SURFACE CONTROL OF CATTLE BONE-ORIGINATED APATITES FOR ADSORPTION OF DIFFERENT PROTEINS BY HEAT-TREATMENTS

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Abstract Cattle bone-originated apatites (r-HAp) powders were prepared by a dissolution-precipitation and subsequent heat-treatments at 673 ~ 1073 K. Adsorption characteristics on the r-HAp powders were investigated for different proteins such as bovine serum albumin (BSA), myoglobin from horse skeletal muscle (MOG), ribonuclease A from bovine pancreas (RBN), lysozyme from chicken egg white (LSZ), and cytochrome c from horse heart (CTC). The adsorption for each of the proteins obeyed the Langmuir equation. The saturated amounts of LSZ, CTC, and RBN (basic proteins with isoelectric points (E_0) > 7.0) adsorbed (A_{SB}) were 2 ~ 10 times larger than those of BSA and MOG (acidic proteins with E_0 < 7.0) adsorbed (A_{SA}). As the heat-treatment temperature was elevated, the A_{SB} and A_{SA} gradually increased, and the ratios of A_{SB} to A_{SA} decreased, and heats of adsorption for basic proteins increased, whereas those for acidic proteins decreased. These characteristic changes can contribute to a design of liquid chromatographic column for adsorption-separation of the proteins.

INTRODUCTION

Hydroxyapatite ($\text{HAp:Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has excellent adsorption characteristics for proteins without denaturation because of high bioaffinity, and thereby has been applied as an adsorbent for high-performance liquid chromatography.¹⁻³ The adsorption sites on the HAp surface are classified into two a C-site on the a-face and a P-site on the c-face. Acidic proteins with a terminal group of $-\text{COO}^-$ (isoelectric point (E_0) = pH 4.2 ~ 4.8) and basic proteins with a terminal group of $-\text{NH}_2^+$ (E_0 = pH 10.0 ~ 11.0) are selectively adsorbed at the C-sites on the a-face and the P-sites

on the c-face of HAp, respectively.^{2,3} The morphology of HAp particles and the proportion of the P- and C-sites on the surfaces should contribute to the selective adsorption for various proteins. Cattle bone-originated apatites (r-HAp) prepared by a dissolution-precipitation method have a structure-sensitive surface containing small amounts of Mg^{2+} ion.^{4,5} The r-HAp powders gave higher specific surface areas and larger total pore volumes than stoichiometric HAp powders prepared from reagents. The adsorption characteristics of the r-HAp powders should be influenced by not only the pore structure but also the total number, proportion and chemical nature of the surface P- and C-sites. Objectives of this study are to clarify adsorption characteristics for each of the five different proteins on the r-HAp powders and to control the proportion of the surface active sites for adsorption of the different proteins by heat-treatments.

EXPERIMENTAL PROCEDURE

Cortical bones in cattle femur (Holstein, bull, Hokkaido, Japan) were used as starting sources. The bones were calcined at 1373 K and then dissolved into a HNO_3 solution. The solution was aged at 298 K and pH 10.5 for 24 h to precipitate the r-HAp powder. The powder was heat-treated at 673 ~ 1073 K for 24 h in a stream of water vapor.^{4,5} Field emission-scanning electron microscopy images of the samples were taken to observe the size and morphology of the r-HAp particles. The BET specific surface area of the powders was measured by N_2 -adsorption at 77 K. Adsorption characteristics on the r-HAp powders were investigated at pH 6.9 ~ 7.1 and 277 ~ 295 K for each of 0.5 ~ 3.0 $\text{mg} \cdot \text{cm}^{-3}$ -proteins solutions such as bovine serum albumin (BSA), myoglobin from horse skeletal muscle (MOG), ribonuclease A from bovine pancreas (RBN), lysozyme from chicken egg white (LSZ), and cytochrome c from horse heart (CTC), whose solutions contained 20 $\text{mmol} \cdot \text{dm}^{-3}$ - KH_2PO_4 and K_2HPO_4 buffers, by a batch method.^{4,5}

RESULTS AND DISCUSSION

The adsorption isotherms obtained for the five proteins on all the r-HAp powders obeyed the Langmuir equation, indicating that the proteins can be adsorbed in a homogeneous monolayer on the r-HAp surfaces. From the equation, the saturated

amounts of proteins adsorbed were calculated and the results were shown in Figure 1 as a function of heat-treatment temperature. The five proteins used have different molecular size and isoelectric point (E_0). The saturated amounts of basic proteins (A_{SB}) and acidic proteins (A_{SA}) adsorbed were related to the E_0 , rather than the molecular size. The A_{SA} values of LSZ, CTC and RBN⁶ with $E_0 > 7.0$ were 2 ~ 8 times larger than those of BSA and MOG^{6,7} with $E_0 < 7.0$. This result suggests that the numbers of the P-sites would be more than the numbers of the C-sites, because basic proteins (LSZ, CTC, RBN) and acidic proteins (BSA, MOG, LSZ) can selectively be adsorbed on the P-sites and C-sites on HAp surface, respectively. At the heat-treatment temperatures lower than 673 K, the A_{SB} and A_{SA} were almost constant. As the heat-treatment temperature was elevated to 873 K, the A_{SB} increased

FIGURE 1. Saturated amounts of proteins adsorbed on the r-HAp powders as a function of heat-treatment temperature. ▲ BSA, ○ MOG, ◇ RBN, □ CTC, △ LSZ.

from $0.063 \sim 0.108$ to $0.079 \sim 0.149 \mu\text{mol}\cdot\text{m}^{-2}$, whereas the A_{SA} increased from $0.014 \sim 0.043$ to $0.042 \sim 0.061 \mu\text{mol}\cdot\text{m}^{-2}$. The increases of A_{SB} and A_{SA} suggest the thermally induced changes in the surface proportion of adsorption active sites for the proteins molecules at the temperatures of $673 \sim 1073$ K, because the specific surface area decreased from 86 to $12 \text{ m}^2\cdot\text{g}^{-2}$ and the morphology of the r-HAp particles varied from microcrystals with about 20 nm in length to sphere-like aggregates with $50 \sim 100$ nm in width. For these adsorption characteristics of basic and acidic proteins on

the r-HAp, one may recognize as changes in the number and nature of the P- and C-sites on the surfaces. The ratios of A_{SB} to A_{SA} , which mean surface proportions of the P- and C-sites,⁵ decreased from 1.5 ~ 7.8 to 1.3 ~ 6.9. This result suggests that the r-HAp powders heat-treated at 673 ~ 1073 K were accompanied with crystal growth along the a-face of hexagonal crystal structure.

Considering that the adsorption heats for basic (Q_B) and acidic (Q_A) proteins reflect adsorption strength of the P- and C-sites on HAp surface,⁵ the isosteric differential heat of adsorption was calculated from the adsorption isotherms at 277 ~ 295 K by the Clausius-Clapeyron equation. Figure 2 shows heats of adsorption for the proteins on the r-HAp powders as a function of heat-treatment temperature. At the temperatures of 673 ~ 1073 K, the Q_A for BSA and MOG decreased from 50 ~ 55

FIGURE 2. Heats of adsorption for proteins on the r-HAp powders as a function of heat-treatment temperature. ▲ BSA, ○ MOG, ◇ RBN, □ CTC, △ LSZ.

to 20 kJ·mol⁻¹, whereas the Q_B for LSZ and CTC increased from 11 ~ 20 to 32 ~ 35 kJ·mol⁻¹. On the surfaces of r-HAp heat-treated at 673 ~ 1073 K, the acid strength on the P-sites for basic proteins-adsorption can be stronger than the basic strength on the C-sites for acidic proteins-adsorption. The r-HAp prepared by the dissolution-precipitation contains foreign metal ions (Mg^{2+} and Na^+) derived from cattle bones.⁴ The proportion of the P- and C-sites on the surfaces should be influenced by the heat-treatment conditions. The acid or basic strength for r-HAp is possibly attributed to the OH⁻-vacancy and the dissolution of Mg^{2+} ion.⁵ When the

r-HAp powders are added to aqueous solutions containing the proteins, it is deduced that the OH^- , Ca^{2+} and Mg^{2+} ions on the surfaces dissolve minutely, and that each of the remaining sites constitutes the active C- and P-sites which are available for adsorption of acidic or basic proteins.

SUMMARY

The physicochemical consideration of the active sites (P- and C- sites) on the r-HAp surfaces was carried out by the adsorption characteristics of basic and acidic proteins; the proportion of the P- and C-sites on the surfaces was evaluated by the ratio of saturated amounts of the proteins adsorbed ($A_{\text{SB}}/A_{\text{SA}}$), whereas the chemical nature of the two sites was represented by the heats of adsorption for the proteins (Q_{B} and Q_{A}). The $A_{\text{SB}}/A_{\text{SA}}$ and the Q_{A} decreased, and the Q_{B} increased with rising heat-treatment temperature from 673 to 1073 K. Based on these results, it was found that the r-HAp prepared by the dissolution-precipitation method gave an advantage for the adsorbent of proteins, because one can easily design the r-HAp surfaces having the requested proportion of the P- and C-sites and the acid-basic strength for adsorption of various proteins by controlling the heat-treatment temperature.

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