Formation of Calcite Biominerals by seed nucleation with 3-amino acid polypeptides: A computational modeling study

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Abstract: Organic and biomolecules gets adsorbed to the growing nuclei in connection with ordered organic /inorganic templates. Organic templates determine the morphology of the biominerals. The interaction of oligomers/polypeptide chains on calcite surfaces as a precursor of biomineralization has been studied in this paper . Molecular Dynamics simulations using Cerius 2 software (Accelrys) were performed at 300K. The most favorable adsorption energy is calculated for $(lys\text{-}gln\text{-}tyr)^{-1}$ to a non-polar step parallel to [48-1] ([4-4-1]), and to a polar step parallel to [010] terminated by Ca^{2+}.$(Phe\text{-}leu\text{-}lys)^{1-}$ exhibits the most favorable adsorption energy on a polar step edge parallel to [42-1] terminated by Ca^{2+} ions. We can conclude from the above results that if the amino acid side chain contains a benzene ring or a cloud of electrons (electron pairs or π-electrons) that can interact with the mineral surface atoms, it will be favorably adsorbed to that surface location, overcoming the counteracting effect or steric hindrance. Among the above-mentioned peptides that favorably get adsorbed to the calcite surface steps, lysine and glutamine contain amide groups that have electron pairs and tyrosine and phenyl-alanine contain benzene rings. The π-electrons of benzene rings interact with calcium ions at the surface steps.

Index Terms **– Calcite, Biominerals, Nucleation, Polypeptides, Amino acids**

I. INTRODUCTION

Organic and biomolecules gets adsorbed to the growing nuclei in connection with ordered organic /inorganic templates determine the morphology of the biominerals. When these interactions are thermodynamically favourable, interactions are stronger and yield more negative energy values.

Most of the minerals that grow as a result of biological processes are comprised of carbonates, silicates, phosphates, and, to a smaller degree, of sulphates **[1]**. Weathering and complex biological and chemical interactions have led to the re precipitation of dissolved minerals into sedimentary rocks. The emergence of primitive organisms promoted the processing of dissolved mineral constituents in the marine environment where both prokaryotic and eukaryotic organisms have the ability to produce mineralized skeletal elements. Thus, the resulting minerals of biogenic origin comprise a large portion of the Earth's crust and represent a large storage of sequestered carbonate, silicate, and phosphate ions. The majority of carbonates occur as calcite, typically produced by plankton and invertebrates. Organic macromolecules appear to be an integral part of most calcium carbonate biominerals.

Organic matrix frameworks can provide binding sites for the components of a mineral, thereby selectively nucleating specific crystallographic faces **[2]**. Organic carrier molecules can help to produce local supersaturation with respect to a certain mineral phase to be precipitated. Organic templates determine the morphology of the biominerals, and the structure of the organic template is responsible for producing growth nuclei in the mineral phase. A well-studied example of this is coccolithophores, which deposit calcite as their mineral phase. Calcite is a rhombohedral polymorph of calcium carbonate ($CaCO_3$). The lowest-energy faces of pure calcite are the {104} family of faces **[3]**. DNA, which is a double-stranded helix consisting of polymers found in the nucleus of the cell of living organisms **[4]**, controls the initial biomineralization process by transferring its genetic information to proteins in the outer cell wall **[5]**.

When an organism forms a template in its exoskeleton, it is thought to be an image of the genetic information carried in its DNA. According to the central dogma of molecular biology, translation of DNA forms RNA, and transcription of RNA forms proteins **[6]**. The synthesis of protein from RNA allows genomic techniques to be applied in protein studies. Therefore, the study of the interaction of polypeptides (containing amino acids) with calcite surfaces can help us understand the structural matching of interfaces between the organic matrix-and the inorganic mineral surface during biomineralization. Combination of our study with the experimental AFM work on thermodynamics of calcite growth will strengthen our understanding of calcite biomineralization at molecular level **[7]**.

The interaction of oligomers/polypeptide chains on (104) calcite surfaces as a precursor of biomineralization has been studied in this chapter. Our main objective is to find suitable orientations of amino-acid residues in these peptide chains, where these peptide chains align themselves parallel to flat calcite surfaces or parallel to a step on these surfaces. The stereochemical relation between the coordination environments of ions on specific crystal faces (Ca^{2+} and CO_3^{2-} in this case) and the arrangement of ligands (i.e., peptide residues, oligomers) around ions bound to the surface is a potential factor for organic nucleation and selectivity of biominerals. If the distance between the repeating unit residues in the adsorbent matches the distance between the repeating units in the adsorbate surface and if the polarities of organic template and mineral surface/step match, the adsorbing long chain polymer or oligomeric organic compound can lie parallel to the surface step and, thus, stabilize the interface. The most favorable interfacial energy between a pair of organic matrix-inorganic nucleating surface leads to formation of a stable biomineral.

II. MATERIALS AND METHODS

As a precursor of biomineralization, we are studying the interaction of oligomers/polypeptide chains on the {104} calcite family of faces. Our main objective is to find suitable orientations of amino-acid residues in these peptide chains and where these peptide

chains align themselves parallel to the calcite surface. Objective of these studies to find most suitable sequence of peptides that will promote control growth of biominerals.

In order to find a suitable polypeptide sequence for optimal adsorption, one has to note that translation from the DNA to polypeptide sequences involves the conversion of a four-base code (ATCG) into twenty different amino acids. A codon or triplet of bases specifies a given amino acid [8]. Most amino acids are specified by more than one codon. The conversion of codon information into proteins is conducted by means of the RNA. Each transfer-RNA (tRNA) has an anticodon, which can base pair with a codon. Some anticodons have modified bases that can pair with more than one codon, specifying the same amino acid; this means that we do not need 61 different tRNA molecules for all 61 codons.

In order to avoid steric hindrances of complex polypeptides, we chose to work initially with 3-amino acid long small-chain oligopeptides to study their interaction with calcite surface steps. Since a given amino acid requires 3 bases to be expressed, we can apply the A-T G-C rule, commonly known as Chargaff's rule [Chargaff et al, 1948]. Chargaff's rule states that the molar ratio of A (adenine) to T (thymine) and of G (guanine) to C (cytosine) is always approximately equal in a DNA molecule. Chargaff's Rule is true as a result of the strict hydrogen bond forming rules in base pairing. For every G in a double-strand of DNA, there must be an accompanying complementary C; similarly, for each A, there is a complementary paired T (U-uracil for RNA).

We are using empirical force-field methods to study the adsorption of peptides containing the above-mentioned amino-acids on calcite (104) surfaces and surface steps. Using the Cerius2 software (Accelrys Inc.), we have created calcite (104) surfaces and surface steps along periodic bond chains parallel to the symmetry equivalent [48-1] and [4-4-1] directions. In this paper, we have studied interaction of negatively charged peptides on non-polar steps, and polar steps parallel to [010] and [42-1] of calcite. These studies are 3-amino acid peptide studies, which in future will be extended to 12-amino acid residue.

Molecular dynamics simulations at 300 K were performed to avoid trapping the adsorbate in a local energy minimum before and during optimization. These dynamics simulations were performed using a constant NVE ensemble. This is the most suitable ensemble for the setup that we used with constant number of atoms (constant N), non-periodic system (thus using V rather than P), and an adiabatic approach (constant E). The structure is allowed to evolve in time, by solving Newton's equation of motion. Every 100 steps, velocities are rescaled if the average temperature goes outside the specific window around the target temperature. The dynamic time step is 0.001 ps, and for each MD run, we run 500 steps. We use quantum mechanical calculations using Gaussian03 [9] to calculate the hydration energy of the 3-amino acid residue peptides. We use a 3-21g basis set and a dielectric continuum approach [10] with a dielectric constant of water of 78.39. A molecular dynamics approach with hundreds of water molecules for tens of different configurations would have been to computationally expensive.

III. RESULTS AND DISCUSSIONS

Interaction of negatively charged peptides on calcite surface: 3 amino acid residue peptides:

We studied interaction between negatively charged peptide residues with non-polar steps on calcite (104) surface and 2) negatively charged peptide residues with polar steps on calcite (104) surface. The calculations are done in alkaline pH condition.

1) Negatively charged peptide residues on non-polar steps:

For the non-polar surface step, there are two different situations. The carbonate on the surface step can form both an acute angle and an obtuse angle with the underlying surface. We have calculated adsorption of the peptides on both types of non-polar surface step (obtuse and acute). The Ca^{2+} - CO_3^{2-} distance is 3.301 Å and the Ca-Ca distance is 6.602 Å. Table 1 shows the corresponding adsorption energies. The average distance between the nitrogen atoms in the peptide bond of peptide residues are 3.458 Å in the investigated peptides. According to Langmuir Blodgett film theory [11] if the distance between the repeating unit's residue in the adsorbent matches the distance between the repeating units in the adsorbate surface, the adsorbent long chain polymer or oligomeric organic compound can lie parallel to the surface step. We can see that the average distance between nitrogen atoms in peptide bond and the distance between Ca^{2+} - CO_3^{2-} ions in non-polar surface steps are comparable, within 0.15 Å. However, we have to consider another factor, which is steric hindrance presented by bulky side chain groups in the amino acids.

Phe-leu-lys, lys-gln-tyr, gly-ser-trp – these 3 peptide residues have a benzene ring in their side chain. Benzene rings are fairly bulky functional groups which can cause steric hindrance with other functional groups that could potentially bind to the surface or step. That is why only the carboxylic groups of these peptides are electrostatically attracted and close to the calcite surface steps. Other side chain residues and —NH- and —N-C=O of the peptide bonds are hindered to bond by the benzene ring, and thus point away from the surface step. The electrostatic interaction between the oppositely charged species at the interface of the peptide and calcite surface step is still strong enough to adsorb these 3-amino acid residue peptides to a non-polar surface step. $(Lys\text{-}gln\text{-}tyr)^{1-}$ has the most favourable adsorption energy (2.87 eV/residue) on a non-polar step with carbonates forming an obtuse angle with the underlying terrace. The adsorption energies are significantly lower when the carbonates make an acute angle with the underlying terrace. The electron density on the oxygen in a carbonate ion is higher, and when it makes an acute angle, the oxygen atoms are exposed to the negatively charged peptides on a step direction (contrary to the obtuse angle case where the carbonate oxygens are on the "other" side of the peptides). For the acute angle case, $(phe\text{-}leu\text{-}lys)^{1-}$ has the most favourable adsorption energy (-1.40 eV/residue).

Table 1: Adsorption energies of negatively charged 3-amino acid residue peptides on non-polar steps parallel to [48-1] ([4-4-1])

3-amino acid residue peptide	**$E_{adsorption}$ per amino acid (eV) (E_1)**	**$E_{hydration/amino\ acid}$ (eV) (E_2)**	**$E_{effective\ adsorption/amino\ acid}$ (eV) (E_1-E_2) (obtuse angle)**	**$E_{adsorption}$ per amino acid (eV) (E_1)**	**$E_{hydration/amino\ acid}$ (eV) (E_2)**	**$E_{effective\ adsorption/amino\ acid}$ (eV) (E_1-E_2) (acute angle)**
arg-val pro (1-)	-2.59	-1.29	-1.30	-2.38	-1.29	-1.09
leu-pro cys (2-)	-4.33	-3.05	-1.28	-2.69	-3.05	0.36
lys-gln-tyr (1-)	-4.42	-1.55	-2.87	-2.78	-1.55	-1.23
phe-leu lys (1-)	-4.22	-1.47	-2.75	-2.87	-1.47	-1.40
leu-ala-pro (1-)	-3.84	-1.54	-2.30	-2.35	-1.54	-0.81
gly-ser-trp (1-)	-3.26	-1.43	-1.83	-2.44	-1.44	-1.01

2) Negatively charged peptide residues with polar steps parallel to [010] and [42-1]:

Polar steps on a calcite surface can be bounded by Ca^{2+} ions or CO_3^{2-} ions. Also, the polar step can be parallel to both [010] and [42-1] direction on calcite surface. Out of these 4 possible scenarios, the interaction between negatively charged peptides with CO_3^{2-} ions terminated polar step is not favorable. We get negative adsorption energies for the peptides in vacuum, but since the peptides are negatively charged, they have high hydration energy also, which eventually makes them unfavorable to get adsorbed. For the polar surface terminated by Ca^{2+}, the closest distance between two Ca^{2+} ions are 4.957 Å. In Table 2, we have listed the interface adsorption energies of each 3-amino acid peptide residue with the polar calcite surface step edge terminated by Ca^{2+} ions. Adsorption energies are little higher on step direction [010] compared to [42-1], although in both cases the adsorption of 3-amino acid residues is highly favorable. $(Leu\text{-}pro\text{-}cys)^{2+}$ is most favorably adsorbed to steps parallel to [010] (-8.27 eV/residue). The presence of sulfur atom in the cysteine residue causes the strong electrostatic interaction with the step terminating Ca2+ ions. The energies of different peptides do not vary much when adsorbed to steps parallel to [42-1]. Here, $(gly\text{-}ser\text{-}trp)^{1-}$ has the most favorable adsorption energy (6.98 eV/residue).

Table 2:Adsorption energies of negatively charged 3-amino acid residue peptides on Ca2+ polar steps parallel to [010] and [42-1]

3-amino acid residue peptide	$E_{adsorption}$ per amino acid (eV) (E_1)	$E_{hydration/amino\ acid}$ (eV) (E_2)	$E_{effective\ adsorption/amino\ acid}$ (eV) (E_1 E_2) (parallel to [010])	$E_{adsorption\ per\ amino\ acid}$ (eV) (E_1)	$E_{hydration/amino\ acid}$ (eV) (E_2)	$E_{effective\ adsorption/amino\ acid}$ (eV) (E_1 E_2) (parallel to [42-1])
arg-val-pro (1-)	-6.46	-1.29	-5.17	-5.51	-1.29	-4.22
leu-pro-cys (2-)	-11.32	-3.05	-8.27	-8.89	-3.05	-5.84
lys-gln-tyr (1-)	-8.91	-1.55	-7.36	-7.81	-1.55	-6.26
phe-leu-lys (1-)	-7.36	-1.47	-5.89	-7.74	-1.47	-6.27
leu-ala-pro (1-)	-6.10	-1.54	-4.56	-7.54	-1.54	-6.00
gly-ser-trp (1-)	-7.94	-1.43	-6.51	-8.41	-1.43	-6.98

Figures

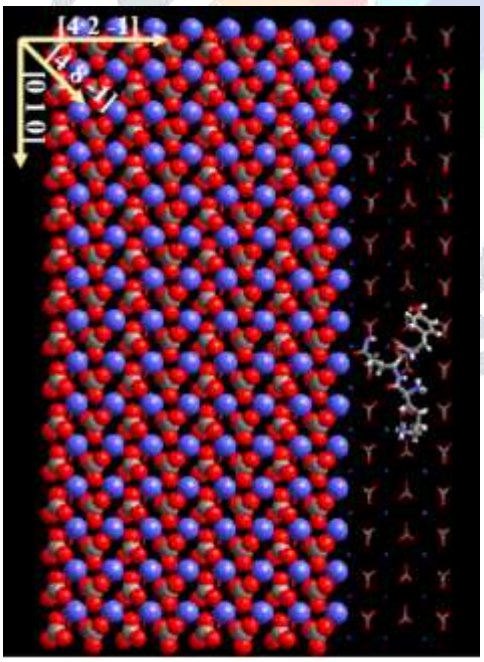

Figure 1: Adsorption site of 3 amino-acid residues on the polar calcite surface step parallel to [010]

CONCLUSION

We have seen that under favorable conditions, peptides are more favorably adsorbed on a calcite (104) surface step parallel to [42-1] direction than [010] direction. The distance between consecutive calcium ions or carbonate ions are 4.36 Á in the step direction parallel to [42-1] compared to 4.96 Á in the step direction parallel to [010]. The smaller distance in [42-1] matches better with the separation of adsorbing functional groups in the peptides investigated. We also observe that on non-polar surface steps, the peptides are more favorably adsorbed when the carbonate ions form an obtuse angle with the underlying terrace rather than an acute angle.

The different side-chains of the amino-acids present in the peptide during peptide-calcite interaction play a significant role in peptide adsorption on the calcite surface. We initially studied the interaction of small 3-amino acid residue peptides with the step edge of calcite surface to establish the role of functional groups while interacting with atoms on such a step-edge. The most favorable adsorption energy is calculated for (lys-gln-tyr)-1 to a non-polar step parallel to [48-1] ([4-4-1]), and to a polar step parallel to [010] terminated by Ca^{2+}. (Phe-leu-lys)1- exhibits the most favorable adsorption energy on a polar step edge parallel to [42-1] terminated by Ca^{2+} ions. We can conclude from the above results that if the amino acid side chain contains a benzene ring or a cloud of electrons (electron pairs or π-electrons) that can interact with the mineral surface atoms, it will be favorably adsorbed to that surface location, overcoming the counteracting effect or steric hindrance. Among the above-mentioned peptides that favorably get adsorbed to the calcite surface steps, lysine and glutamine contain amide groups that have electron pairs and tyrosine and phenyl-alanine contain benzene rings. The π-electrons of benzene rings interact with calcium ions at the surface steps.

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