Protein Corona on Hydroxyapatite Nanoparticles

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Abstract

Bioceramic nanoparticles have a variety of characteristics that make them an ideal material for hard tissue engineering. However, knowing their success within the fluids of the human body is vital to fully comprehend their behaviour. The interaction of hydroxyapatite nanorods (HA) and bovine serum albumin (BSA) was investigated in order to accomplish this task. Using a variety of techniques, the key features of the physiological interaction between BSA and hydroxyapatite nanoparticle are discussed. Experimental data were collected using specific approaches to determine critical parameters including rates, affinities, and stoichiometries.

Materials and Methods

Preparation of HA and ABS samples

Bone-like HA nanoparticles of 8 ± 1 nm diameters and 28 ± 3 nm length were synthesized by a previously explained methodology [1]. Special focus has been put in their surface microrough properties, as it is an effective platform to control its bioactivity. [2] In order to prepare the dispersions of HA, the proper amount was weighed added to vials with 0.015 L of distilled water. Solutions were strongly sonicated for 10 minutes until to obtaining homogeneous dispersions. Protein solutions were freshly prepared in triple distilled water and stored at 0 – 4 º C. Before the beginning of each experiment, BSA solutions were exposed to room temperature (RT) for a maximum of 1 h.

Characterization of the interaction

Isothermal titration calorimetry. Experiments were carried out in a VP-ITC microcalorimeter (MicroCal Inc., Northampton, U.S.) [3]. In order to determine the binding isotherms, the hydroxyapatite were introduced into the syringe (296 μL), while the BSA solutions were introduced into the sample cell (1.4166 mL). To avoid HA precipitation, the stirring was maintained steady throughout all the experiment at 416 rpm. Injections of 10 μL at a constant rate of 0.5 μL s⁻¹ were performed every 300 s. To eliminate negative signals, a reference power of 25 μJ s⁻¹ was applied. Following the explained methodology, tests were performed at a temperature of 25 ºC.

CD spectroscopy. Far-UV circular dichroism (CD) spectra were measured using a JASCO-715 spectropolarimeter (Japan) with a JASCO PTC-343 Peltier-type thermostated cell holder. CD spectra of pure BSA and nanoparticle/BSA solutions were obtained from 190 to 270 nm. The following setting was used: resolution, 1 nm; bandwidth, 1 nm; sensitivity, 50 mdeg; response time, 8 s; accumulation, 3; and scan rate, 50 nm/min.
Results and Discussion

Isothermal titration calorimetry (ITC) was used to study and characterize the interaction of Hydroxyapatite nanorods with Bovine Serum Albumin. A 0.05 mM solution of the protein was inserted in the calorimetric cell and a 0.5 mM dispersion of HA in water was added to the syringe. Figure 1 depicts the results of the titration, showing the dependence between the molar ratio and the amount of heat released. Each dot corresponds to an injection. Values were obtained after subtracting the blank, in order to neglect dilution heats. As it can be seen in the Figure, the binding interaction between the two compounds is exothermic and its values gradually diminished with increasing HA/BSA ratio. This result is in good accordance with the standard, as most of the nanoparticle-protein interactions are monotonically exothermic, just some of them are endothermic [4].

![Figure 1](image)

**Figure 1.** a) Heat of interaction for titration of HA nanoparticles into BSA solution at 298.15 K. Solid lines represent the fit using a one-site binding model. b) Zeta potential of HA nanoparticles as a function of BSA/HA ratio.

The fitting of the results into isothermal functions is a helpful way of quantifying the thermodynamic parameters. The binding constant ($K$), enthalpy change ($\Delta H$), and binding stoichiometry ($n$) were determined from fitting analyses. The entropy changes ($\Delta S$) were also obtained by means of standard thermodynamic equations. Respective values were; $K = (4.87 \pm 1.23) \times 10^4$ M$^{-1}$; $\Delta H = -1093 \pm 280$ kJ·mol$^{-1}$; $n = 1.1 \pm 0.3$ and $\Delta S = -9.23 \pm 3.67$ kJ·mol$^{-1}$·K$^{-1}$. The negative value of enthalpy is directly related to the electrostatic and hydrophobic interactions, formation of hydrogen bonds and π-π interaction. On the other hand, hydration and conformational restriction of the amino acid residues on protein’s surface following the adsorption contribute to unfavorable entropy loss. Therefore, for this particular bioceramic-protein system it is safe to assure that the main driving forces involved in the adsorption process were non-covalent bondings [5].

To complete ITC data, z-potential measurements also served to probe an evolution of the protein corona with the increasing ratio. As the BSA concentration increased a quick decrease in the
Zeta potential appeared, suggesting the binding of BSA. After this minimum, a small raise and a final plateau is observed. In this particular case, both the HA and BSA have negative charge, though, it was previously reported that protein adsorption onto nanoparticles lead to a shift in zeta potential regardless of original surface charge [6]. In terms of variations in z-potential when considering the isotropic surface of nanoparticles, the observed changes were logically attributed to BSA’s special structural characteristics.

Another important step to complete the characterization of the system was to probe whether BSA adsorption onto HA resulted in any structural improvements. The CD spectra of BSA in the absence of HA and at varying molar ratios are seen in Figure 2. Free BSA has a high α-helix content of 63% and a low β-sheet content of 2%, according to the CD result, which is consistent with previous research [7]. In the presence of HA, the ellipticity values in the CD spectra decrease significantly.

Secondary structure of BSA is mostly α-helical [8]. In the presence of the nanoparticles, the negative peaks at 209 and 222 nm in the spectrum increase, suggesting a loss of BSA α-helical structure and an increase in the β-shift. Minor changes indicate that conformational changes are localized and minimal. Nanoparticles cause minor disruption to the secondary structure of proteins [9]. This finding is consistent with the fact that BSA’s structure is relatively stable due to its high α-helix nature. The versatility allows BSA to be adsorbed onto the nanoparticle surface while retaining the 3D structure [10] [11]. At low nanoparticle concentrations, substantial changes in the secondary structure of BSA can also be noticed. Changes in the secondary structure are greatly reduced as the nanoparticle concentration is increased. It is clear, thus that HA nanoparticles have only a minor impact on BSA conformation, demonstrating excellent biocompatibility.

Figure 2. Circular Dichroism (CD) spectra of BSA under different molar ratios of HA:BSA as marked in the figure.
References.