Role of proteins in controlling nanoparticle size distribution

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Nanomaterials are present in an increasing array of consumer goods, electronics and industrial applications. However, a drawback to currently available methods for the synthesis of nanoparticles is the cost both economic and environmental due to the use of toxic reagents and solvents. Bacteria have been shown to produce nanominerals in natural settings and may be harnessed for the production of nanoparticles with industrial applications.

Here, we report on work carried out to evaluate the role of proteins in the formation and the control of size distribution of Se nanoparticles (SeNPs). The goal of the work is to (1) characterize the role of the cell proteome on SeNP formation and size distribution and (2) identify specific proteins involved in controlling nanoparticle size distribution.

The chemical reduction of selenious acid to elemental selenium carried out in the presence of cell free extract from *Escherichia coli* strand K-12 (*E. coli*) lead to the formation of a SeNPs with a narrow size distribution around 106.7 +/- 8.7 nm whereas, in the absence of cell free extract, the size distribution was much broader (10-150 nm). In order to identify specific proteins responsible for the narrowing in size distribution, we developed a method to purify proteins strongly associated with the SeNPs. We identified several proteins -including a majority of enzymes- and proceeded to overexpress and purify one protein: alcohhol dehydrogenase popanol prefering – AdhP. This protein was confirmed to bind strongly to SeNPs and its presence during the formation of SeNPs lead to a narrowing of the size distribution of the product albeit to a lesser extent than that observed with cell free extract.

Thus we showed that some proteins exert a fundamental control over the size distribution of SeNPs product despite their lack of programmed involvement in mineral formation. Furthering our understanding of the role of proteins during nanoparticle production is an important contribution to promote the protein-mediated nanomaterial manufacturing.

Characterization of a silica-induced protein in *Thermus thermophilus* related to biosilicification

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Thermus thermophilus TMY, an extreme thermophile, was isolated from a siliceous deposit formed from geothermal water at a geothermal power plant [1]. At concentrations higher than the solubility of amorphous silica (400 to 700 ppm SiO_2, at 75°C), a silica-induced protein (Sip) was expressed in the cell envelope of log-phase cells grown in the presence of supersaturated silicic acid. Molecular weigt and pI of Sip were about 35 kDa and 9.5, respectively. Induction of Sip expression occurred within 1 h after the addition of a supersaturating concentration of silicic acid to broth. Production of Sip and its upregulation were in excellent temporal agreement with the silica precipitation, which was observed at silicic acid concentrations greater than 400 ppm in the medium at 75°C.

The amino acid sequence of Sip was similar to that of the predicted solute-binding protein of the Fe^{3+} -ABC transporter in *T. thermophilus* HB8 and HB27 [2]. Within the genome, *sip* is situated as a component of the Fbp-type ABC transporter operon, which contains a palindromic structure immediately downstream of *sip*. This structure is conserved in other *T. thermophilus* genomes and may function as a terminator that causes definitive Sip expression in response to silica stress.

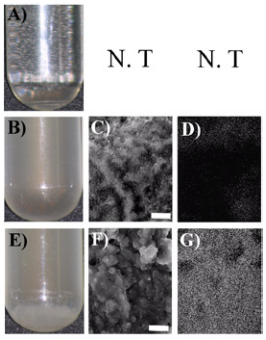

Figure 1: Biosilicification with *E. coli*.
A) Control, B)〜D) Non recombinants, E)〜G) Recombinants. C), F) and D), G) SEM images and Si distribution illustrated by EPMA. Bar: 10 mm, N.T=not tested

The *sip* gene was cloned in pET32 and expressed in *E. coli*. Transformant grown in LB containing 600 ppm silica precipitated silica during their growth (Fig. 1). Purified and heat-treated Sip protein also precipitated silica effectively. Therefore Sip might exhibit their silica precipitation ability.

[1] Fujino *et al*. (2008) *J. Appl. Microiol*. **104**, 70–78. [2] Doi *et al*. (2009) *Appl. Environ. Microbiol*. **75**, 2406–2413.