Discrimination of Albumin and Lysozyme-Adsorption Characteristics on Two Differently-Originated Apatites

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(Received May 30, 1996)

Two kinds of hydroxyapatite (HAp) originated from cattle bones and reagents displayed different adsorption behavior of albumin on the C-sites and lysozyme on the P-sites of the HAp surface, which was strongly influenced by the basic strength of the C-sites and the acid strength of the P-sites as well as the total amount or surface proportion of the two sites, depending on the heat-treatment temperature of the HAp's.

Hydroxyapatite (HAp : $Ca_{10}(PO_4)_6(OH)_2$) has excellent characteristics for adsorbing acidic and basic proteins, which are originated from biocompatibility.[1] The surface characteristics of HAp as an adsorbent for the proteins are possibly influenced by the size and morphology of crystallites.[2,3] The adsorption sites on the hexagonal HAp's surface are classified into a C-site, which means the position of OH^- on the a-face, and a P-site, which corresponds to the columnar Ca I site on the c-face.[4] Bovine serum albumin (BSA)[5] and lysozyme from egg white (LSZ)[6] are selectively adsorbed on the C- and P-sites on HAp, respectively.[4] On the two differently-originated apatites, which are Ca^{2+}-deficient HAp (r-HAp) containing small amounts of impurity ions derived from cattle bones, and stoichiometric HAp (s-HAp) prepared from reagents,[7] they will necessarily constitute different fine surface structure. The proportion and chemical nature of the C- and P-sites on the two HAp's can be strongly influenced by the heat treatment conditions. The aim of this study is to discriminate clearly the adsorption characteristics of BSA and LSZ on the two HAp's by changing the heating temperature.

r-HAp was prepared by a consecutive procedure of cattle bones such as calcination, dissolution to a 0.2 mol·dm^{-3} HNO_3 solution and reprecipitation at 298 K and pH 10.5 .[7] s-HAp was synthesized from guaranteed grade $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ reagents by the wet method. Adsorption characteristics of BSA and LSZ on these HAp's were compared at the heating temperatures of 273 ~ 1473 K in a stream of water vapor. The HAp powders were added into the solutions containing the 0.3 ~ 3.0 mg·cm^{-3} of BSA or LSZ which were adjusted at pH 6.9 ~ 7.1 in a mixed solution of 20 mmol·dm^{-3} KH_2PO_4 and K_2HPO_4 , and they were agitated at 282 or 295 K.

The adsorption of BSA or LSZ on all the HAp powders obeyed Langmuir's isotherm equation, and the saturated amount of adsorption (designated by A_{SA}(B) for BSA and A_{SA}(L) for LSZ) per surface area of HAp was calculated. The heat of adsorption (Q_B for BSA and Q_L for LSZ) was easily determined from the two adsorption isotherms obtained at 282 and 295 K using the Clausius-Clapeyron equation.[7] Figure 1 shows three dimensional correlations among A_{SA}(B), A_{SA}(L), Q_B , Q_L and the heating temperature (T) of s-HAp and r-HAp powders, respectively. For each of the two HAp's, two characteristic curves were obtained for BSA and LSZ adsorption and they clearly exhibited the difference in the adsorption behavior of BSA on the C-sites and LSZ on the P-sites of the HAp surface. From these results, one can recognize that the physicochemical nature of the two HAp's surfaces is drastically varied by raising the heating temperature, and that the adsorption parameters of BSA and LSZ independently move on their own locuses of the three dimensional space. Since the big differences between Q_B and Q_L are responsible for high chromatographic separation efficiencies of the proteins on HAp, it is found that r-HAp has a more effective surface for liquid chromatography to separate BSA and LSZ than s-HAp. The geometry of each of the locuses should reflect the surface characteristics of the two HAp's such as the total number of occurrences and the proportion and chemical nature of the P- and C-sites.

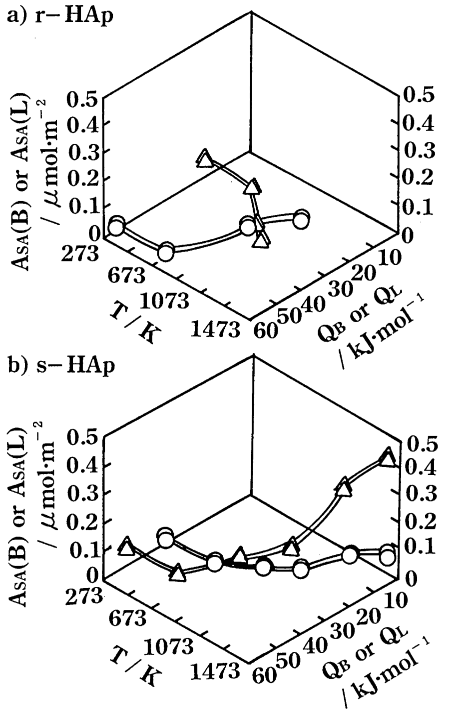

Figure 1. Three dimensional correlations among the saturated amount of adsorption, the heat of adsorption and the heating temperature for the two HAp's. (○) BSA, (△) LSZ.

The surface proportion of the P- and C-sites on HAp can be evaluated by the ratio (A_{SA}(L) /A_{SA}(B) = R_A). Figure 2 shows R_A as a function of the heating temperature of the two HAp's powders. For r-HAp, one can recognize two temperature regions on the characteristic R_A variation, almost constant at 273 ~ 673 K (Region Ⅰ) and steeply decreasing at 673 ~ 1073 K (Region Ⅱ). The XRD patterns and the FE-SEM photographs of the HAp's powders separately demonstrated change in the microstructure. In the Region Ⅰ, the crystallite size (C_S) which was evaluated by using XRD patterns and the morphology of particles for the HAp's varied little. However, in the Region Ⅱ, the crystal of r-HAp grew isotropically, while that of s-HAp grew on the a-face along the c-axis, so that C_S gradually increased. The microstructure became sphere-like aggregates with 50 ~ 100 nm in width for r-HAp and prismatic particles with 3 ~ 5 aspect ratios for s-HAp. Based on these results, the reduction of R_A for r-HAp in the Region Ⅱ can attribute to a decrease in surface proportions of both the sites which can be related to the surface structure variation such as an increase of OH^--vacancy and a loosening effect due to segregation of the substituted metal ions. For s-HAp, R_A abruptly increased at 1073 ~ 1473 K. At the temperature region, the microstructure of s-HAp transformed to plate-like particles, whereas r-HAp partially decomposed to β-$Ca_3(PO_4)_2$ because of Ca^{2+}-deficient structure (Ca/P=1.65) by heat treatment at temperatures higher than 1073 K.[8]

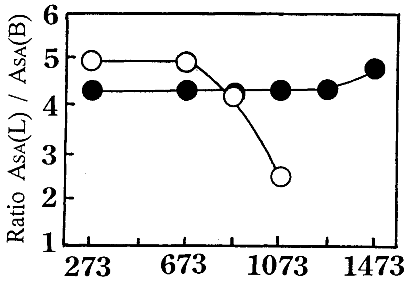

Figure 2. Ratios of the saturated amounts of adsorption for LSZ and BSA as a function of the heating temperature of the two HAp's. (○) r-HAp, (●) s-HAp.

Concerning the nature of the adsorption sites, provided that the values of Q_L and Q_B, respectively, indicate adsorption strength of the P- and C-sites, the ratio of acid and basic strength on the surfaces can be represented as the ratio (Q_L/Q_B =R_Q). Figure 3 shows R_Q as a function of the heating temperature of the HAp's powders. One can recognize again the two temperature Regions in R_Q value, for r-HAp, Region Ⅰ (273 ~ 673 K) in which it shows almost constant, and Region Ⅱ (673 ~ 1073 K) in which it shows significant increase and, for s-HAp, it shows gradual decrease in Region Ⅱ (673 ~ 1473 K). Additionally, in the Region Ⅱ, Q_L for r-HAp and s-HAp, respectively increased and decreased, but Q_B for both the HAp's decreased. As for an explanation for the R_Q variation in the Regions Ⅱ, it may be proposed that the acid strength of the P-sites and basic strength of the C-sites considerably changed on the HAp surface, together with the variation in total number or surface proportion of the two sites. Especially, on the surface structure of r-HAp with 30 ~ 40 nm in C_S, in the Region Ⅱ, the acid strength on the P-sites for LSZ adsorption would be stronger than the basic strength on the C-sites for BSA adsorption. Because the crystallites of r-HAp grow isotropically by heat treatment, r-HAp can have different acid strength from s-HAp. Moreover, the P-sites on r-HAp are partially substituted by impurities such as 0.6 % Mg^{2+} and 0.04 % Na^+ ions. If the r-HAp powders obtained at the higher temperatures than 673 K are suspended in the LSZ solution, the impurity ions segregated on the HAp surface will dissolve minutely because of the loosening surface structure, so that the remaining sites make up the active P-sites having the high acid strength for LSZ adsorption.

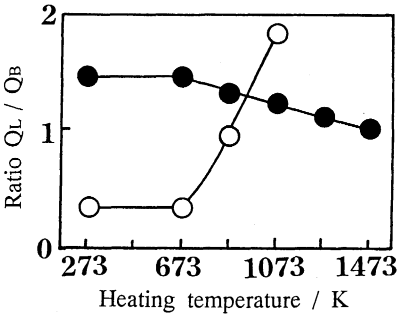

Figure 3. Ratios of the heats of adsorption for LSZ and BSA as a function of the heating temperature of the two HAp's. (○) r-HAp, (●) s-HAp.

Accordingly, one can conclude that the adsorption characteristics of BSA and LSZ are clearly discriminated on the two originated HAp's, and that the adsorption behavior is strongly influenced by the basic strength of C-sites and the acid strength of P-sites on each the HAp's. The total number and surface proportion of both the sites are strongly reflected by the size and morphology characteristics of crystallites depending on the heating temperatures and starting materials.

References and Notes

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