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Use of specific metal binding of self-assembling S-layer proteins for metal bioremediation and recycling

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Abstract. Most bacteria and all archaea possess as outermost cell envelope so called surface-layers (S-layers). These layers were formed by self-assembling proteins having a number of habitat depending interesting intrinsic properties. As example, S-layers from bacterial isolates recovered from heavy metal contaminated environments have outstanding metal binding properties and are highly stable. Thus they selectively bind several metals with different affinity. For using S-layer proteins for metal bioremediation and recycling three aspects of the metal-interactions with S-layer proteins must be taken into account. First, S-layers possess different functionalities, e.g. carboxyl, phosphoryl groups, binding toxic metals and metalloids, like U(VI) and As(V), nonspecifically depending on pH. Second, precious metals like Au and Pd are likewise nonspecifically bound to functional groups, but presumably covalently, making the binding irreversible. Third, intrinsic specifically bound metals, e.g. Ca^{2+}, are needed for native protein folding, self-assembly, and the formation of highly-ordered lattices. Their binding sites also allow selective binding of chemical-equal elements including the trivalent rare earth elements, possessing comparable ionic radii. Thus this study combines older and recently generated results regarding the metal dependent binding behavior of the S-layer proteins. It enables the development of biohybrid materials for the separation, removal or recovery of strategic relevant metals from natural occurring or industrial waste waters using pH-value as regulating parameter for selective metal binding and also conceivably release.

Introduction

Environmental aspects, increasing prices of metals, strategic issues, such as independent resource access and increasing demand, give rise to investigate and develop newly metal remediation and recycling strategies. Nature, and by this biological components, may often offer potential environmentally friendly and cost efficient solutions for those issues. This study should give one example of bioligand which can be used directly or as biocomposite material for metal bioremediation and recycling.

Most bacteria and all archaea possess as outermost proteinaceous cell envelope so called surface-layers (S-layer). S-layers are comprised of identically (glyco)protein monomers with a molecular weight ranging from 40 to 200 kDa and feature the intrinsic property of being able to spontaneously self-assemble in suspension, at interfaces, or on technical surfaces, thereby forming two-dimensional highly regular paracrystalline lattices [1]. These S-layer lattices possess regular arranged pores with defined size and different kinds of also regular arranged functional groups. The formation of stable and functional S-layer lattices via self-assembly on surfaces or interfaces is a dynamic and complex process. Despite the fact that S-layer proteins have been investigated for over 30 years, the full reaction cascade of self-assembly, which includes the role of different bivalent cations such as Ca^{2+} and Mg^{2+}, is still not fully understood. Furthermore, these S-layers have a number of intrinsic properties and largely determine the cells interaction with the environment including selective exchange of nutrients and the interaction with external stressors, e. g. heavy metals. Interestingly, S-layers from bacterial isolates recovered from heavy metal contaminated environments have outstanding metal binding properties and are highly stable. They show potential for selective binding of several metals, some of them with high affinity [2]. Therefore, for using S-layer proteins directly or as biocomposite material for metal bioremediation and recycling three aspects of the metal-interactions with S-layer proteins (prepared as described in [3]) must be taken into account. These already published as well as recently observed metal-S-layer-interactions will be described in the following section.

Metal binding of S-layer proteins and biocomposites

S-layers possess different functionalities, e.g. carboxyl, phosphoryl groups, nonspecifically binding toxic metals and metalloids, like U and As. This interaction process is strongly driven by pH-value as for metal-binding the functionalities need to be deprotonated. Raff et al. [4] demonstrated that cells and S-layers of the bacterial waste pile isolate *Lysinibacillus sphaericus* JG-A12 have high binding capacities for U and Cu. As another new example in Figure 1 the sorption behavior of different S-layers for As(V) is shown. It can be seen that some of the S-layers show a better sorption of As(V) compared to the iron hydroxide reference material FerroSorp®.

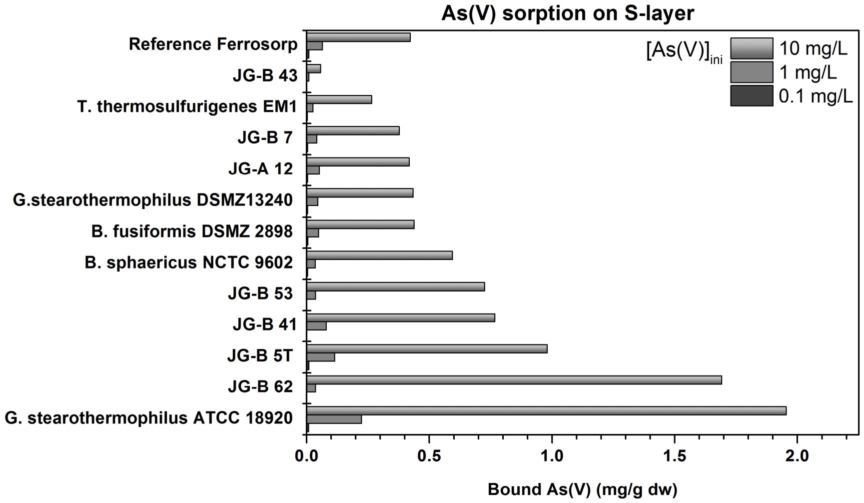

Figure 1: As(V) sorption by S-layer proteins with a concentration of 1 mg/mL in comparison to reference material Ferrosorp®.

A second type of metal binding by S-layer proteins is demonstrated with precious metals like Au(III) and Pd(II). They are likewise nonspecifically bound to functional groups, but presumably covalently, making the binding irreversible unless the S-layer protein is destroyed completely. Examples for binding of those metals by S-layer proteins from *L. sphaericus* JG-A12 and JG-B53 are given in several studies [3,5,6,7].

In a third case, intrinsic and highly specific metal binding sites being relevant for native protein folding of the S-layer protein monomer, self-assembly, and the formation of highly-ordered lattices are involved. In the native S-layer proteins bivalent cations such as Ca^{2+} are bound. As known from titration experiments, there are at least two different binding sites for these bivalent cations showing different binding affinities. Important is that these binding sites not only allow selective binding of calcium, but also of chemical-equal elements including the trivalent rare earth elements (REE), possessing comparable ionic radii. REE were bound with higher affinity due to their higher charge, what was also proven by titration and laser fluorescence spectroscopic experiments (data not shown). Recently, sorption experiments were conducted to investigate whether there is a selectivity of S-layer proteins for a certain REE by using a multielement REE-model solution at pH 3.5 containing several REE in varying amounts. As can be seen in Fig. 2 (left) all REE with around 15%

are bound to the S-layer JG-A12 in similar amount. So there is no preferred binding of one REE of the tested ones under those conditions. The quite low pH value was chosen to favor the selective binding via Ca^{2+} binding sites over deprotonated functional groups.

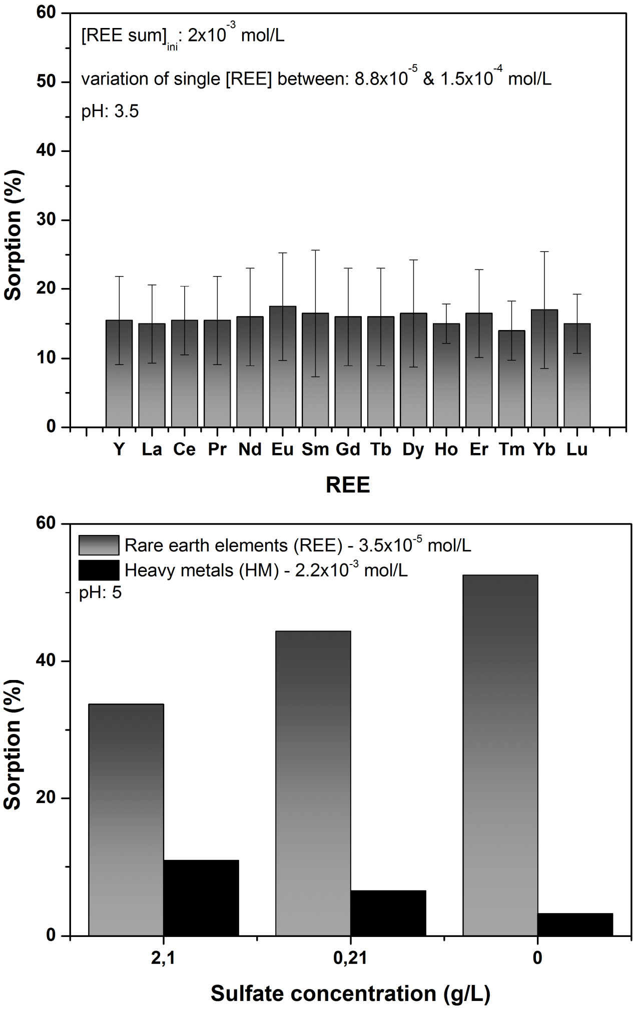

Figure 2: Sorption of rare earth elements (REE) by S-layer protein JG-A12 (1 g/L). Left: Model solution with random distribution of REE-concentrations in the range of $8.8x10^{-5}$ to $1.5x10^{-4}$ mol/L at pH 3.5. Right: Model solution of REE ($3.5x10^{-5}$ mol/L) mixed with heavy metals (HM, 2.2 x 10^{-3} mol/L) and varying sulfate concentration at pH 5.

Additionally, selective binding of REE compared to heavy metals (HM) by S-layer proteins could be shown (Fig. 2 right) from a model solution containing 60 times more HM than REE. This study also showed that the selectivity of the metal sorption to S-layer proteins is influenced by competing factors like sulfate concentration as shown in Fig. 2 (right). With increasing sulfate concentration the selectivity of REE over HM is decreasing. Most likely, sulfate increases the hydrophobicity of the proteins (Hofmeister series) and by this may alter the protein structure, e.g. the specific binding sites, leading to a decreased specific binding of REE.

Whereas the experiments demonstrate the general suitability of S-layer proteins for the selective enrichment of industry relevant metals in natural and industrial water samples, the results demonstrate coevally the interference of the metal binding with other substances, e. g anions, dissolved in the water. Thusly further experiments have to be carried out to identify all water dissolved matter influencing the selective metal binding by S-layers and to identify the real application potential of S-layer based materials.

Nevertheless, there are already some experiments where the S-layers were used as biological components for the production of (semi)metal-binding biohybrid materials [4,8]. As one example in this study nanoparticulate metal oxide materials like magnetite and titanium dioxide were immobilized on the S-layers of JG-A12 for As(V) sorption [9]. The biohybrid materials showed better binding of As(V) than the protein and the nanoparticulate materials alone and surpass also the binding capacity of the reference material FerroSorp®. Additionally, it could be shown that there is a kind of synergistic effect between the two components of the biocomposite materials as the As(V) overall sorption capacity of the biohybrid materials is better than the sum of the sorption capacity of the two single components (Fig. 3).

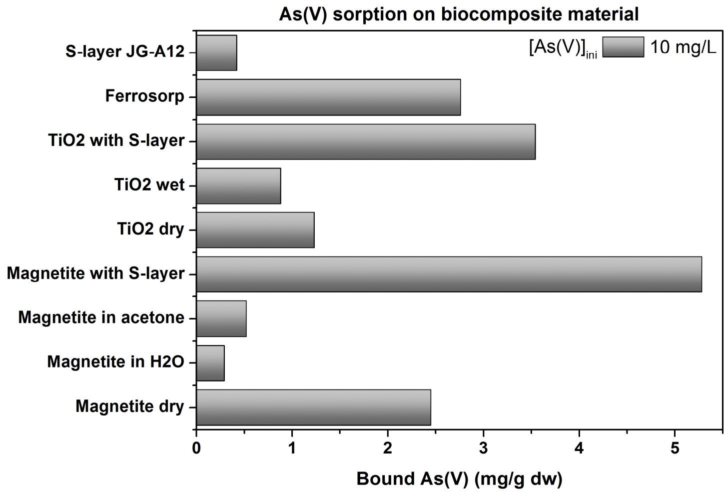

Figure 3: As(V) sorption by biocomposite materials with S-layer JG-A12 as biocomponent, the single components and the reference material Ferrosorp® each in a concentration of 1.7 mg/mL (for experimental details see [9]).

Summary

This study shows that the intrinsic properties and physiological functions of the S-layer proteins build the base for its selective metal binding behavior and its potential for fabrication of biohybrid materials. So by combination of S-layers with well-known sorption materials, functional coatings or composite materials with improved properties can be developed. The produced biohybrid materials can be directly used as selective metal filter material for the removal or recovery of strategic relevant metals using pH-value as regulating parameter for selective metal binding and also a conceivably release (Fig. 4).

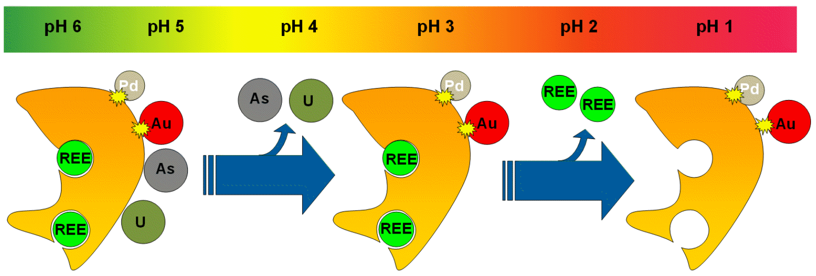

Figure 4: Schematic drawing of selective metal binding and conceivable release by the S-layer protein monomer.

Thus, the knowledge of the metal dependent binding behavior of the proteins not only contributes to the development of biohybrid materials for the separation, removal or recovery of strategic relevant metals, but also allows a biochemical and mechanistic understanding of the interaction of different metal ions with S-layer proteins.

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