Conformational Switching between Carboxylic Acid and Carboxylate Anion States by NH···O Hydrogen Bonding

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Summary: Biologically important Ca-proteins and Ca-biominerals as metal-polymer complexes are often regulated by the complexation and demetalation with the biopolymers. Metal-oxygen bond is supported by NH···O hydrogen bonds between coordinating oxyanion and neighboring amide NH and by the successive hydrogen bonding networks. Carboxylate anion under hydrophobic conditions has a high basicity that leads to a covalent Ca-O bond character that is significantly affected by the NH···O hydrogen bond. The hydrogen bonds in Asp-containing tripeptide fragments in Ca-proteins presumably control coordination/dissociation of metal-oxygen bonds. Our systematic studies of carboxylate, sulfonate and phosphate Ca(II) complexes demonstrate a relationship between the basicity of oxyanion in carboxylate and hydrogen bonds as cooperating with the oligopeptide conformation in Asp-containing Ca(II) complexes. Hydrogen bonds between carboxylate oxyanion and amide NH, controlled by a conformational switching of oligopeptide fragments, seem to be one of essential factors for the regulatory formation of Ca-proteins and nano-architectures in connection with the interface structure of inorganic and organic phases in biominerals.

Keywords: biomineralization; metal-polymer complexes; peptides

Introduction

Biomineralization of inorganic-organic materials has been studied in biological systems,[1,2] especially for bone and teeth, or shell and pearl which are composed of $Ca(PO_4)_2(OH)_2$ or $CaCO_3$ crystals, respectively, in the combination of organic polymer ligands such as proteins, polysaccharides and lipids. Highly acidic polypeptides having a repeated Gly-Xaa-Asn fragment (Xaa = Glu, Asp, Asn) are thought to be involved in the controlled crystallization of aragonite phase[3] having a tightly connected carboxylate-Ca(II) bond as schematically drawn in Fig. 1a. Hydroxyapatite crystals in bone and teeth is arranged by organic matrices, such as collagens, acidic peptides and peptideglycans.[4,5] The acidic peptide in phosphoryn containing repeated Asp-Ser-Ser sequence in which Ser residues are totally phosphorylated.[6] Similarly, large

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amount of Glu, Asp and Ser residues are found in peptideglycan and dentine sialoprotein. These proteins are known to be essential for the calcification of hydroxyapatite crystals.[4]

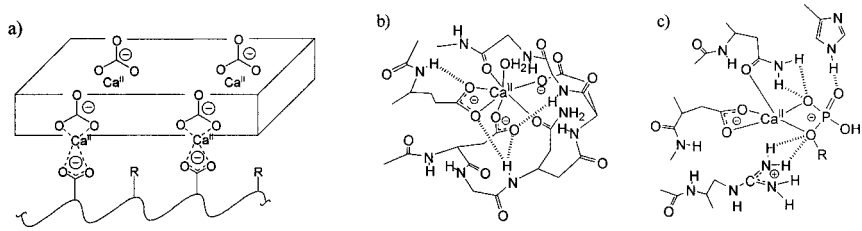


Fig. 1. Proposed drawing of a) $CaCO_3$/protein microcrystal composite in biominerals. Structures of a) Ca-binding site in troponin C and b) Ca center of mammalian phosphoinositide.

These polymer ligands also play important roles for the dissociation of Ca(II) ion from $CaCO_3$ crystals in some biological systems such as gastroliths containing chitins and proteins which have Glu-rich.[7] The organic matrix seems to be involved in the regulation of Ca ion transport. Therefore, these biopolymer ligands play a crucial role for both mineralization and demineralization, although they are quite opposite function with each other.

Formation and dissociation of calcium-oxygen bonds are known precisely to be controlled by calcium-binding proteins participating in the regulation of concentration of calcium ion in troponin C or calmodulin with the loop region of EF-hand structure.[8] Aspartate and glutamate are observed to bind to calcium ion and the oxygen atoms of these residues are almost hydrogen-bonded with the main chain amide NHs as shown in Figure 1b. In the active site of mammalian phosphoinositide, specific phospholipase C, the phosphate groups coordinating to Ca(II) ion have several NH···O hydrogen bonds with surrounding peptide NHs, as shown in Figure 1c.[9] These NH···O hydrogen bonds to the coordinating oxo acid groups are thought to be functionally essential especially as a hydrogen bond network. The hydrogen bond network is thought to contribute to the rigid folding of the protein chain.

However, we have emphasized the importance of NH···O hydrogen bonds in chemical functions as well as the roles of NH···S hydrogen bonds in metal-thiolate complexes as models of sulfur-containing metalloenzymes. This paper presents weak or strong NH···O hydrogen bonds in simple Ca(II) complexes with bulky carboxylate ligands, and a conformational switching in oligopeptides between carboxylic acid and carboxylate upon formation of the NH···O hydrogen bonds.

Regulation of pK_a for Ligand by NH···O Hydrogen Bond

Various metal complexes of carboxylate with intramolecular NH···O hydrogen bond were synthesized.[10,11] The carboxylate ligand with the hydrogen bond promotes a ligand exchange reaction in the following equation. The reaction proceeds quantitatively because of the difference of each pK_a value in a less polar solvent.

$$M^{II}(OCO\text{-}2,4,6\text{-}i\text{Pr}_3\text{Ph})_n(OH_2)_{4-n} + ArCOOH \rightarrow M^{II}(OCOAr)_n(OH_2)_{4-n} + 2,4,6\text{-}i\text{PrPhCOOH}$$
$$(Ar = 2,6\text{-}(t\text{-BuCONH})_2C_6H_3COO^-)$$

Thus, Ca-O bonds between crystals and biopolymers in biominerals can be also controlled by the hydrogen bond networks formed with polypeptides. The regulation of calcium-oxygen bonds in biomineral systems is assumed to be achieved by the transformation of the peptide structure correlated with rearrangement of the hydrogen bonding network. A series of Ca complexes with oxo acids, such as phosphate, sulfonate and carboxylate ligands, having strategically-oriented bulky amide groups have been studied in order to elucidate the roles of NH···O hydrogen bonds as models of the biologically important hydrogen bond networks.[12,13] Novel analogues with the hydrogen bonding networks in calcium cluster with designed bulky amide ligands were synthesized to investigate the transformation of the calcium cluster cooperating with the reorganization of the hydrogen bonding networks.[14]

Functional role of the NH···O hydrogen bonds on the pK_a shift was examined in an aqueous micellar solution using simple carboxylate and carboxylic acid. Although, in general, pK_a value is obtained in an aqueous solution, the micellar solution allowed us to measure it under the hydrophobic conditions which maintain the intramolecular NH···O hydrogen bond. The pK_a data correspond to the dissociation of metal-carboxylate bonds. The dissociation is prevented by the NH···O hydrogen bonds for coordinating carboxylate groups, because the NH···O hydrogen bonds lower the pK_a value of the corresponding carboxylic acid.[10,11,15] The hydrogen bonds toward the coordinating oxygen or sulfur atom of phenolate[16] or thiolate have a similar function with each other.[17,18]

Mononuclear Ca(II) Complexes Using Bulky Ligands

Correlation between the calcium–oxygen bond and the NH···O hydrogen bonds can be discussed only in case of the mononuclear structure of Ca(II) complexes, because the Ca-O bond properties including a bridging O atom is not able to be evaluated for the oxy anion basicity. The properties of the NH···O hydrogen bonds in phosphoric acid, 2,6-$(Ph_3CCONH)_2C_6H_3OPO_3H_2$, mononuclear Ca(II) complexes with a phosphate monoanion, $(NMe_4)[Ca^{II}\{O_2P(OH)OC_6H_3\text{-}2,6\text{-}(NHCOCPh_3)_2\}_3(N{\equiv}CMe)_3]$, and with a phosphate dianion, $[Ca^{II}\{O_3POC_6H_3\text{-}2,6\text{-}(NHCOCPh_3)_2\}\ (H_2O)_3(MeOH)_2]$ as illustrated in Fig. 2. Spectroscopic results indicate that the NH···O hydrogen bonds are not formed in the phosphoric acid state, formed strongly in the monoanion state and do very strongly in the phosphate dianion state.

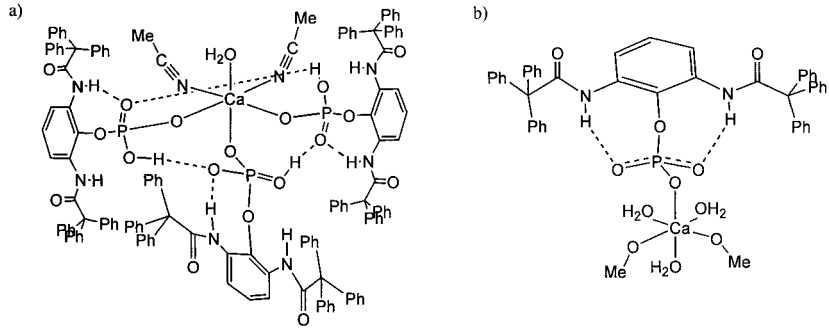


Fig. 2. Molecular structures of a) phosphate monoanion, $(NMe_4)[Ca^{II}\{O_2P(OH)OC_6H_3\text{-}2,6\text{-}(NHCOCPh_3)_2\}_3(N{\equiv}CMe)_3]$ and b) phosphate dianion, $[Ca^{II}\{O_3POC_6H_3\text{-}2,6\text{-}(NHCOCPh_3)_2\}\ (H_2O)_3(MeOH)_2]$.

The difference in Ca–O bond between carboxylate and sulfonate Ca(II) complexes is related to the regulatory properties of NH···O hydrogen bonds. The NH···O hydrogen bonds to the coordinated oxygen atoms prevent the Ca–O bonds from dissociation by a ligand exchange reaction due to lowering pK_a value of the ligands. The regulation of the Ca–O bond using NH···O hydrogen bonds was proposed.[11,19,20] The ^1H NMR results for carboxylate Ca(II) complex indicate that the NH···O hydrogen bonds can decrease the basicity on the oxy anion which is correlated with the charge transfer from $RCOO^-$ to Ca(II) ions.

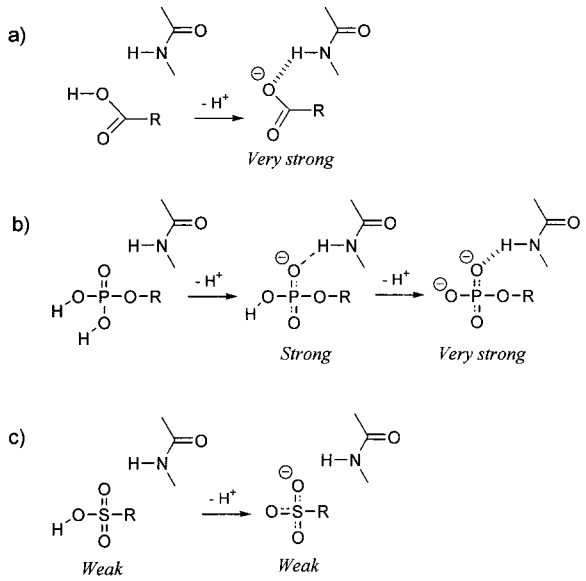


Fig. 3. Strength of NH···O hydrogen bond in a) carboxylate, b) phosphate and c) sulfonate.

The NH···O hydrogen bonds weakly form not only in the sulfonic acid state but also in the sulfonate–Ca(II) complex. There is a little difference in the strength of ionic Ca–O(sulfonate) bond character with the NH···O hydrogen bonds between the two states. Therefore, the regulation of Ca–O bond character with the NH···O hydrogen bond is not available for the case of the highly ionic Ca–O bonds with sulfonate due to the strong delocalization in RSO_3^-. Thus, carboxylate and phosphate, which have a higher basicity, are different in the properties of hydrogen bonds from sulphonate. The systematic investigation of the properties of the hydrogen bonds reveals that the strong NH···O hydrogen bonds are formed only with O atoms of high basicity coordinating to the metal ions.

NH···O hydrogen Bonding Networks Invariant Asp-containing Oligopeptide Fragment

Carboxylate with NH···O hydrogen bond is found in a resting form of aspartic proteases. The crystal structure of pepsin indicates the formation of hydrogen bonds from amide NHs of Gly33

and Ser34, and OH of Ser34 to aspartate oxygen.[21] The hydrogen bonding is similar to the Ca(II)-binding form in Ca-proteins as described above. A conformational switching was found in Asp-containing oligopeptides analogous to the resting form of the pepsin active site. Benzyloxycarbonyl-Phe-Asp-Thr-Gly-Ser-Ala-NH-cyclohexane in chloroform or aqueous micellar solution forms an extended form in the carboxylic acid state and a hairpin turn form with three NH···O hydrogen bonds in the carboxylate state. A short tripeptide, adamantly-CO-Asp-Val-Gly-$NHCH_2Ph$ also shows a similar conformational change in chloroform during deprotonation, as shown in Fig. 4. The solution structure analysis for these peptides in both states was carried out using a ^1H NMR simulation annealing (SA), IR and CD spectroscopic methods. Although the hydrogen-bonded form in the carboxylate state decreases the basicity of anion, it increases the binding constant to Ca(II). A bisamidated Kemp's acid derivatives were synthesized as simple models of the tripeptides. The acid form has a pair of antiparallel amide planes whereas its carboxylate form shows a pair of parallel ones due to the formation of two intramolecular NH···O hydrogen bonds. The deprotonation accompanies with a structural change of the cyclohexane skeleton from chair to boat besides the change in the orientation of amide NHs.

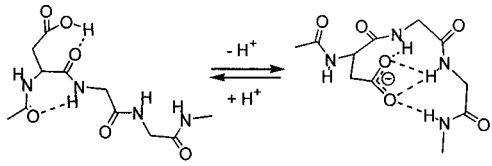


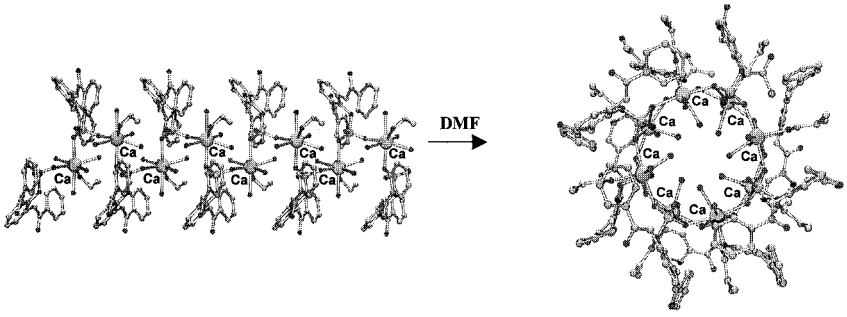
Fig. 4. Conformational switching between aspartic acid and aspartate in AdCO-Asp-Val-Gly-$NHCH_2Ph$. The both solution structures were determined by a SA method using ^1H NMR spectroscopy.

Structural Transformation of Calcium Phosphate Clusters by the Rearrangement of Inter- and Intramolecular Hydrogen Bonding Networks

Many synthetic metal phosphate complexes with small ligands are known to have an open-framework structure and then intermolecular hydrogen bonding networks. We have studied the structure of Ca(II) complexes with extremely bulky and less bulky amide ligands. For example, $(NMe_4)[Ca^{II}\{O_2P(OH)OC_6H_3\text{-}2,6\text{-}(NHCOCPh_3)_2\}_3(N{\equiv}CMe)_3]$ with an extremely bulky

triphenylacylamino group is enforced to form a mononuclear Ca(II) core by the steric congestion.

Less bulky ligands enable to restrict the coordination mode to the side of Ca cluster. A less-bulky phosphoric acid, 2,6-$(PhCONH)_2C_6H_3OPO_3H_2$, give three novel polynuclear Ca(II)- and Na(I)-phosphate complexes.[14] One is a zigzag-chain Ca cluster , $[Ca^{II}\{O_3POC_6H_3\text{-}2,6\text{-}(NHCOPh)_2\}(H_2O)_4(EtOH)]_n$ as shown in Fig. 5. Second is a cyclic-octanuclear form, $[Ca^{II}{}_8\{O_3POC_6H_3\text{-}2,6\text{-}(NHCOPh)_2\}_8(O{=}CHNMe_2)_8(H_2O)_{12}]$ and third is a hexanuclear complex, $(NHEt_3)[Na_3\{O_3POC_6H_3\text{-}2,6\text{-}(NHCOPh)_2\}_2(H_2O)(MeOH)_7]$. The crystallographic structures reveal that all have an *unsymmetric* ligand position due to the less-bulky amide groups. A dynamic transformation of the zigzag-chain Ca structure to the cyclic-octanuclear Ca complex is induced by the addition of *N,N*-dimethylformamide (DMF) due to the coordination of DMF molecules.[14] The transformation occurs with a reorganization of the intermolecularly and intramolecularly hydrogen bonding networks.



Zigzag-chain calcium complex Cyclic-octanuclear calcium complex

Fig. 5. Structural transformation of Ca(II)-phosphate cluster involving rearrangement of hydrogen bond networks.

Thus, a rearrangement of hydrogen bond network derived from solvent as well as deprotonation of carboxylic acid leads to the change of coordination geometry besides the regulation of Ca-O bond.

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