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Original Research Paper

Selective adsorption of acidic protein of bovine serum albumin onto sheet-like calcium hydroxyapatite particles produced by microreactor

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ABSTRACT

The protein adsorption behavior onto sheet-like calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (Hap) particles produced by microreactor was examined by using typical acidic bovine serum albumin (BSA) and basic lysozyme (LSZ). Since the sheet-like Hap particles were highly growth through *b*- (or *a*-) and *c*-axes, the particles had larger fraction of *bc* (or *ac*) crystal face. The saturated amounts of adsorbed BSA (n_s^{BSA}) for the sheet-like Hap nanoparticles were increased with increase in the particle size; i.e., n_s^{BSA} showed a good linear relationship with the total surface area of *bc* and *ac* faces of each particle. This result strongly suggested that the sheet-like Hap particles progressed in *b*- and *c*-axes with large fraction of **C** sites on *bc* and *ac* particle faces and exhibit a high selective adsorption of BSA. On the contrary, very few LSZ molecules adsorbed onto sheet-like Hap particles. Hence the present study developed that the amounts of adsorbed protein, especially for BSA, can be controlled optimally by altering the size of sheet-like Hap particles. It was also found that the sheet-like Hap particles can be applied to separate completely BSA molecules from BSA–LSZ mixed solution.

1. Introduction

It is well known that a synthetic calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, designated as Hap, shows high affinity for proteins and much attention has been focused on Hap nanocrystals as sorbents to remove pathogenic proteins from blood in blood purification therapy [1,2] or to acts as a carrier for protein delivery [3,4]. Hap is in the space group $P6_3/m$; its unit cell parameters are $a = b = 0.943$ nm and $c = 0.688$ nm, and it possesses two different binding sites (**C** and **P** sites) on the particle surface as is depicted the crystal structure of Hap in Fig. 1. Thus, it contains a multiple-site binding character for proteins [5–7]. After dispersing Hap particles in aqueous media, calcium atoms (Ca(II) atoms in Fig. 1a are exposed on the Hap surface by dissolution of OH^- ions at the particle surface to produce rich in calcium ions or positively charged sites to bind to acidic groups of proteins, so called as **C** sites. These **C** sites are arranged on *ac* or *bc* crystal face in a rectangular manner with the interdistances of 0.943 nm and 0.344 nm (c/2) for the *a* (or *b*) and *c* directions, respectively (Fig. 1a). The **P** sites, negatively charged adsorbing sites, each formed by six oxygen atoms belonging to three crystal phosphate ions, are arranged hexagonally on the *ab* crystal face with a minimal interdistance in both *a* and *b* directions equal to $|a|(=|b|) = 0.943$ nm (Fig. 1b). In addition, Hap is the most stable calcium phosphate under physiological conditions. Hence, Hap is widely applied for separating various proteins using as a column for a high-performance liquid chromatograph (HPLC) apparatus and many essential studies have been reported [6,8,9]. In the past decades, the authors' group has been conducted fundamental studies on the adsorption of acidic bovine serum albumin (BSA: isoelectric point 4.7, molecular weight 67,200 Da, size 4×14 nm^2), neutral myoglobin (MGB: isoelectric point 7.0, molecular weight 17,800 Da, size 3.5×4.5 nm^2) and basic lysozyme (LSZ: isoelectric point 11.1, molecular weight 14,600 Da, size 3×3.5 nm^2) onto various kinds of synthetic Hap particles [10–19].

Recently, micromachining technologies have been applied to design miniaturized devices for synthetic applications; i.e., microreactors [20,21]. A microreactor is a device in which chemical reactions can be carried out on a microscale [21], and possesses some beneficial effects, e.g., the temperature can be controlled effectively in a microreactor, and mixing progresses rapidly because of the short diffusion length of the materials in a microreactor. Because of these advantages, microreactors have recently been used in various fields. Recently our research group reported on the production of sheet-like Hap particles using a microreactor [22]. The size of the sheet-like Hap particles increased and the length of *b*- and *c*-axes of Hap particles was elongated as the mixing efficiency of the microreactor increased, whereas the length of *a*-axis remained almost unchanged. Since the **C** sites are arranged on *bc* or *ac* crystal face as described before, the sheet-like Hap particles

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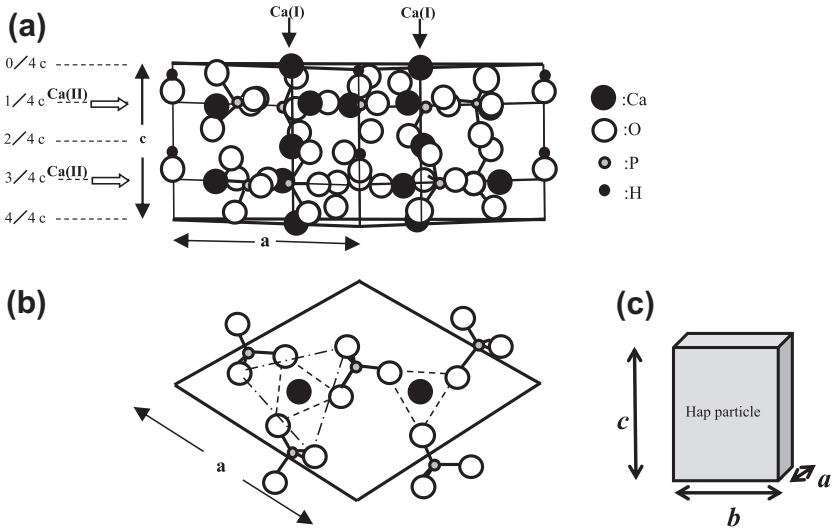

Fig. 1. (a) Projection of unit cell of Hap crystal viewed along [1 1 0] face, (b) surface structure of the *c* crystal surface viewed along [0 0 1] face and (c) diagram of sheet-like Hap particle.

with large fraction of *bc* crystal face are suitable for adsorption of BSA molecules (Fig. 1c). It can be therefore expected that sheet-like Hap particles can adsorb selectively BSA molecules rather than LSZ.

Accordingly, the objectives on the present study are to disclose whether the sheet-like Hap particles possess the selective adsorption behaviors of BSA or not. The results obtained in the present study must serve not only to elucidate the interaction of proteins to sheet-like Hap particles but also the researchers in the fields of biomaterials, biomineralization and biosensor.

2. Experimental section

2.1. Materials and their characterization

Eight kinds of sheet-like Hap-A∼H particles with various sizes as shown in Fig. 2 were produced in our previous paper by the microreactor (Micro Process Server™) [22], a laboratory microreactor system produced by Hitachi Plant Technologies, Ltd. as is illustrated in Fig. 3, under various conditions. For details of the reaction our previous paper should be referred [22]. The shape, specific surface area, crystal phase, and Ca^{2+} and PO_4^{3-} contents of Hap particles were determined by a transmission electron microscope (TEM; JEOL JEM-2100), N_2 and H_2O adsorption measurements, X-ray diffraction (XRD; Rigaku Rad-RC, Ni-filtered Cu Kα radiation, 40 kV, 120 mA), Fourier-transform infrared spectrometer (FTIR; Nicolet Protégé 460) and inductively coupled plasma atomic emission spectroscopy (ICP-AES; SII SPS 3520UV-2). The adsorption isotherm of N_2 was measured at the boiling point of liquid nitrogen with the use of a computerized automatic volumetric apparatus built in-house. Adsorption isotherms of H_2O were also determined at 25 °C by a gravimetric apparatus built in-house. Specific surface areas were obtained by fitting the BET equation to these N_2 and H_2O adsorption isotherms and were abbreviated as S_N and S_W, respectively. Prior to these gas adsorption measurements, the samples were evacuated at 100 °C for 2 h.

2.2. Protein adsorption measurement

The amounts of typical acidic (BSA) and basic (LSZ) proteins adsorbed on the Hap particles were measured by a batch method as following the method employed in our previous papers [16–19]. This measurement was conducted at 15 °C employing a 1×10^{-4}-mol/dm^3 KCl solution of the protein with 100 mg sheet-like Hap particles in 10 cm^3 Nalgen polypropylene centrifugation tubes.

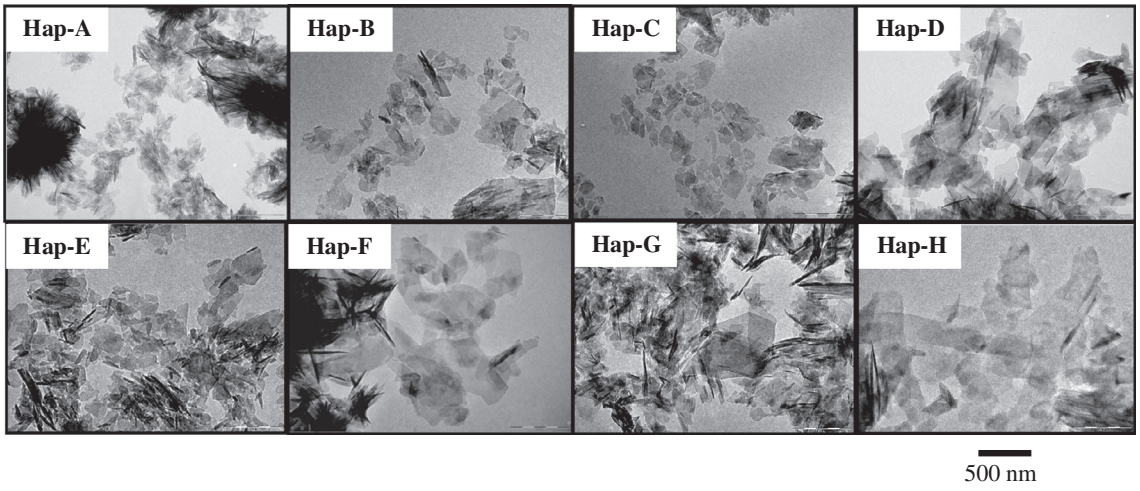

Fig. 2. TEM micrographs of the sheet-like Hap particles produced by microreactor (From Ref. [22]).