

## SPECTROSCOPIC INVESTIGATION OF MYOGLOBIN CONFORMATIONAL CHANGE ON HYDROXYAPATITE NANOCRYSTALS FUNCTIONALIZED WITH ALENDRONATE

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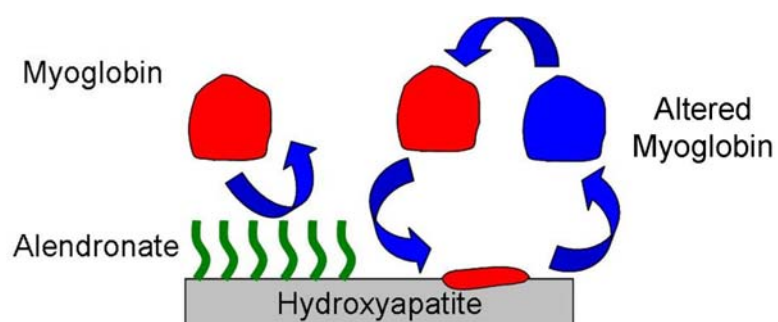
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The interaction of proteins with solid inorganic surfaces is not only a fundamental phenomenon but is also the key to several important and novel applications. In the biomaterials field, protein-surface adhesion is the first event in the integration of an implanted device or material with biological tissues. In nanotechnology, protein-surface interactions are pivotal for the assembly of interfacial protein constructs, such as sensors, activators, and other functional components at the biological/electronic junction.

In the biomaterial field, calcium phosphates, which are the inorganic components of bone, are widely used as bone grafts. Synthetic hydroxyapatite [ $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ] nanocrystals (nHA) represent an elective material for bone substitutes, and moreover, their surface functionalization with bioactive molecules makes them able to transfer information to and to act selectively on the biological environment. The chemical conjugation of bisphosphonates (BPs), specifically alendronate, to hydroxyapatite could be an effective means to impart to it fine-tuned bioactivity. The obtained materials have potential use in bone implantation and as prospective drug-delivery devices [1].

Horse heart myoglobin (Mb), a well-characterized protein, has been adsorbed onto biomimetic hydroxyapatite nanocrystals and onto the nHA/alendronate conjugate powdered samples. The kinetic adsorption of Mb onto nHA is dramatically affected by its functionalization with alendronate. The covering of the nHA surface by alendronate inhibits the adsorption of myoglobin. The adsorption mechanisms of the protein were studied by spectroscopic techniques (UV-vis and surface-enhanced Raman spectroscopy).

The results indicate that the protein changes conformation upon adsorption on the inorganic substrate. In particular, the interaction with nHA alters the coordination state of the iron in the heme through the formation of a hexacoordinated low-spin Mb heme, possibly involving the distal histidine. Instead, the covering of the nHA surface by alendronate does not adsorb the protein but preserves the coordination state of the heme moiety (Figure 1) [2]. The spin state of myoglobin is relevant in relation to the catalytic activity of the protein. Myoglobin behavior toward alendronate-grafted nHA shows that this functionalization imprints surface selectivity to nHA and drives the biological environmental response toward them.



**Figure 1.** Representation of the interaction between Mb and HA/alendronate nanocrystals

## References

1. Palazzo B., Iafisco M., Laforgia M., Margiotta N., Natile G., Bianchi C.L., Walsh D., Mann S., Roveri N., *Adv. Funct. Mater.*, 2007, 17, 2180.
2. Iafisco M., Palazzo B., Falini G., Di Foggia M., Bonora S., Nicolis S., Casella L., Roveri N., *Langmuir*, 2008, 24, 4924.