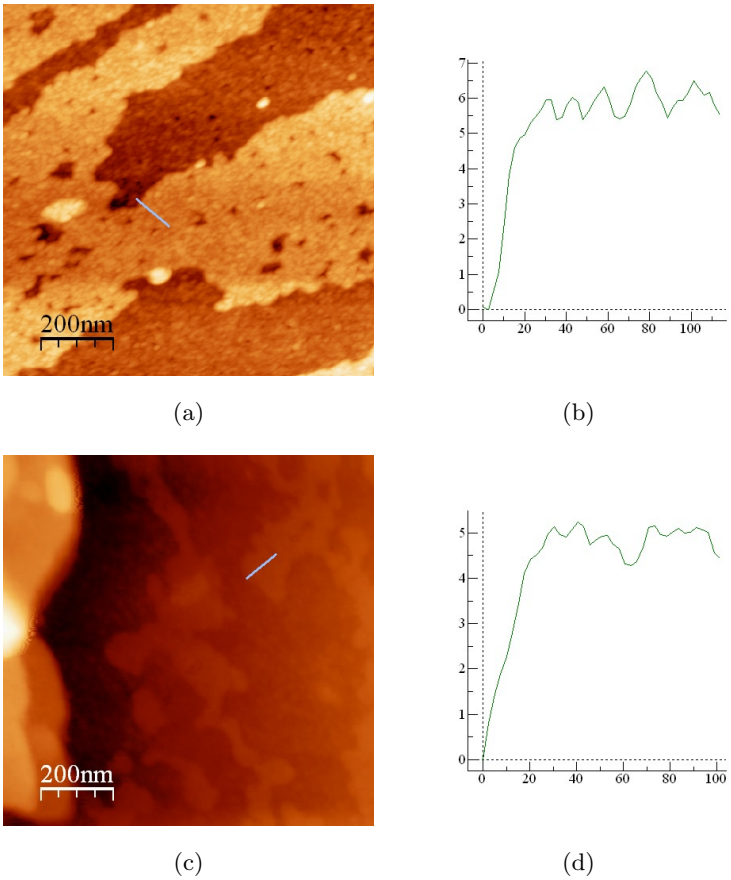
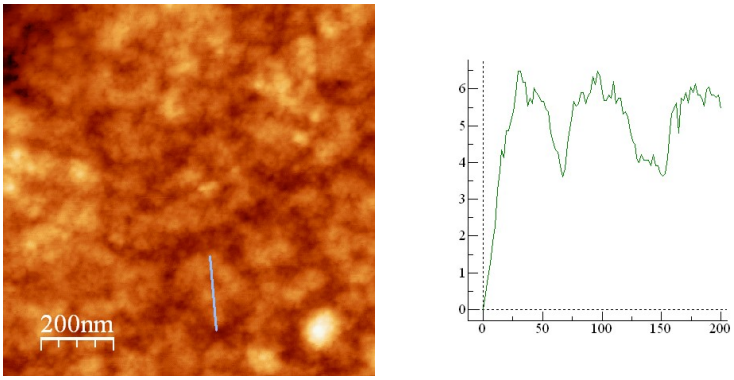


Figure 3.2: (a) and (c) are AFM images of isolated s-layer proteins from S. solfataricus placed on a silicon surface. (c) and (d) are profiles of (a) and (d) respectively

Both AFM images of the s-layer proteins from *S. solfataricus* shown a distinct lattice pattern. The profiles from both images indicates that the height of the s-

layer lattice is between 5-6 nm thick. This corresponds with the description of s-layer proteins in general.

Figure 3.3 shows the AFM image and profile of s-layer proteins isolated from *h. salinarium*.



(a) (b)

Figure 3.3: (a) is the AFM image of isolated s-layer proteins from h. salinarium placed on a silicon surface. (b)is the profile of (a).

Part IV

Conclusion and Perspective

CHAPTER 4
CONCLUSION AND PERSPECTIVE

4.1 Conclusion

From the three different isolation protocols in Section 2.2 the most useable protocol is found to be the one with *S. solfataricus* and *H. salinarum*. This protocol is chosen both from the result in Section 3.1 and from other studies of s-layer proteins. Other studies describes this to be the standard protocol for isolating various types of s-layer proteins. The results from the SDS-PAGE in Section 3.1 also shows that the visible bands are from *S. solfataricus* and *H. salinarum*. The next step in the isolation process is to modify the selected protocol to match the selected s-layer proteins. This could for instance be to change the dialysis solution.

From the AFM results in Section 3.2 it is shown the s-layer proteins from *S. solfataricus* and *H. salinarum* form monolayer self-assemble point on a unmodified silicon surface. From this it can be concluded that the s-layer proteins has been isolated from the cell surface and that they self-assemble on solid surfaces. *Sulfolobus solfataricus* was also tested on a mica surface but no useable images has been produced yet. The s-layer protein from *D. radiodurans* was tested on a mica surface but no useable images was produced. For future work in the analysis part the surfaces need to be modified (hydrophobic or hydrophillic). The immobilization process should also be modified. Other studies mention that a sample of s-layer proteins are left on the surface for 3-6 hours before drying. Some studies even left the sample for more than 24 hours. For comparison the samples in these experiments was left on the surface for 10 minutes.

4.2 Perspectives

The next period of this master will not only include the mentioned modifications for the isolation protocol and for the surfaces used in analysis of immobilized s-layer

proteins. It will also include the study of other surfaces such as gold. The analysis of the s-layer lattice should also be studied more thorough in the AFM. It might be a good idea to use SEM to support the AFM analysis. More studies of the proteins with for instance SDS-PAGE would also be required. A more accurate size ladder should be used for this.

The immobilization of an enzyme onto the s-layer lattice would require the use of surface plasmon resonance (SPR) to follow the binding process. The enzyme activity from both bulk and immobilized enzymes should be studied. Studies of the protein concentration would also be necessary in this regard.

As these experiments should be comprehensive and could easily turnout to be time-consuming only one or two s-layer proteins should be studied at first. More s-layer proteins can be added as the experiments are progressing.

BIBLIOGRAPHY

[A. Agah et al., 2005] A. Agah, A. Hassibi, J.D. Plummer, and P.B. Griffin (2005). Design requirements for integrated biosensor arrays. *Proceedings- SPIE The International Society for Optical Engineering*, 5699:403–413.

[D. Pum et al., 2004] D. Pum, B. Schuster, M. Sará, and U.B. Sleytr (2004). Functionalisation of surfaces with s-layers. *IEE proceedings. Nanobiotechnology*, 151:83–86.

[E.S. Györvary et al., 2003] E.S. Györvary, O. Stein, D. Pum, and U.B. Sleytr (2003). Self-assembly and recrystallization of bacterial s-layer proteins at silicon supports imaged in real time by atomic microscopy. *Journal of Microscopy*, 212:300–306.

[M. Sará and U.B. Sleytr, 2000] M. Sará and U.B. Sleytr (2000). S-layer proteins. *Journal of Bacteriology*, 182:859–868.

[M. Sumper et al., 1990] M. Sumper, E. Berg, R. Mengele, and I. Strobel (1990). Primary structure and glycosylation of the s-layer protein of *Haloferax volcanii*. *Journal of Bacteriology*, 172:7111–7118.

[U.B. Sleytr et al., 2007] U.B. Sleytr, C. Huber, N. Ilk, D. Pum, B. Schuster, and E.M. Egelseer (2007). S-layers as a tool kit for nanobiotechnological applications. *FEMS Microbiology Letters*, 267:131–144.

[U.B. Sleytr et al., 1986] U.B. Sleytr, M. Sará, Z. Küpcü, and P. Messner (1986). Structural and chemical characterization of s-layers of selected strains of *Bacillus stearothermophilus* and *Desulfotomaculum nigrificans*. *Archives of Microbiology*, 146:19–24.