Communication

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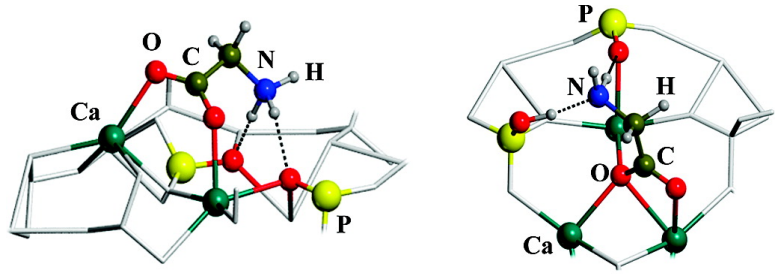

Gly-HA(001) **Gly-HA(010)**

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Ab Initio Modeling of Protein/Biomaterial Interactions: Glycine Adsorption at Hydroxyapatite Surfaces

Albert Rimola,[†] Marta Corno,[†] Claudio Marcelo Zicovich-Wilson,[‡] and Piero Ugliengo*,[†]

Dipartimento Chimica IFM, NIS Centre of Excellence and INSTM (Materials Science and Technology) National Consortium, University of Torino, Via P. Giuria 7, 10125 Torino, Italy, and Facultad de Ciencias, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, 62209 Cuernavaca, Morelos, México

Received August 16, 2008; E-mail: piero.ugliengo@unito.it

Hydroxyapatite (HA), $[Ca_{10}(PO_4)_6(OH)_2]$, is the natural major inorganic constituent of bone and teeth and the system of choice to study biomolecule/biocompatible-surface interactions. Interest in this topic is growing dramatically due to implications in many fields,[1] including nanotechnology,[2] biomaterials,[3,4] biomineralization,[5−7] biotechnology,[8] drug delivery systems,[9,10] bone tissue engineering,[11,12] and bioseparations.[13] Among biomolecules, proteins are by far most interesting because of their adhesion to HA surfaces. Indeed, several studies (spectroscopic analyses and empirical model potential calculations) were reported,[14−24] concluding that proteins are generally adsorbed by electrostatic forces of different strength depending on the protein structure. Particularly, the salivary statherin protein in contact with HA is a well-documented system.[14−19] Recent works have invoked the interaction of proteins with HA as a novel strategy to induce well-defined protein folded conformations,[17−20] which would allow the retention of the peptide biological functionality upon adsorption.

Despite great efforts in investigating protein/HA systems, a detailed atomistic picture of binding mechanisms and anchoring points occurring at the HA surfaces is still missing. *Ab initio* techniques are suitable to this purpose. However, modeling protein/HA interactions at a quantum mechanical level remains a daunting task, so that single amino acids are often adopted to model separate functionalities available in the real protein.[25] This communication reports on *ab initio* results for the adsorption of glycine (Gly) at (001) and (010) HA surfaces, providing a detailed molecular picture of the Gly/HA interface.

The two HA crystal faces shown in Figure 1 are the most relevant ones from a biological point of view. Indeed, crystal growth occurs overall at the (001) plane during biomineralization, which implies the (010) face becomes very extended in the final HA crystal.[26−28] Additionally, *ab initio* results indicate that (001) is the most stable HA surface,[29] whereas the (010) HA surface exhibits a higher reactivity because of water spontaneous dissociation when adsorbed.[30] The (001) and (010) surfaces of hexagonal HA were recently simulated at the B3LYP level by some of us.[29,30] Both surfaces are treated within the slab approach by means of a two-dimensional slab (no image replicas in the third direction) ∼14 Å thick. The (001) surface unit cell exhibits two Ca ion types (Ca1 and Ca3), whereas at the (010) surface three types (Ca1, Ca2, and Ca3) exist (see Figure 1). Gly was adsorbed at all Ca ions in various configurations. In the present work a number of points are addressed: (i) determination of the most stable Gly adduct at each HA crystal face (neutral/zwitterionic/anion); (ii) analysis of the influence of different HA surface topologies and functionalities on the adsorbed Gly structure; (iii) nature of the interactions responsible for the Gly−HA contact; (iv) estimate of the strength of Gly interaction as a function of the HA crystal face; (v) extrapolation of results for Gly to more realistic protein/biomaterial systems. Periodic B3LYP calculations with a polarized double-ζ basis set have been run by using the CRYSTAL06 code.[31] Details on computational parameters are reported as Supporting Information (SI).

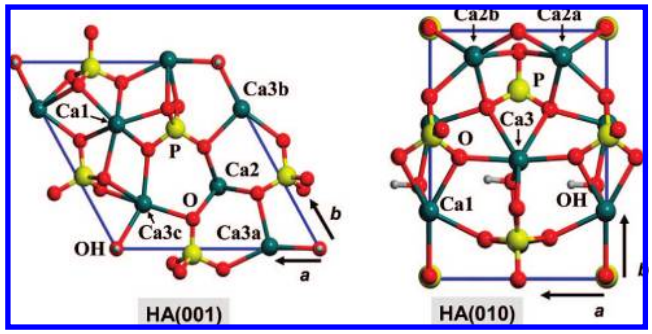

Figure 1. B3LYP-optimized geometries of the (001) and (010) hydroxyapatite (HA) surfaces.

Table 1. Interaction Energies (ΔE_{int}) and Corresponding Relative Energies (ΔE_{rel})[a]

structure	ΔE_{int}	ΔE_{rel}
001-Gly1	−248.4	0.0
001-Gly2	−207.0	41.4
001-Gly3 (SI)	−206.8	41.6
001-Gly4 (SI)	−182.2	66.2
001-Gly5 (SI)	−154.0	94.4
010-Gly1	−448.9	0.0
010-Gly2	−440.0	8.9
010-Gly3 (SI)	−388.9	60.0
010-Gly4 (SI)	−363.6	85.0
010w-Gly	−322.2	

[a] Values in units of kJ mol^{-1}.

The most stable adduct for the Gly/HA(001) system, with Gly adsorbed as a zwitterion (**001-Gly1**), exhibits an interaction energy (taking the neutral structure of Gly in the gas phase as state of reference) as large as −248 kJ mol^{-1} (see Table 1 and Figure 2). Several other adducts were characterized and reported in the SI. These results confirm the great affinity of the NH_3^+ and COO^- groups to HA (although incorporated in a simple amino acid like Gly), which is in agreement with the estimations reported for different protein/HA systems.[14−23] Zwitterions are known to be stable forms in solution[32] or when interacting with multiply charged metal ions in the gas phase[33] (due to NH_3^+ and COO^- charge stabilization by H-bond and electrostatic interactions, respectively), but to our knowledge, there is no evidence that Gly may adsorb at solid surfaces from the gas phase as a zwitterion. In **001-Gly1** the zwitterion stability arises from COO^-/Ca^+ electrostatic interactions

[†] University of Torino.
[‡] Universidad Autónoma del Estado de Morelos.

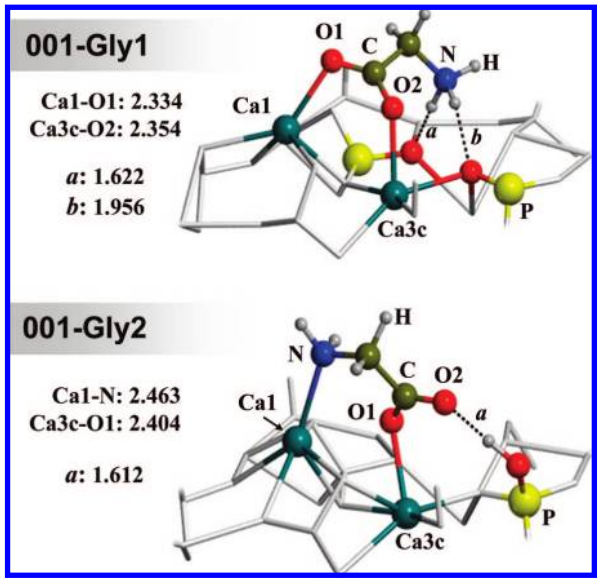

Figure 2. B3LYP-optimized geometries of the different adducts computed for the Gly/HA(001) system. Bond lengths in Å.

and H-bonds between NH_3^+ protons and surface oxygen atoms of the PO_4 group. At the HA (001) surface, however, Gly interacts simultaneously with two Ca ions (Ca1 and Ca3c), at variance with $[Ca(Gly)]^{2+}$ in gas phase.[34] This results in an extra stabilization of 95 kJ mol^{-1} with respect to **001-Gly5** (shown in the SI), which only interacts through the Ca1 ion as in the case of the gas-phase $[Ca(Gly)]^{2+}$ compound.

The basic character of the HA (001) surface is revealed by the **001-Gly2** adduct (ΔE_{int}= −207 kJ mol^{-1}), in which a proton transfer occurs from the acidic COOH group of Gly to the basic PO_4 group of the surface. In this case, Gly acts as a negative ion tightly bound to the HA surface positive sites, i.e., two Ca ions (Ca1, Ca3c) and the acidic POH proton. Close in stability to that of **001-Gly2**, **001-Gly3** may be regarded as the neutral form of structure **001-Gly1**, whereas **001-Gly4** exhibits features similar to those of the **001-Gly2** structure but for an extra energy penalty of ∼22 kJ mol^{-1} due to the missing proton transfer toward the HA surface (both structures shown in the SI). In summary, Gly is adsorbed at the HA(001) surface in its zwitterionic form, whereas the anionic form due to proton transfer toward the surface occurs in just one case which is, however, ∼40 kJ mol^{-1} higher in energy than the zwitterionic one. The situation is rather different for the (010) surface, the latter being more reactive than the (001) one, and recent periodic B3LYP calculations[30] showed that even water dissociates spontaneously giving rise to new functionalities at the surface, i.e. $CaOH_w$ and POH_w groups.

In line with this, once adsorbed at the (010) face, Gly transfers its acidic proton to the surface, confirming the higher reactivity of this surface compared to the (001) one (see Figures 3 and 4).

ΔE_{int} values (see Table 1) for the Gly/HA(010) system are all indeed higher (by a factor of 1.8) than that for adsorption at HA(001), in agreement with what is found for water adsorption. Because of the proton transfer occurrences from Gly to HA(010) surface, Gly cannot be adsorbed as a zwitterion and forms an ion pair (Gly^-/HA^+). Because of the adopted Gaussian basis set, ΔE_{int} values are overestimated (∼40%; see SI) by the basis set superposition error (BSSE). Dispersive forces are not accounted for by B3LYP so that ΔE_{int} are somehow underestimated. However, these energy contributions (which tend to cancel) to the ΔE_{int} are similar in each structure so that relative stabilities are almost unaffected (for further details see SI).

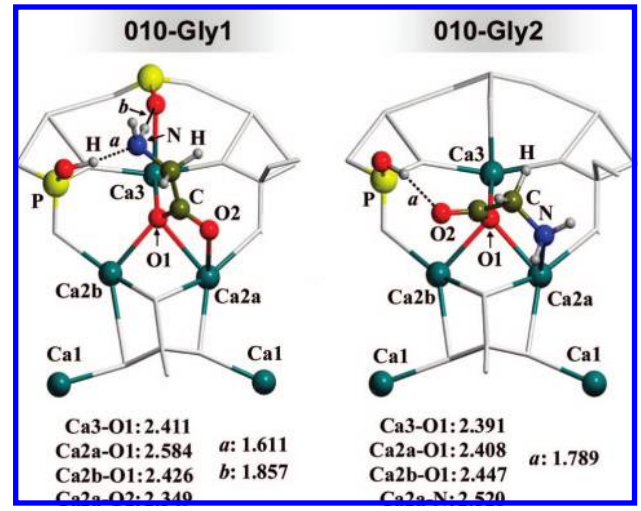

Figure 3. B3LYP-optimized geometries of the different adducts computed for the Gly/HA(010) system. Bond lengths in Å.

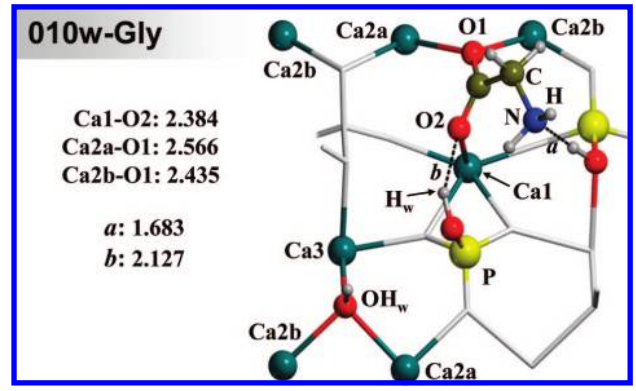

Figure 4. B3LYP-optimized geometry of the adduct found for the Gly/HA(010)w system. Bond lengths in Å.

The most stable adduct, **010-Gly1** exhibits a structural feature not found on the (001) surface: the O1 atom of the COO^- group is shared among three Ca ions, namely Ca3, Ca2a, and Ca2b, whereas the second oxygen of the carboxylate (O2) is engaged in a rather strong interaction with the Ca2a ion and the NH_2 group H-bonds with the surface POH moiety, the latter resulting from the proton transfer.

The **010-Gly2** structure is only 9 kJ mol^{-1} lower in stability compared to the **010-Gly1** one, the main difference being the swapped positions of NH_2 and COO^- groups (see Figure 3), showing a rather similar behavior as far as the formation of one strong bond between the carbonyl C═O1 group and three Ca ions (Ca2a, C2b, and Ca3). Both the **(010)-Gly3** and **(010)-Gly4** structures (shown in SI) are similar to **(010)-Gly1** and **(010)-Gly2**, respectively, except for a bond between the C═O1 group and the Ca1, Ca2a, and Ca2b ions. For this reason they are unlikely formed.

Until now, the Gly adsorption process has envisaged an unreacted HA (010) surface as the pristine material. As we have reported[30] (vide supra), the (010) surface is unlikley to exist as an "as cut" surface from the HA bulk, owing to fast reaction with water, ubiquitously present during crystal growth. For this reason, the interaction of Gly was also considered with the (010) hydrated surface. **010w-Gly** (see Figure 4) shows the structure of Gly adsorbed at the hydrated surface (H_w and OH_w were already present at the surface as a result of water dissociation). Gly, adsorbed initially as a zwitterion, rapidly transfers a proton from a NH_3^+

group (strong acid) to the surface PO_4 group (strong base) giving rise again to a Gly^-/HA^+ ion pair. Because OH_w is bound to the Ca2a, Ca2b, and Ca3 ions (which were the most favorable Gly anchoring sites for the **010-Gly1** and **010-Gly2** cases) the carboxylate interacts with the remaining Ca1, Ca2a, and Ca2b ions less efficiently. In that respect, ΔE_{int} for **010w-Gly** (see Table 1) is less favorable than that for **010-Gly3** (the analogous structure with an "as cut" (010) HA surface; see SI) because of the decreased basic character of the water-reacted HA(010)w surface in comparison to the pristine (010) one.

In conclusion, adsorption of Gly at HA surfaces under strict gas-phase conditions studied at B3LYP in a periodic approach reveals the following: (i) Gly is adsorbed as a zwitterion at the HA (001) surface, the COO^- group interacting with two Ca ions and the NH_3^+ protons H-bonding the oxygen surface atoms; (ii) the acidic COOH proton is transferred to the surface PO_4 group in only one case (**001-Gly2**) at the HA (001) surface giving rise to a Gly^-/HA^+ ion pair; (iii) on both the pristine HA (010) surface and the one reacted with water (010)w, a proton (from either COOH or the NH_3^+ groups) is transferred from Gly to the surface; (iv) the Gly carboxylate interacts with three Ca ions at the same time at the pristine (010) face. These results indicate that (i) the HA(001) surface behaves as a "solid solvent" capable of stabilizing the zwitterionic form of Gly with no need of liquid water and (ii) the HA(010) surface leads to easy Gly deprotonation showing a strong basic character which is responsible of its chemical activity. These theoretical results also provide clues about protein/biomaterial surface interactions. The fact that both COO^- and NH_3^+ (or NH_2) groups are responsible for Gly HA contact indicates that amino acid side chains carrying either acidic (namely, aspartic and glutamic acids) or basic (namely, lysine and arginine) residues are very prone to strongly interacting with HA surfaces with the same mechanism proposed here. This is indeed in line with literature spectroscopic and classical modeling results, showing that carboxylic and basic-containing residues are usually found in close proximity to the HA surfaces.[14−23] Furthermore, COO^- and NH_3^+-rich peptides are induced to become folded in an α-helix conformation by interaction with the HA surfaces, as a consequence of the rather strong interactions of these groups with the HA surface.[17−20]

Further studies are currently in progress in our laboratory to simulate the adsorption of different amino acids as a way to gauge the influence of the side chain residues on the adsorption strength. Along the same line, but requiring a significantly larger computational effort, is the role of water as a crucial solvent for understanding protein−HA interactions in biological conditions.

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Supporting Information Available: Surface models, computational details, absolute energies, and fractionary coordinates of all the optimized structures. This material is available free of charge via Internet at http://pubs.acs.org.

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