JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Engineered Biomimetic Polymers as Tunable Agents for Controlling $CaCO_3$ Mineralization

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Supporting Information

ABSTRACT: In nature, living organisms use peptides and proteins to precisely control the nucleation and growth of inorganic minerals and sequester CO_2 *via* mineralization of $CaCO_3$. Here we report the exploitation of a novel class of sequence-specific non-natural polymers called peptoids as tunable agents that dramatically control $CaCO_3$ mineralization. We show that amphiphilic peptoids composed of hydrophobic and anionic monomers exhibit both a high degree of control over calcite growth morphology and an unprecedented 23-fold acceleration of growth at a peptoid concentration of only 50 nM, while acidic peptides of similar molecular weight exhibited enhancement factors of only ~2 or less. We further show that both the morphology and rate controls depend on peptoid sequence, side-chain chemistry, chain length, and concentration. These findings provide guidelines for developing sequence-specific non-natural polymers that mimic the functions of natural peptides or proteins in their ability to direct mineralization of $CaCO_3$, with an eye toward their application to sequestration of CO_2 through mineral trapping.

CO_2 emission is one of the most important anthropogenic sources of global warming. Moreover, even the most optimistic estimates of energy production from renewable sources and nuclear power over the next century show that the burning of fossil fuels will continue to be the dominant source of worldwide energy production.[1] Consequently, research to develop new materials that can reduce atmospheric CO_2 concentrations by efficiently capturing CO_2 at the source has drawn intense interest[1,2] and has recently been directed toward synthesis of porous materials such as zeolites[3] and metal−organic framework compounds.[1,4] However, most scenarios for transforming captured CO_2 into a sequestered product rely primarily on storage in nano-to-mesoporous underground geologic reservoirs where, over time, dissolution of supercritical CO_2 into pore-space brines will lead to permanent trapping through precipitation of thermodynamically stable carbonate minerals.[5,6] Even during the period of time when most of the CO_2 remains in supercritical form, partial mineralization of pore throats, where the curvature is greatest, can serve to immobilize supercritical CO_2 through capillary trapping. Conversely, premature mineralization near the injection zone can prevent adequate utilization of the reservoir. As a result, chemical agents that can inhibit carbonate mineralization at certain stages of sequestration but serve as catalysts to accelerate mineralization in other stages have the potential to significantly improve the utility and integrity of underground reservoirs for CO_2 storage.[7]

In nature, many marine organisms use proteins to induce mineralization of carbonates from aqueous precursors[7−9] on such a vast scale that they impact global seawater chemistry.[10] In doing so, over time, these organisms have produced a sedimentary record that represents the single largest terrestrial reservoir of CO_2. While organisms use the process of biomineralization to produce well-defined carbonate minerals, both crystalline and amorphous, for routine functions such as mechanical support and protection, *in vitro* experiments have demonstrated that some proteins found in association with $CaCO_3$ biominerals, as well as shorter-chain peptides with sequences similar to those found in the proteins, can promote or inhibit $CaCO_3$ nucleation and growth absent any biological context.[11] These findings suggest that synthetic molecules able to provide similar controls over nonbiological crystallization processes might be discovered. Moreover, the design of molecules that mimic the action of these natural biopolymers, but are much more stable against high pressures, temperatures, and acidic conditions, might result in a technology that is broadly applicable to industrial crystallization, including CO_2 sequestration.

Here we report that peptoids, a novel class of sequence-specific non-natural biomimetic polymers, can be designed and synthesized to mimic peptides and proteins for the mineralization of $CaCO_3$. We identified a set of amphiphilic peptoids that dramatically alter calcite growth morphology and accelerate mineralization rates by more than an order of magnitude, even at very low (nM) concentrations.

Peptoids, or poly-N-substituted glycines, were recently developed to mimic both the structure and functionality of peptides and proteins, and bridge the gap between biopolymers and bulk polymers.[12−14] As with peptides, sequence-specific peptoids can be efficiently and cheaply synthesized by using automated solid-phase synthesis starting from a large number of chemically diverse amine building blocks.[15] Moreover, peptoids exhibit much higher protease stability[16] and structural stability[17] than peptides or proteins. In view of the ability of peptides and natural proteins to enhance carbonate mineralization, the interesting structural features and properties of peptoids prompted us to tackle the challenge of developing high-performance synthetic alternatives.

Numerous discoveries within the field of biomimetic mineralization of $CaCO_3$ indicate that the specific amino acid sequence, the number of carboxylic acid groups (or the number of glutamic

Received: January 20, 2011
Published: March 21, 2011

dx.doi.org/10.1021/ja200595f | *J. Am. Chem. Soc.* 2011, 133, 5214–5217

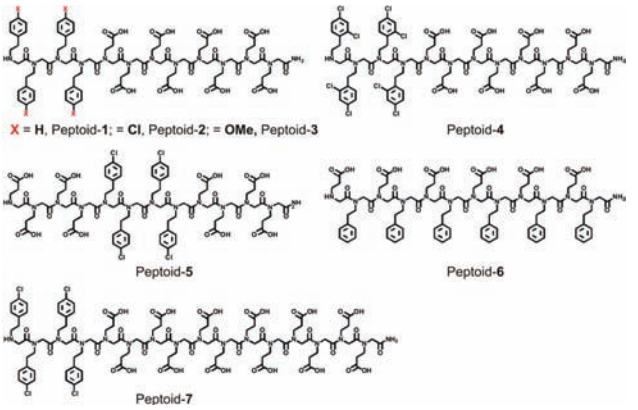

Figure 1. Structures of peptoids **1**−**7**.

or aspartic acids), and the overall hydrophilicity of the proteins or peptides play important roles in the control of nucleation and growth of $CaCO_3$ minerals.[11,18−20] Therefore, we began by designing and synthesizing peptoids using a minimalist monomer set consisting of the hydrophobic monomers *N*-[2-(X-phenylethyl)]glycine (NXpe) (X = 4-H, 4-chloro, 4-methoxy, or 2,4-dichloro) and the hydrophilic monomer *N*-(2-carboxyethyl)glycine (Nce). A small library of peptoids (Figure 1) was made by varying the X groups, the number of carboxylic acid residues, the position of the hydrophobic monomers, and the main-chain length (12 or 16 monomers). All of the peptoids were synthesized on an automated robotic synthesizer using a solid-phase submonomer cycle as described previously.[15]

To demonstrate the ability of these designed peptoids to direct $CaCO_3$ mineralization, we first studied their effect on $CaCO_3$ growth morphology. We reasoned that any peptoid that could strongly affect $CaCO_3$ nucleation and growth should certainly alter the morphology of $CaCO_3$ grown at high peptoid concentration. $CaCO_3$ mineralization was performed within a sealed desiccator by slow diffusion of CO_2 and ammonia vapor into a solution containing calcium chloride and peptoid.[21] We found that the morphological features of the $CaCO_3$ crystals grown in the presence of some peptoids at 51 μM concentrations were very similar to those observed in crystals grown from solutions containing natural acidic proteins associated with biominerals.[22] As shown in Figure 2, the obtained $CaCO_3$ crystals exhibited a number of unique morphologies ranging from elongated spindles and twisted paddles to crosses and spheres. This high degree of morphological control demonstrates that peptoids are capable of strongly interacting with the $CaCO_3$ surface and affecting nucleation and growth. Interestingly, peptoid-**6**, which is composed of closely related NXpe (X = 4-H) and Nce monomers, exhibited almost no effect on the crystal morphology. The $CaCO_3$ crystals grown in the presence of peptoid-**6** at 51 μM were very similar to the rhombohedral calcite crystals grown in the peptoid-free control solution (Figure S1 in the Supporting Information).

Further comparison of peptoid-induced control over the calcite morphology showed that the choice of the X group of the hydrophobic monomer has a significant effect. For example, although peptoid-**1**, -**2**, -**3**, and -**4** have very similar structures, they exhibited distinct effects on the crystal morphology. Each of these sequences contains four NXpe at the N-terminus and eight Nce at the C-terminus. Peptoid-**4** (X = 2,4-dichloro) gave twinned spheroidal shapes (Figure 2c), whereas peptoid-**2** (X = 4-chloro) caused the formation of elongated spindles (Figure 2a) and crystals

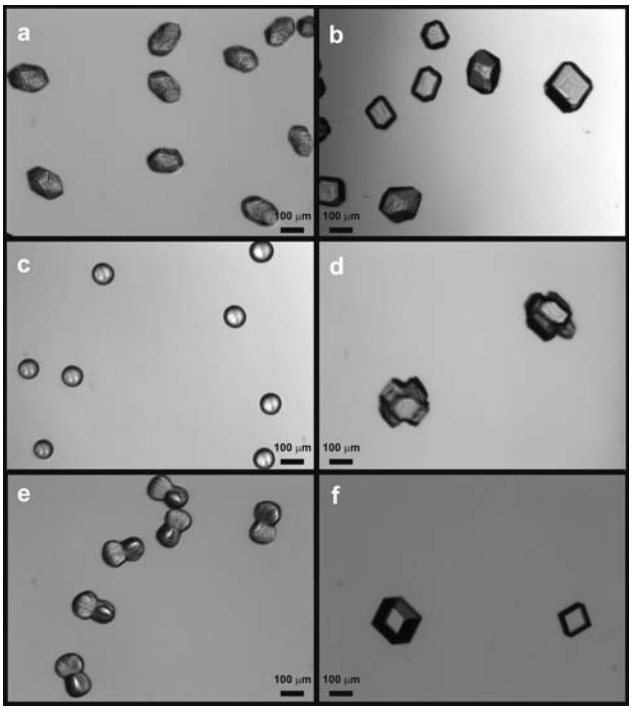

Figure 2. Morphology of $CaCO_3$ crystals grown in the presence of (a) peptoid-**2**, (b) peptoid-**3**, (c) peptoid-**4**, (d) peptoid-**5**, (e) peptoid-**7**, and (f) control.

grown in the presence of peptoid-**1** (X = 4-H) and peptoid-**3** (X = 4-methoxy) produced truncated rhombohedra (Figure S1 and Figure 2b). These results indicate that variation of the X group is sufficient to tune the peptoid−$CaCO_3$ interactions and thus affect $CaCO_3$ nucleation and growth.

We also examined the impact of the number of carboxylic acid groups on the crystal morphology. While elongated spindles were obtained in the presence of peptoid-**2** (Figure 2a), peptoid-**7**, which has four more Nce residues at the C-terminus than does peptoid-**2**, induced the formation of "twisted paddles" (Figure 2e). Interestingly, when we moved the group of four hydrophobic monomers NXpe (X = 4-chloro) to the middle position to make peptoid-**5**, the result was the formation of cross-shaped crystals (Figure 2d). These results demonstrate that both the number of carboxylic acid groups and the peptoid sequence can also be used to tune the peptoid−crystal interactions.

To determine whether these peptoids also had significant effects on the calcite growth kinetics, as was observed previously for acidic peptides and proteins,[22−24] we measured the dependence of the molecular step speed on the peptoid concentration using *in situ* atomic force microscopy (AFM). At low to moderate supersaturations, calcite crystals grow on (104) faces through the advance of atomic steps of two crystallographically distinct types (Figure S2), commonly known as positive and negative in reference to the angle formed by the crystal face and the nominal plane of the step riser. In situ AFM measurement of step speed is a well-established method for probing the molecular-scale effects of modifiers on the crystal growth kinetics.[25,26]

Figure 3 and Figure S3 show the measured enhancement of step speed as a function of peptoid concentration at a supersaturation of 0.14 (supersaturation is defined in the Supporting Information). These figures show that in addition to altering the calcite growth morphology, some peptoids dramatically

dx.doi.org/10.1021/ja200595f | *J. Am. Chem. Soc.* 2011, 133, 5214–5217

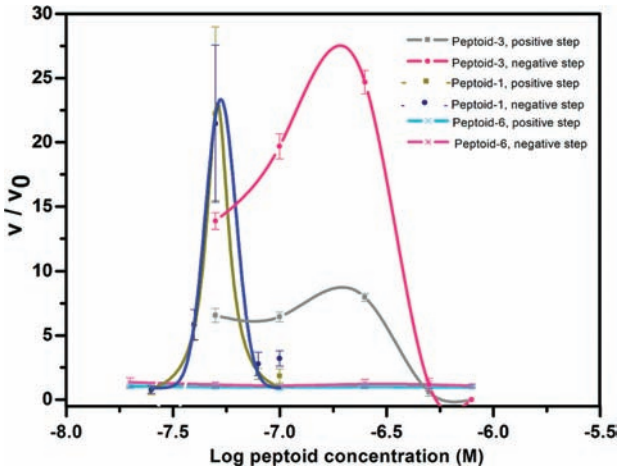

Figure 3. Measured enhancement of calcite step speed as a function of peptoid concentration. Normalized propagation rates are shown as the ratio of step velocity in the presence of peptoid (v) to that without peptoid (v_0).

impacted the step speeds at low concentrations ($\leq$200 nM). For example, peptoid-**1** accelerated both the positive and negative step speeds by nearly 23-fold at a concentration of only 50 nM. Peptoid-**3** accelerated the speed of the negative step much more than that of the positive step, giving accelerations of nearly 28-fold and 8-fold, respectively, at ~200 nM. These extreme values of acceleration stand in stark contrast to those previously reported for acidic peptides of similar molecular weight, which exhibited enhancement factors of 1.64 or less (Table S1).[23] As was previously observed with acidic peptides, increasing the peptoid concentration beyond the point of maximum acceleration reversed the effect, and the step speed rapidly decreased, likely as a result of binding of peptoid to the calcite step.[27]

Interestingly, we further found that addition of peptoid-**6**, which exhibited almost no effect on the $CaCO_3$ morphology at 51 μM (Figure S1a), showed almost no changes in either the positive or negative step speed from 20 to 800 nM, despite the fact that peptoid-**6** and peptoid-**1** both contain 12 monomers and the same NXpe (X = 4-H) and Nce building blocks. These large differences show that the peptoid-block sequence patterning plays an important role in determining the effect on the calcite growth kinetics.

The observations presented here combined with previous experimental and computational results suggest three possible sources of growth acceleration. The first is that peptoids effectively raise the local supersaturation. All of these peptoids are rich in carboxylic acid groups and thus could act as a Ca(II) transporters, which could affect the available Ca(II) concentration near the crystal surface. This mechanism of enhancement was predicted for barite growth in the presence of aspartic acid on the basis of molecular dynamics simulations.[20,23,28] However, an examination of the dependence of the step speed on the Ca^{2+} concentration rendered this an unlikely explanation. The step speed, V, is given by

$$V = \beta_0(C - C_e)\exp(-E/k_BT) \quad (1)$$

where C and C_e are the actual and equilibrium concentrations, E is the activation barrier to desolvation and attachment at the step, and β_0 is a concentration-independent parameter that includes a number of factors. However, because the step speed is linear in $(C - C_e)$, for this mechanism to explain the >10-fold enhancements seen here, the peptoids would have to increase the local concentration by an equivalent factor.

An order of magnitude estimate illustrates the difficulty in achieving this increase. For the Ca^{2+} concentrations used in this study, the Ca^{2+} number density was $\sim 1.0 \times 10^{-4}$ ions/nm^3. A 10-fold increase in Ca^{2+} concentration would require an additional 9.0×10^{-4} Ca^{2+} ions/nm^3. For the most potent peptoid, there are eight carboxylic side chains. If every side chain weakly complexed a Ca^{2+} ion, one would require a peptoid concentration of 0.20 mM (1.2×10^{-4} peptoids/nm^3) near the crystal surface to get this increase in Ca^{2+} concentration. Since the bulk peptoid concentration was only 50 nM, achieving a 10-fold increase in Ca^{2+} levels would thus require a 4.0×10^3 enhancement in the peptoid concentration, which seems unlikely. Nonetheless, this estimate shows that a high affinity of the peptoids for the surface could have a moderate impact on near-surface concentrations

The second potential mechanism for the observed acceleration is that peptoid interactions with solvated ions lower the activation barrier to desolvation and/or orientation of the solute ions prior to attachment to the crystal. This mechanism was proposed by Elhadj et al.,[23] who used an analysis of eq 1 to show that E was reduced in the presence of carboxyl-rich peptides. As with the peptides in that investigation, all of the peptoids studied here have large numbers of carboxyl groups. These should lead to strong interactions with solvated cations in solution such as Ca^{2+} and thereby weaken the bonds of Ca^{2+} hydration.[29] Because the value of E in the pure system is ~33 kJ/mol,[30] producing a 10-fold enhancement through a reduction of the barrier would require a decrease in E of less than 6 kJ/mol. However, as discussed above, the degree of acceleration is much larger for the peptoids, despite the fact that they are of comparable size to the most effective of the peptides.[23] Consequently, applying this same explanation to both the peptides and peptoids is problematic.

The third potential mechanism is that the activation barrier to solute attachment to the crystal is lowered through disruption of the water layer adsorbed to the crystal surface. A number of simulations have predicted that displacing these surface-bound waters from the crystal is the most energetically costly step in adsorptions of solute ions to the crystal surface.[31,32] All of these peptoids are amphiphiles containing a polar block of multiple carboxylic acid residues and a hydrophobic block of multiple aromatic residues. The carboxyl-rich block is structurally similar to previously studied peptides, which are known to bind to the calcite surface. The polar block may serve to recruit the hydrophobic block to the proximity of the surface, which may then assist in the disruption of the surface-adsorbed waters. The hydrophobic block may also serve to modulate the overall affinity of the peptoid for the surface. Because these peptoids are active over a relatively narrow range of peptoid concentration, it is very likely that reversible binding to the calcite surface is important. The magnitude of the binding energy per water molecule is ~90 kJ/mol,[31,32] so the 6 kJ/mol drop in E required to obtain a 10-fold enhancement requires a relatively small reduction in the binding energy.

In conclusion, anionic and amphiphilic peptoids exhibited both a high degree of control over the calcite growth morphology and extreme levels of growth acceleration. Both the morphology and rate controls were observed to depend on the peptoid sequence, side-chain chemistry, chain length, and concentration. Because large peptoid libraries can be built from a broad set of chemically diverse amine building blocks, we expect more effective peptoids to be discovered in the near future. Because

subtle changes in peptoid structure generated large changes in the degree of control over growth, this approach also has the potential to offer new insights into how biopolymers precisely control mineral growth in natural systems. Moreover, because of their high protease resistance[16] and structural stability[17] as well as their ability to greatly accelerate $CaCO_3$ mineralization rates at very dilute concentrations, peptoids may be useful in CO_2 sequestration by enhancing rates of mineral trapping and the extent of capillary trapping in geologic reservoirs.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures, Figures S1−S3, and additional supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported as part of the Center for Nanoscale Control of Geologic CO2, an Energy Frontier Research Center, and performed as a User project at the Molecular Foundry, Lawrence Berkeley National Laboratory, both of which are funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-05CH11231.

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