doi:10.1246/cl.2011.387
Published on the web March 12, 2011

Improved Adsorption of Basic Protein on Hydroxyapatite Nanoparticles Synthesized Using a Lamellar Template of Sodium Dodecylphosphate

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We synthesized hydroxyapatite (HAp) nanoparticles using a lamellar phase, which was formed using a phosphorous surfactant, sodium dodecylphosphate (SDP). The HAp nanoparticles synthesized using SDP, after calcination, adsorbed more egg white lysozyme compared to that synthesized without SDP.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is a primary mineral component of natural bone and tooth, and it has attracted much attention because of its physicochemical and biological properties. It is widely applied as an adsorbent for proteins,[1–3] catalysts[4,5] and in water purification.[6]

HAp particles can be synthesized by various routes such as chemical precipitation, hydrothermal reactions, sol–gel synthesis, and mechanochemical synthesis. Recently, a surfactant-based template method has shown promise for the synthesis of HAp or calcium phosphate nanoparticles with unique morphology[7] and mesostructures.[8–12] In view of previous reports on the synthesis of HAp mesostructures using surfactant self-assembly,[8–11] Yao et al. have reported the formation of hexagonal mesostructured HAp using cationic surfactant as a template.[8] On the other hand, several research groups have reported the synthesis of a stable lamellar mesostructured HAp using various kinds of surfactants as templates.[9–11] We are certain that an effective surface-modification is possible in the synthesis of a stable lamellar mesostructured HAp because entire HAp layer surfaces can be surrounded by surfactant molecules. Therefore, the selection of an appropriate surfactant for the formation of a lamellar mesostructure is also important for the enhancement of the interaction between the surface of HAp and the surfactant molecules.

In this work, we synthesized highly ordered lamellar HAp nanoparticles using sodium dodecylphosphate (SDP, Nikko Chemicals Co., Ltd.). SDP containing a phosphate group was used as a template to form a lamellar structure. The adsorption properties of the basic and acidic proteins of the calcined HAp nanoparticles were examined in order to clarify the effects of surface modification of HAp synthesized using a lamellar template of SDP.

Hydroxyapatite nanoparticles were synthesized using SDP by the template method. $Ca(NO_3)_2 \cdot 4H_2O$ (5.31 g) was dissolved in water (7.5 mL). $(NH_4)_2HPO_4$ (1.78 g) and 0.185 M aqueous SDP solution were added to the above solution. $(NH_4)_2HPO_4$ was added at a Ca/P molar ratio of 1.67. The pH of the mixed solution was adjusted from 10 to 12 using an NH_4OH solution. The mixed solution was then heated to 353 K under reflux for 24 h. After washing with water, the obtained white precipitate was dried at 333 K for 24 h and then calcined at 823 K for 6 h to remove the surfactant template.

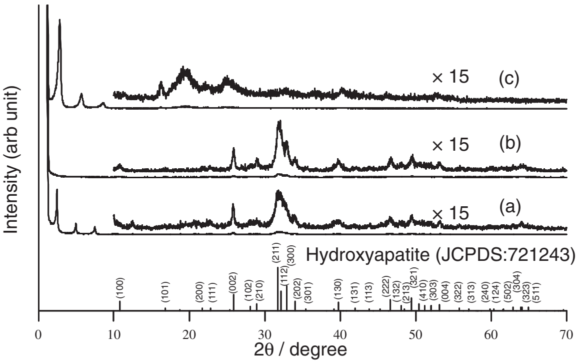

Figure 1. XRD patterns of the products synthesized in the presence of SDP: (a) dried product, (b) calcined product, and (c) dried product synthesized without $(NH_4)_2HPO_4$.

The adsorption properties of the egg white lysozyme (LSZ) of basic protein and bovine serum albumin (BSA) of the acidic protein were also examined. Various weights of protein/K_2HPO_4–KH_2PO_4 as buffer solutions (pH 7) were prepared. HAp particles (10 mg) were added to the solution under agitation at 295 K for 72 h. After centrifugation, the concentration of protein in the solution was estimated by measuring the absorbance with a UV spectrophotometer.

Figure 1 shows XRD patterns of the products synthesized at pH 12 in the presence of SDP. The product has a single crystal phase of HAp (JCPDS card No. 721243) as shown in Figures 1a and 1b. In the low-angle region, three sharp peaks at $2\theta = 2.5$, 5.0, and 7.5° are clearly observed, as shown in Figure 1a. These peaks correspond to a periodic distance of 3.53 nm, which was calculated using Bragg's formula. After calcination, these peaks disappeared, as shown in Figure 1b, and this is due to the removal of SDP molecules. Similar results were obtained for the syntheses at pH 10 and 11. We are sure that these three peaks come from the lamellar phase formed by the self-assembly of SDP molecules.

Figures 2a–2d show SEM images of the dried and calcined products synthesized at pH 12. Spherical and some short rod-like particles were obtained in the products synthesized without SDP, as shown in Figures 2c and 2d. In contrast, many particles with a rod-like morphology and with a higher aspect ratio of 10 to 15 were clearly observed in the product synthesized in the presence of SDP, as shown in Figures 2a and 2b. The typical

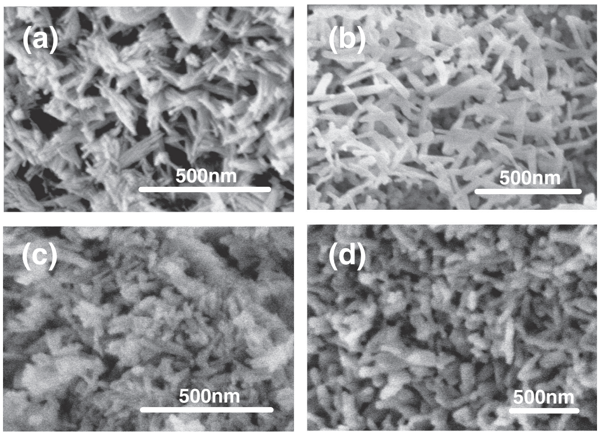

Figure 2. SEM images of (a) dried and (b) calcined products synthesized in the presence of SDP, (c) dried and (d) calcined products synthesized without SDP.

size of the rod particles is ca. 20 nm in diameter and 200–250 nm in length (Figure 2a) although both the diameter and length increased after calcination (Figure 2b). Before calcination, the relative intensity of the (002) diffraction peak (70–80%) in the XRD pattern (Figure 1a) was much higher than that (38%) in the standard XRD pattern of HAp (JCPDS card No. 721243). That is, this synthesis using a lamellar template favors the HAp crystal growth along the *c* axis. It is assumed that the rod-like particles formed within the two-dimensional water layer in the lamellar phase, which is consistent with that reported in a previous study.[11] Zhang et al. have reported that rod-like HAp particles were formed using a lamellar phase of phosphoric acid monododecyl ester in the presence of ethanol and that the long axis of HAp crystals were aligned parallel to the direction of the *c* axis.[11] Therefore, it is obvious that the morphology of the HAp crystals is strongly affected by the SDP molecules in our synthesis.

To investigate the interaction between the HAp crystals and the SDP molecules, we carried out a synthesis without $(NH_4)_2HPO_4$, which had been used as a PO_4^{3-} source. As a consequence, a calcium dodecylphosphate crystal[9] with a lamellar structure was observed, as shown in Figure 1c. This strongly suggests that SDP molecules have a good affinity for the calcium ion because a calcium phosphate compound formed despite a lack of $(NH_4)_2HPO_4$. It is likely that SDP molecules play important roles in the lamellar template and also in the substrate for the nucleation and growth of HAp crystals. XPS measurements were used to obtain further information about the surface of the HAp particles. Table 1 presents the molar ratio of Ca to phosphate (Ca/P) at the surface of the calcined HAp particles measured by XPS and BET surface area measured by N_2 adsorption. The Ca/P molar ratios of the HAp particles that were synthesized at any pH in the presence of SDP were apparently lower than those of the HAp particles synthesized without SDP. These results indicate that the many phosphate ions that were derived from the SDP molecules exist at the surface of the HAp particles synthesized in the presence of SDP, even after the removal of SDP molecules by calcination.

The adsorption properties of LSZ and BSA on the calcined HAp particles were also determined. All the adsorption isotherms were of Langmuir type. Figures 3a and 3b show the saturated amount of adsorbed LSZ (n_s^{LSZ}) and BSA (n_s^{BSA}) on the HAp particles synthesized at different pH values. In all cases the n_s^{LSZ} values of the HAp particles synthesized in the presence of SDP were significantly larger than that of the HAp particles synthesized without SDP while little difference was found for the n_s^{BSA} values. In addition, the n_s^{LSZ} values of the HAp particles synthesized with SDP at pH 12 were larger than that of the other particles.

A remarkable difference was found for the n_s^{LSZ} values of the HAp particles synthesized in the presence of SDP and those in the absence of SDP. The adsorption of proteins on HAp will be related both to the surface properties of the HAp and to the nature of the proteins. The surface properties of HAp can be changed by the existence of various ions that are included in HAp. According to previous reports,[1–3] since the isoelectric point of the LSZ is 11.1, LSZ was positively charged in the solution that was adjusted to pH 7. Therefore, LSZ molecules adsorb onto the phosphate ions and/or negatively charged sites because of the electrostatic attractive force. As I mentioned above, it is likely that the phosphate groups of the SDP molecules serve as a substrate for the nucleation and growth of HAp crystals, and many phosphate ions exist at the surface of the HAp particles because of the formation of lamellar mesostructure, which can be judged from the results of low Ca/P molar ratios as shown in Table 1. This would be a reason why HAp crystal surfaces become more negative charged and thus show better adsorption for LSZ. It has also been reported that the n_s^{LSZ} on HAp particles increased with a decrease in the Ca/P molar ratio,[1,2] which supports our results. On the contrary, since the isoelectric point of the BSA is 4.7, BSA has been charged

Table 1. Molar ratio of Ca to phosphate (Ca/P) and BET surface area of the calcined HAp samples synthesized under various conditions

Sample	pH	Ca/P	BET surface area/$m^2\,g^{-1}$
HAp[a]	10	1.31	37
	11	1.33	37
	12	1.32	34
HAp-SDP[b]	10	1.02	21
	11	1.05	30
	12	0.99	19

[a]The calcined HAp particles synthesized without SDP. [b]The calcined HAp particles synthesized in the presence of SDP.

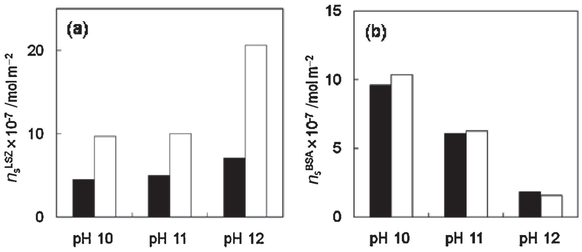

Figure 3. The saturated-adsorbed amounts of each protein on the calcined HAp particles synthesized at various pH values, (a) LSZ, (b) BSA; □: in the presence of SDP, ■: without SDP.

negatively in the solution adjusted to pH 7 and then BSA molecules adsorb onto the calcium ion or OH vacancies and/or positively charged site.[1–3] Thus it is assumed that n_s^{BSA} has no relationship with the effects of SDP molecules. In addition, n_s^{LSZ} and n_s^{BSA} seem to depend on the pH values in the HAp synthesis regardless of the existence of SDP molecules. We consider that this is because OH vacancies would be poor in the HAp particles synthesized at higher pH, which results in the higher adsorption of LSZ and lower adsorption of BSA.

In summary, we developed HAp nanoparticles with improved adsorption for basic proteins using a lamellar template of SDP molecules. These HAp nanoparticles can be used as adsorbents or catalysts because of their unique surface properties.

This work was supported by a Grant-in-Aid for Scientific Research (A) KAKENHI No. 20241020 to Shigeru Sugiyama.

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