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Design of a doubly-hydrophilic block copolypeptide that directs the formation of calcium carbonate microspheres†

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Received (in West Lafayette, IN, USA) 2nd March 2004, Accepted 27th April 2004
First published as an Advance Article on the web 25th June 2004

The crystallization of calcium carbonate into microspheres has been accomplished using the rationally-designed, doubly-hydrophilic block copolypeptide poly{N_ε-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine}$_{100}$-*b*-poly(L-aspartate sodium salt)$_{30}$ as a structure-directing agent.

The finely-tailored properties of biominerals and biocomposites reflect a high level of control over the size, morphology, and orientation of crystalline constituents. In large part, this control is exerted by specialized proteins that interact with inorganic building blocks in a structure-directing fashion.[1] Previous studies have shown that these proteins retain structure-directing abilities *ex vivo*, and even more remarkably, that these functions can be mimicked by synthetic additives.[2] For example, block copolypeptides can direct the assembly of silica into spherical and columnar morphologies at room temperature and neutral pH.[3]

In this communication, we extend similar biomimetic concepts to the crystallization of calcium carbonate. Our interest in this material is motivated by *(i)* the importance of calcium carbonate as a biomineral in many organisms,[4] and *(ii)* the commercial applications of calcium carbonate in the paper and paint industries.[5] Specifically, we report that calcium carbonate microspheres can be prepared with the help of a doubly-hydrophilic block copolypeptide, poly{N_ε-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine}$_{100}$-*b*-poly(L-aspartate sodium salt)$_{30}$ (Fig. 1), as the structure-directing agent in the crystallization process.

The design of polypeptide **1** was inspired by previous crystallization studies.[6] We incorporated the poly(L-aspartate) block because domains of anionic aspartate residues in proteins are known to nucleate calcium carbonate crystallization.[2] This effect is believed to be caused by matching interactions between aspartate and the atomic spacings of certain crystal faces in the growing mineral. In addition, highly-charged proteins isolated from *Haliotis rufescens* (red abalone) provide control over calcite crystal phase,[2] and numerous studies have shown that it is possible to tune the morphology of calcite into rod, dumbbell, ellipsoid, or spherical shapes using charged synthetic additives.[7] We chose the second block of methylated diethyleneglycol-functionalized lysine because of its strong tendency to form hydrophilic α-helical structures,[8] which potentially have the ability to direct the spatial arrangement of calcium carbonate nucleation sites [*i.e.*, along the poly(L-aspartate) block of **1**].

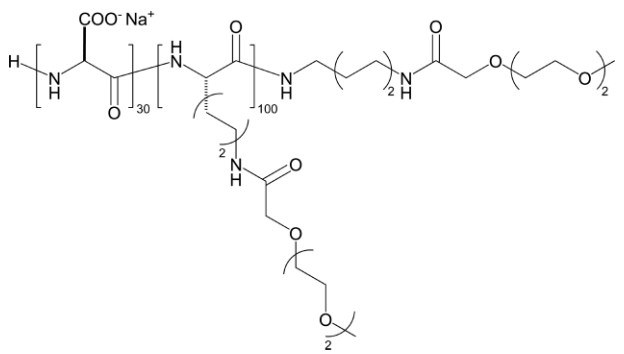

Fig. 1 Doubly-hydrophilic block copolypeptide **1**.

Polypeptide **1** was prepared by the metal-mediated polymerization of α-amino acid *N*-carboxyanhydride monomers.[8,9] The circular dichroism spectrum (ESI†), which provides information about the secondary structure of **1**, exhibits double minima and indicates the presence of α-helical domains in the uncharged blocks. The controlled crystallization of calcium carbonate was achieved by adding 54 μL of an aqueous 0.5 M Ca^{2+} solution (pH 7) and 54 μL of an aqueous 0.5 M CO_3^{2-} solution (pH 10) to 50 μL of an aqueous 0.125 mg mL^{-1} solution of **1** (pH 7). The resulting solution (pH 7) was diluted to 2.7 mL (optimized concentration) and placed in a polystyrene petri dish containing a 2.2 × 2.2 cm glass cover slip. This dish was allowed to sit undisturbed for 48 hours at ambient temperature. Then the cover slip was removed from the solution, rinsed with water, and dried. Scanning electron microscopy (SEM) and light microscopy revealed the presence of calcium carbonate microspheres on the cover slip (Fig. 2).‡ The spheres range from 30 to 50 μm in diameter.

The microspheres were scraped off the cover slip and ground into a powder for characterization by X-ray diffraction. The diffraction pattern (Fig. 3) shows the presence of both calcite and vaterite. This mixture of polymorph phases is common in biomimetic crystallization experiments.[10] A reasonable explanation for this phenomenon is that the aspartic acid segment of **1** destabilizes the nucleation of calcite, such that the less thermodynamically stable phase of vaterite forms first. However, during the 48 hours of the crystallization experiment, the majority of the initially formed vaterite transforms into calcite, which is reflected in the final relative amounts of the two phases.

It is important to identify those interactions between the structure-directing agent and the inorganic components that influence crystallization, in order to ultimately apply these principles to the rational design of new materials. When the homopolymer poly{N_ε-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine}$_{100}$ (**2**) is present during calcium carbonate crystallization,

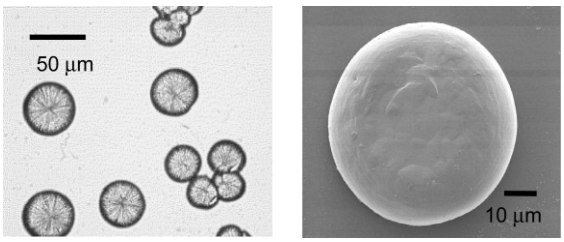

Fig. 2 Light microscopy (left) and SEM (right) images of calcium carbonate microspheres.

† Electronic supplementary information (ESI) available: circular dichroism spectrum of **1** and a table summarizing the effects of different block copolypeptides on the morphology of calcium carbonate crystallization. See http://www.rsc.org/suppdata/cc/b4/b403211j/

DOI: 10.1039/b403211j

only calcite rhombohedra form (Fig. 4b), similar to control experiments (Fig. 4a). In contrast, irregularly-shaped, polycrystalline calcite forms (Fig. 4c) when poly(aspartic acid) (**3**) is present.

Based on these results, we propose that the aspartate block of **1**, which has a high affinity for Ca^{2+} ions, provides sites for Ca^{2+} concentration. This mechanism induces the local supersaturation of Ca^{2+} that is necessary for mineral nucleation. At the same time, the hydrophilic, α-helical poly{N_ε-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine} segments of **1** aid in keeping the growing crystal nucleus in solution, resulting in a spherical morphology. In this way, the block copolypeptide plays a critical role in organizing the inorganic material on the atomic, microscopic, and macroscopic levels. If a 50/50 mixture of the homopolymers **2** and **3** is used instead, only irregularly-shaped, polycrystalline calcite forms. This result confirms that the two amino acids must be present in the same polymer chain to direct microsphere formation. Additional results with a myriad of polypeptides (ESI†) also are consistent with this mechanism. Compared to other methods of microsphere preparation, the use of **1** in a simple, one-pot procedure is quite effective at low concentrations and under mild crystallization conditions.[6] This approach toward controlling the formation and properties of inorganic microstructures is promising, particularly because of the wide range of accessible block copolypeptides.[9]

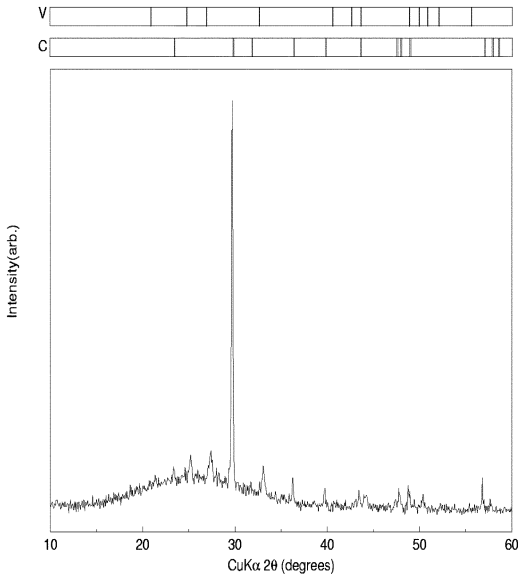

Fig. 3 X-ray diffraction pattern of calcium carbonate microspheres (V = vaterite, C = calcite).

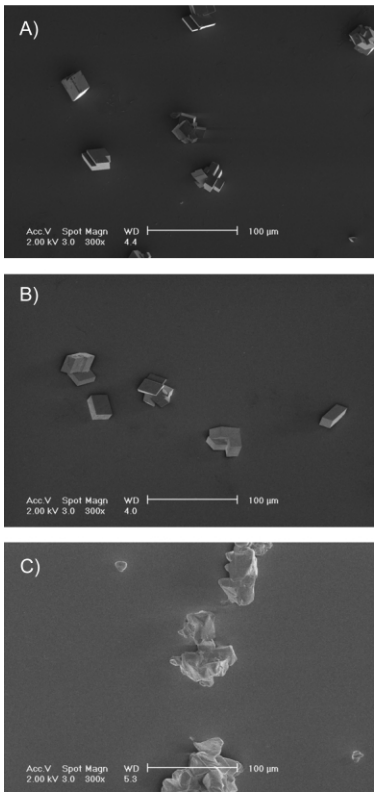

Fig. 4 SEM images that illustrates changes in the morphologies of calcium carbonate grown in the presence of A) no additive, B) poly{N_ε-2[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine}$_{100}$ (**2**) (2 μg mL^{-1}), and C) poly(L-aspartate sodium salt)$_{30}$ (**3**) (2 μg mL^{-1}).

We thank Professor Ram Seshadri, Aditi S. Risbud, Eric S. Toberer, Mark D. Cornish, Lucas Brown, and Dr. Andrew P. Nowak for helpful discussions. We also acknowledge Anthony J. Morfa for assisting with the crystallization experiments. This work was partially supported by the MRSEC Program of the National Science Foundation under Award No. DMR00-80034. L. E. E. thanks the NSF for an IGERT graduate fellowship (DGE-9987618). T. M. T. is a Breetwor Family Fellow supported by the Damon Runyon Cancer Research Foundation (DRG-1778-03).

Notes and references

‡ *Characterization of calcium carbonate microspheres:* The powder sample was analyzed using a Scintag X-Ray Diffraction X2 instrument with Cu Kα radiation (XRD). The SEM samples were coated with gold, and micrographs were obtained with a FEI Sirion SEM microscope (2 KeV). The bright field light micrograph was taken with a Nikon optical light microscope equipped with a Sony DXC-970MD CCD video camera.

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