Ca²⁺ and Phosphate Ion Transport To and Calcium Phosphate Cluster Nucleation Within Collagen Fibrils In Bone Biomineralization

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Bone is a composite material consisting of collagen, an insoluble, fibrillar, protein; a non-stoichiometric calcium phosphate (Ca-P) phase idealized to hydroxyapatite (HAP); and soluble proteins. Based on TEM observations for six decades, collagen fibrils are known to be arranged in a hierarchical, "staggered" array, which controls the locations of the earliest nucleation in "hole zones" of the "a and e bands" within the fibril. However, the mechanisms for Ca^{2+} and inorganic phosphate (P_i) ion transport into the fibrils and for Ca-P nucleation in specific locations remain unknown.

We used the structure of collagen mimetic peptides and the 3-D packing structure of collagen molecules within a fibril to construct and optimize the entire collagen fibril structure with regular Molecular Dynamics (MD) simulations. Furthermore, Ca-P cluster formation and water diffusion and density distributions within the fibril were determined using Hamiltonian Replica Exchange Molecular Dynamics (HREMD), which captures even rare nucleation events. Significantly, we found that the lateral space between two adjacent collagen molecules is too small to allow prenucleation clusters larger than ~1nm to enter the fibril and reach the hole zones near the a and e bands where the earliest nucleation is observed by TEM. This result provides constraints on previously proposed mechanisms for calcium phosphate prenucleation cluster transport into the intrafibrillar space. Further, the charged amino acid side chains (glutamate, aspartate, lysine, arginine) of the e bands are oriented to point into the hole zones, and attract Ca²⁺ and P_i ions electrostatically to form the earliest Ca-P clusters. We have shown for the first time the mechanisms by which the 3D heirarchical structure of collagen controls mineral nucleation from the Å - 10s of nm length-scale.

Carbonate speciation in depolymerized silicate melts (glasses): New evidence from ab initio calculations and ¹³C MAS and static NMR measurements

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Knowledge of the dissolution mechanisms of CO_2 in silicate melts and glasses is indispensible for understanding its effects on physical and thermodynamic properties. For depolymerised silicate compositions, previous IR, Raman and ¹³C MAS NMR studies of quenched glasses all revealed CO_3^{2-} as the dominant species. However, no consensus has been reached as to whether CO_3^{2-} are linked to network-formers (e.g. Si) or bonded to network-modifiers (e.g. Ca) only (referred to as free carbonates hereafter), due to the lack of direct information for spectroscopic features of the former.

Here we report ab initio calculation results (using Gaussian 09) of vibrational frequencies (at B3LYP/6-31+G(d,p), scaled by 0.9685) and ¹³C chemical shift tensors (at HF/6-311+G(2df,p)) for CO₃²⁻ groups bonded to one and two SiO₄/AlO₄ tetrahedra. We also report ¹³C MAS and static NMR results for several ¹³CO₂-bearing depolymerized silicate glasses, e.g. diopside (CaMgSi₂O₆) and Ca-melilite (Ca_{1.5}AlSi₂O₇), prepared by quenching melts at 1.0-1.5 GPa and 1400-1600 °C in a piston cylinder apparatus. The ab initio calculation revealed that the splittings of the v_3 (asymmetric stretching) doublets for CO32- bonded to one or two tetrahedral Si/Al are all large (around 180-480 cm⁻¹). In contrast, experimental data for CO₃²⁻ (bonded only to metal cations) in minerals show v_3 splitting from zero to moderate, depending on local geometry. Thus, the moderate v_3 splitting (70-100 cm⁻ ¹) reported for many depolymerized silicate glasses, which was used as evidence for CO_3^{2-} bonded to one Si (e.g. [1]), should be better viewed as evidence for free carbonates. Our calculations also showed that carbonates bonded to one or two Si/Al all show distinctly different characteristics of ¹³C chemical shift tensors compared to free carbonates, similar to experimental observations for organic carbonates (bonded to one or two C). Our ¹³C MAS and static NMR data for depolymerized silicate glasses are consistent with free carbonates as the dominant species. Its formation would lead to polymerization of the silicate structure.

[1] Blank, JG & Brooker, RA (1994) Rev. Mineral. 30, 157-186.