

論文の要旨

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論文題目 **Molecular studies on the formation of silica layer in bacterial spores and its application**
(細菌孢子におけるシリカ層の形成に関する分子生物学的研究とその応用)

Silicon (Si), the second most abundant element in the Earth's crust, is an important mineral for living organisms. In eukaryotes ranging from microorganisms to higher plants, silicon is taken up as silicic acid, then condensed as biogenic silica through natural process, named biosilicification. In the work described in this thesis, the author identified the first prokaryotic protein involved in biosilicification. Moreover, this protein has been utilized for engineering silica-binding tags for protein immobilization and purification.

The first objective of this study is to identify the protein causing the formation of silica layer in and around *Bacillus cereus* spore coat has been determined. Several peptides and proteins, including diatom silaffin and silacidin peptides, are involved in eukaryotic silica biomineralization (biosilicification). Homologous sequence search revealed a silacidin-like sequence in the C-terminal region of CotB1, a spore coat protein of *B. cereus*. The negatively charged silacidin-like sequence is followed by a positively charged arginine-rich sequence of 14 amino acids, which is remarkably similar to the silaffins. These sequences impart a zwitterionic character to the C-terminus of CotB1. Interestingly, the *cotB1* gene appears to form a bicistronic operon with its paralog, *cotB2*, the product of which, however, lacks the C-terminal zwitterionic sequence. A $\Delta cotB1B2$ mutant strain grew as fast and formed spores at the same rate as wild-type bacteria, but did not show biosilicification. Complementation analysis showed that CotB1, but neither CotB2 nor C-terminally truncated mutants of CotB1, could restore the biosilicification activity in the $\Delta cotB1B2$ mutant, suggesting that the C-terminal zwitterionic sequence of CotB1 is essential for the process. The author found that the kinetics of CotB1 expression, as well as its localization, correlated well with the time course of biosilicification and the location of the deposited silica. This is the first report of a protein directly involved in prokaryotic biosilicification.

The second aim of this research is to engineer novel silica-binding tags for protein purification and immobilization. The author demonstrated that *B. cereus* CotB1 (171 amino acids [aa]) and its C-terminal 14-aa region (corresponding to residues 158-171, designated CotB1p) show strong affinity for silica particles, with dissociation constants at pH 8.0 of 2.09

and 1.24 nM, respectively. Using CotB1 and CotB1p as silica-binding tags, a silica-based affinity purification method has been developed in which silica particles are used as an adsorbent for CotB1/CotB1p fusion proteins. Small ubiquitin-like modifier (SUMO) technology was employed to release the target proteins from the adsorbed fusion proteins. SUMO-protease mediated site-specific cleavage at the C-terminus of the fused SUMO sequence released the tagless target proteins into the liquid phase while leaving the tag region still bound to the solid phase. Using the fluorescent protein mCherry as a model, this purification method achieved 85 % recovery, with a purity of 95% and yields of 0.60 ± 0.06 and 1.13 ± 0.13 mg per 10-mL bacterial culture for the CotB1-SUMO-mCherry and CotB1p-SUMO-mCherry fusions, respectively. CotB1p demonstrated high affinity for silica and is a promising fusion tag for both affinity purification and enzyme immobilization on silica supports.

Furthermore, the author developed a method utilizing an even shorter silica-binding tag and mild elution conditions for silica-based affinity purification. The C-terminal 7-aa region of CotB1p peptide (designated SB7-tag), a short silica-binding peptide, has been used as an affinity tag with L-arginine as an eluent. The combination of the protein-stabilizing effect of L-arginine and small size of the tag would help minimize alteration of the intrinsic properties of target proteins. The author also demonstrated that “shirasu”, volcanic ash broadly deposited in Southern Kyushu, can be used as an affinity support in the developed method, thus enabling purification of recombinant proteins at much lower cost compared to the commercially available silica particles. This affinity method enables purification of recombinant proteins at low cost with the purity and yield comparable with the commonly used His-tag purification method.

In conclusion, this research includes the first identification and characterization of prokaryotic protein involved in biosilicification, and the application of this protein (as well as the peptides derived from CotB1) to protein purification and immobilization. However, further study is needed for the complete understanding of the molecular mechanism of biosilicification. In particular, identification of the genes and proteins that control the transport of silicon in its soluble form (silicic acid) into the bacterial cell is important for fully understanding of biosilicification.