Self-assembly of *Deinococcus radiodurans* S-layer Proteins

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ABSTRACT

Proteins capable of self-assembly are crucial to many bio-architectures and have potential applications in nanotechnology. An example is the group of proteins used to construct crystalline surface layers (S-layers) in nearly all archaebacteria and many eubacteria. One extensively studied S-layer is the hexagonally packed intermediate layer, which represents the major cell envelope protein of the radiation-resistant bacterium *Deinococcus radiodurans*. Here, we demonstrate the *in vitro* self-assembly capacity of S-layer proteins isolated from *Deinococcus radiodurans* IR. Our results showed that the self-assemblies were micron-scale sheet structures when observed under light microscope and electron microscopes, including SEM, TEM, and Field Emission SEM.

Keywords: self-assembly, S-layer, *Deinococcus radiodurans*

1 INTRODUCTION

The outer surface of a number of species of bacteria comprises of a porous crystalline membrane called an S-layer, which is made up of protein monomers called S-layer proteins (SLPs) [1]. SLPs have the intrinsic property to reassemble *in vitro* into S-layer on the surface of a broad spectrum of materials such as wafers, metals and biomembranes [2-5]. S-layers exhibit p2, p3, p4, or p6 symmetry and have lattice constants of 3 to 30 nanometers, a thickness of 5 to 15 nm, and a central cavity with identical pore size of 2 to 6 nm, depending on specific SLP from particular bacteria [1, 6]. Several important applications can be derived from these unique properties of S-layer [7]. Ultrafiltration membranes can be made from S-layer. This membrane will have isoporous structure in contrast to conventional membranes which are made of amorphous polymers. The advantage of S-layer membrane is that the isoporous membrane will give a very sharp molecular exclusion limits. Functional ligand can be immobilized to S-layer site-specifically to be utilized in biomedical devices such as DNA detection and analysis, immunoassays and proteomics. S-layer can be reassembled onto liposomes to strengthen and to improve the physicochemical resistance of liposomes. This will enhance the usage of liposomes in medicine. Because of the periodic structure and constant spacing of the identical pores, S-layer can be used as the template for the *in vitro* deposition of metal nanoparticles. This will give rise to a devise with controlled arrangement of metal particles with a defined size and interparticle distance. Such a device is crucial in the fabrication of ultrasmall electronic components.

SLPs have shown the intrinsic tendency to reassemble into two-dimensional arrays after removal of the disrupting agent, such as guanidine hydrochloride (GHCl) and urea, used in the dissolution procedure [8]

One extensively studied S layer is the hexagonally packed intermediate layer of the major cell envelope protein of the radioresistant bacterium *Deinococcus radiodurans* [9]. The cell envelop of this bacterium is unique in having both a Gram-positive-specific trait (thick peptidoglycan layer) and a Gram-negative-specific trait (outer membrane). We have found that *D. radiodurans* is capable of forming multi-cell-forms [10] which are made possible by producing many cross-walls and a large S-layer in a bacterial particle. We have recently isolated a novel slow-growing, radiation- and desiccation-intolerant mutant that appears to have defect

in construction of its S-layer (manuscript in preparation).

D. radiodurans is distinguished by its extraordinary ability to tolerate the lethal effects of DNA damaging agents, particularly those of ionizing radiation. The physiological basis of the extreme radiotolerance has never been adequately explained [11]. Although available evidence indicates that efficient repair of damaged DNA is, in large part, responsible for the radioresistance in this bacterium, it is interesting to know if the S-layer, the outermost layer of a *D. radiodurans* bacterial particle, plays a role in this radiation resistance

Here, we report our study on the self-assembly capacity of *D. radiodurans* SLPs *in vitro*.

2 METHODS

2.1. Bacterial strain and growth conditions.

D. radiodurans IR, originally isolated from γ –irradiated chicken meat, has been characterized previously [10, 12-14]. Cells were routinely propagated with shaking at 160 rpm in plate count broth (PCB; Difco) containing 5 g yeast extract, 10 g tryptone, and 2 g dextrose per liter. phospahte buffer (67 mM, pH 7.0) containing 4.73 g Na_2HPO_4 and 4.54 g KH_2PO_4 in 1 liter distilled water was used for washing, suspending, and diluting bacteria.

2.2. Preparation of S-layer fraction

Stationary-phase cells of *D. radiodurans* IR, washed and suspended in phosphate buffer, were broken by sonication. The cell-wall fraction, collected by centrifugation, washed and suspended in Tris-HCl buffer (50 mM, pH 7.4), was treated with deoxyribonuclease I (1 mg/ml), ribonuclease A (0.5 mg/ml), and Triton X-100 (0.5%). The partially purified S-layer preparation was suspended in Tris- HCl buffer for further use.

2.3. Reassembly of SLPs in suspension

The S-layer preparation was treated with 5 M GHCl to dissolve the SLPs. The GHCl extract was dialyzed against distilled water to allow the reassembly of the SLPs. The self-assemblies in water were stored at 4℃ for further analysis.

2.4. Microscopy

The self-assemblies were collected by centrifugation and suspended in Tris-HCl buffer. A 5-μl aliquot was transferred onto a glass slide and after air dried the self-assemblies were stained with coomassie blue dye before observed under a phase-contrast microscope. The self-assemblies were micrometer-scale in size. Sample of the self-assemblies was also observed under a scanning electron microscope (SEM) (Hitachi S3500N, 15 kV, gold coated), a transmission electron microscope (TEM) (Hitachi H-7500, 100 kV, uranyl acetate stain), and a field emission scanning electron microscope (FE-SEM) (GEOL LSM-6340F)

3 RESULTS AND DISCUSSION

Our results showed that the *D. radiodurans* SLPs self-assemblies were (i) micron-scale sheet structures when observed under light microscope (Fig. 1) and electron microscopes (Figs. 2, 3 & 4). The TEM micrograph showed a surface arrangement of SLPs' self assembly. The result of FE-SEM suggested a multi-layered structure.

In summary, we have demonstrated the *in vitro* self-assembly capability of the SLPs isolated from *D. radiodurans*,

In our laboratory, the self-assemblies of *D. radiodurans* SLPs obtained by various different protocols, will be further characterized in size, physicochemical stability, and potential application as materials in the area of "bottom-up" bio-fabrication technology. An electronic particle-size analyzer is being used to monitor the self-assembly processes of the SLPs.

In addition, we have isolated from *D. radiodurans* IR a number of novel mutants, including those with alteration in bacterial particle size, radiation-sensitivity, "mutator" phenotype, cell division cycle control, or multicell formation effect. These various mutant strains are currently being investigated to help understand a possible role of S-layers and/or S-layer proteins in the unusual radiation survival strategy in *D. radiodurans* These biological studies will also help our characterization of SLPs with regard to their use in the area of nanotechnology.

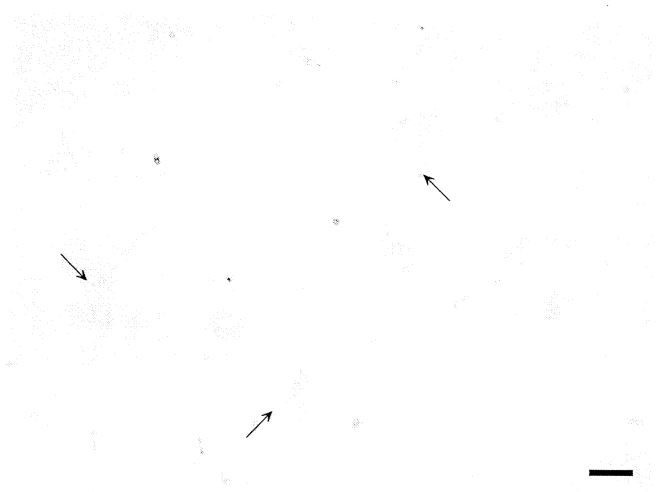

Fig. 1. Phase-contrast micrograph of the self-assemblies of *D. radiodurans* S-layer proteins. Bar = 12.5 μm

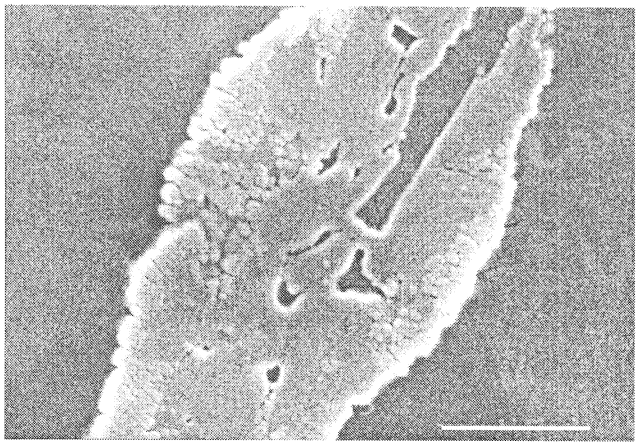

Fig. 2. SEM micrograph of a self-assembly of *D. radiodurans* S-layer proteins. Bar = 1 μm

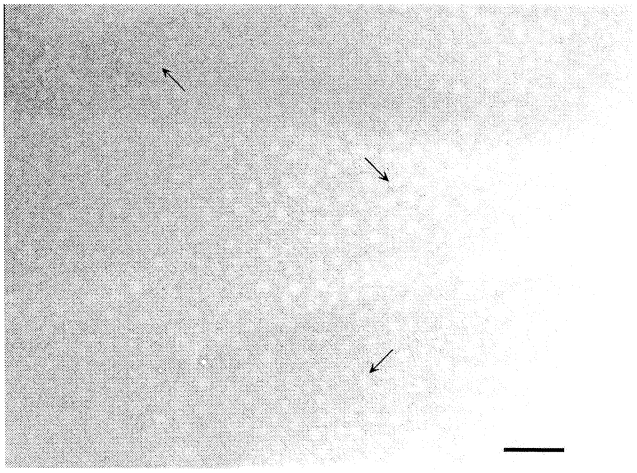

Fig. 3. TEM micrograph of a self-assembly of *D. radiodurans* S-layer proteins. Bar = 100 nm

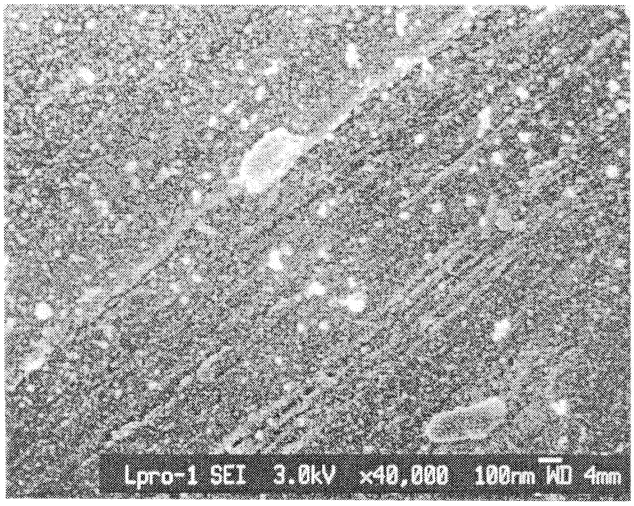

Fig. 4. Field emission SEM micrograph of a self-assembly of *D. radiodurans* S-layer proteins. Bar = 100 nm

ACKNOWLEDGEMENT

This work was partially supported by Department of Education of Taiwan, R.O.C. (DOE 91-E-FA04-1-4/ NTHU 91I0037J4).

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