Effect of Genetically Engineered Materials Specific Peptides on Calcium Carbonate Morphologies

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Statement of Purpose: In shells, there are two polymorphs of $CaCO_3$: calcite, a very stable form, and aragonite, which is normally only stable at high temperatures or with addition of magnesium. In literature, it was shown that the proteins in the nacre were the controlling factor for which polymorph was selected.[1] The process by which nacre proteins are able to control the stabilization of a thermodynamically unfavorable polymorph, is not yet understood fully. Since many of the nacre proteins are present during mineralization simultaneously, it is still uncertain which are involved in stabilizing the polymorph.[1] Control of inorganic materials shapes and crystal morphologies might be achieved either through a templating process or by specific proteins that bind to inorganic surfaces and ions.[2] To mimic these processes, we as well as other groups have been selecting peptides that binds specifically to inorganic surfaces.[2] Here, we selected peptides that bind specifically to the two polymorphs of $CaCO_3$, using cell surface display, and investigated how each such peptide affects biomineralization. So far our aragonite binding peptides are noticeably slowing the transition from vaterite to calcite in our biomineralization experiments.

Methods: *Cell Surface Display:* We used the FliTrx bacterial library, which contains randomized constrained peptides that are 12 amino acids long, in the active site of E. coli thioredoxin (TrxA).[2] We performed four rounds of selection for calcite and three rounds for aragonite, and applied "counter selection" to insure that the peptides are specific to one polymorph of $CaCO_3$ only. For the counter-selection we used the amplified cells from the last round of selection and incubated them with the opposite polymorph of $CaCO_3$. *Characterization:* Selected bacterial clones were incubated with calcite or aragonite substrate, unbound cells were washed away, bound cells were detected by fluorescent microscopy using DNA-binding fluorescent dye SYTO9 (Molecular Probes, UK). The selected clones were classified into three groups: strong, moderate and weak binders accordingly to the respective number of adhering cells. Specificity experiments were also done where the strong binders of each polymorph were incubated with a different polymorph of calcium carbonate and if they did not bind they were considered specific. *Biomineralization: We applied a modified biomineralzation method reported by Colfen`s group.*[4] We put 20ml of a 20mM $CaCl_2$ solution in a petri dish and covered it with parafilm with three holes punched in the top. To this solution we added varying amounts of our peptides. The solutions were then placed in a desiccator along with 2g of (NH4)2CO3 to create a carbon dioxide rich atmosphere. We then allowed the mineral to form overnight at room temperature.

Results: *Selection:* We selected 52 clones for calcite and 35 clones for aragonite. Based on the characterization results we found 5 strong binders to calcite, 4 of which were specific. We found 7 specific strong binders for aragonite. From each one of these two sets of specific binders a sequence was chosen to be synthesized, named as 4Ara33 and 1Cal13.
Biomineralization: In the course of the biomineralization experiments we observed interesting morphology of the crystals in the presence of peptides (Fig. 1). At 0.05 mM of 4Ara33 most of the crystals were completely spherical unlike the control, which contained only rhombohedral crystals. 0.1mM of 4Ara33 produced platelet like growths that were agglomerated as well as rhombohedra shaped calcite. 1Cal13 peptides lead to a morphology similar to that of the control without any peptide. Along with affecting the morphologies of the crystals the aragonite binding peptides also delayed the transition of $CaCO_3$ from vaterite, the least stable polymorph, to calcite as seen from the XRD results (Fig 1).

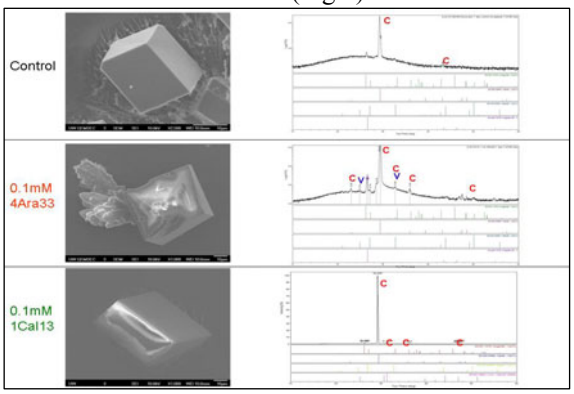

Figure 1. SEM and XRD results from biomineralization experiments by 0.1mM peptide concentrations.

Conclusions: From our experimental results, it is clear that the selected peptides are stabilizing vaterite and slowing down its change to calcite. This would be an opportunity to stabilize vaterite using biocombinatorially selected peptides. Also, our studies on aragonite peptides are ongoing to determine their affect on aragonite formation. Our studies may find substantial use in different areas of biomineralization, with extention to tissue restoration and regeneration.

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References:

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4) Page et al. *Cryst Grow and Des.* **2006;** 6, 1915-1920