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Dynamic adsorption and desorption behavior of protein on carbonate-containing apatite

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A new flow experimental system was employed in order to obtain relevant information about the capability of hydroxyapatite as a drug-delivery carrier in vitro rather than in batch systems. Using our system, we were able to estimate and monitor continuously both the adsorbed and desorbed amounts of protein and the dynamic adsorption and desorption behavior, indicating that this system is a promising technique for evaluating the drug-release properties of ceramic materials.

Key-words : Carbonate ion-containing apatite, Drug-delivery carrier, Adsorption and desorption behavior of protein, Flow system, Bovine serum albumin

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Drug delivery systems (DDSs) have been a key medical technology used for improving the quality of life in patients by prolonging the effective period and decreasing side effects of a drug. There are three main approaches to DDS: (1) target the affected part with a *pro*-drug, which is a chemically-modified drug precursor (2) improve drug absorption with a adsorption promoter, an inhibitor of digestive enzyme and (3) control drug release by chemical modification or by loading onto a carrier.[1] Research into the use of ceramic materials as drug carriers was initiated in the 1980s,[2] and recently, hydroxyapatite (HAp) has been investigated as a controlled-release carrier for various drugs such as proteins,[3),4] hormones,[5),6] bisphosphonates[7] (for use in osteoporosis), antimicrobial metal ions,[8] and antitumor drugs.[9] In most studies, *in vitro* drug-release behavior was examined in batch systems. We followed the adsorption and desorption behavior of proteins on HAp with different carbonate-ion contents (CHAp)[10] in a batch system, and observed that re-adsorption occurred several hours after desorption from CHAp. This static situation, however, differs greatly from the real-life biological environment, for example, in which a drug is exposed to fluid in the vascular system. Therefore, we attempted to apply an *in vitro* flow system, in which protein-loaded CHAp powder was exposed to fluid, to allow us to follow the desorption behavior of proteins in order to investigate the drug-release capability of CHAp. We also compared the results from the flow system with those from the batch system.

The different carbonate-ion content CHAps were prepared by adding 0.06 M $(NH_4)_2HPO_4$ and 0.06 M NH_4HCO_3 aqueous solution to 0.1 M $Ca(NO_3)_2{\cdot}4H_2O$ aqueous solution under controlled pH levels of 6.0, 7.0, 8.5, or 10.5. The detailed procedure has been described in a previous paper.[11] The carbonate-ion content was less than 10 mass % in each case, which is comparable to the content in biologically occurring HAp.[12] The CHAps containing the lowest and highest carbonate-ion content were chosen in order to compare the effect of modifying the carbonate ion on the adsorption and desorption properties of the protein. Two physical and chemical differences were observed between the two CHAp types: the CHAp with the higher carbonate-ion content showed a 40% smaller crystallite along the c–axis and contained more OH-substituted carbonate than the CHAp with smaller carbonate-ion content.[11] The carbonate-ion contents were 1.1 and 8.6 mass % for the flow system and 0.9 and 6.3 mass % for the batch system.

Bovine serum albumin (BSA; Sigma-Aldrich Co. A4503) with an isoelectric point of 4.7–4.9 was used as a model protein drug. Its adsorption and desorption behavior was followed at pH 7.0 ± 0.1 using an AC-5300 Bio-Chromatograph II (ATTO Co.). The apparatus shown in **Fig. 1** was composed of a control unit attached to six electric valves for automatic switching of solution composition, a monitor, a CHAp-packed column, a peristaltic pump to introduce the solution into the CHAp-containing column, a UV detector, and a recorder. CHAp powder (0.5 g) was packed into a layer 1.25 cm thick.

The adsorption and desorption behavior of BSA on CHAp was followed as a function of time using 1.0 g BSA/dm^3 and successively switching to 5 mM phosphate-buffered solution (PBS) and 20 mM PBS as the eluent into the column. In the human vascular system, the blood velocity varies from 0.1 to 30 cm/s depending on section of the body.[13] However, as our apparatus could operate only under ambient pressure, the volumetric flow rate was limited to several cm^3/min because of a large pressure loss. Therefore, a volumetric flow rate of 1.0 ± 0.05 cm^3/min, corresponding to about the velocity of 0.5 cm/s, was employed in this study.

Curve 1 in **Fig. 2** shows the behavior of BSA on CHAp of 1.1 mass % carbonate-ion content, in which the response curve was redrawn in order to eliminate the time lag caused by switching the solutions of different chemical composition (the time lag to be subtracted from the original response time was 2 min 20 s). Furthermore, the effect on the response curve of the time to replace one solution in the column by another was

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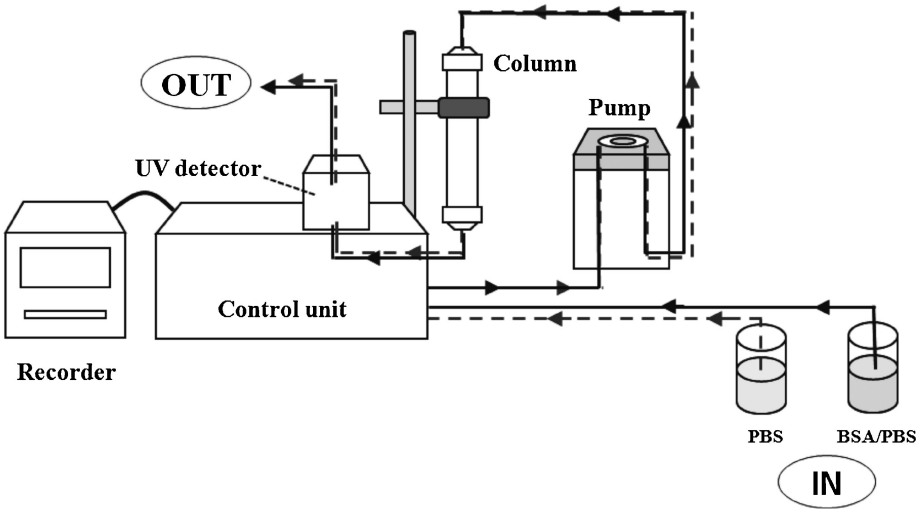

Fig. 1. Schematic diagram of experimental apparatus in this study.

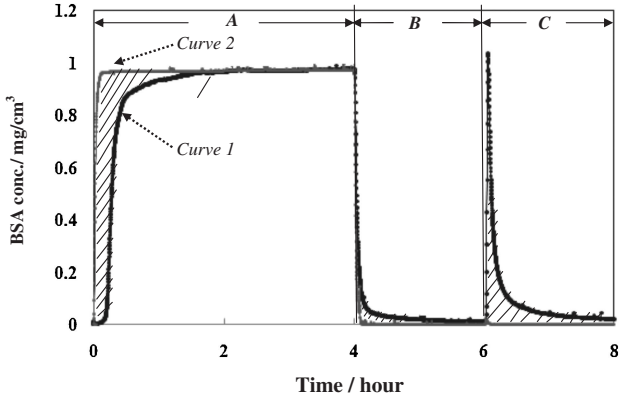

Fig. 2. Adsorption and desorption behavior of BSA on CHAP of 1.1 mass %-content of the carbonate ion with a flow system. *A* 1.0 g/dm^3-BSA/5 mM-PBS; *B* 5 mM-PBS; *C* 20 mM-PBS; *Curve 1* CHAP; *Curve 2* polystyrene beads as a blank; loaded amount 0.5 g; flow rate 1 cm^3/min.

Table 1. Comparison of Q and r_{de} of BSA on the CHAP of different contents under two exprimental systems

	Flow system*1)		Batch system*2)	
Carbonate content (mass %)	1.1	8.6	0.9	6.3
Adsorbed amount (mg-BSA/m2-HAp): Q	1.1	0.072	1.68	0.44
r_{de}*3) in 5 mM-PBS (%)	20.3	21.2	14.9*4)	9.6*4)
r_{de} in 20 mM-PBS (%)	40.1	76.7	—	—
Total r_{de} (%)	60.4	97.9	—	—

*1)1 g-BSA/dm^3-5 mM PBS; *2)2.5 g-BSA/dm^3-5 mM PBS for 72 hour-adsorption; *3)r_{de} is defined as the ratio of the desorbed amount to the initially adsorbed one. *4)The maximal desorbed amount before readorption occurrence was employed for estimating r_{de}.

correlated by the graphical difference between the response for the CHAp-loaded column and that in which styrene-divinylbenzene copolymer beads (20% linked) (Strem Chemicals Co.) was loaded as a blank (shown as Curve 2 in Fig. 2). Therefore, the adsorbed and desorbed amounts of BSA were estimated by graphical integration of the shaded areas in Fig. 2, the former being the lag area up to the plateau of the response in BSA–PBS flow (A), and the latter being the lag area down to zero in the PBS flow (B and C).

In the flow of BSA/PBS (A in Fig. 2), BSA increased steeply for about 30 min and then did slowly. It took about 3 h to reach saturation, that is, completion of BSA adsorption. In 5 mM-PBS (B in Fig. 2), BSA decreased steeply for about 10 min and then declined steadily over about 1 h to zero. In 20 mM-PBS following 5 mM-PBS (C in Fig. 2), desorption of BSA was again observed with an initial maximum. Proteins adsorb on apatite with an electrostatic attraction:[14] the negatively-charged C terminal, the positively-charged N terminal, or negatively- or positively-charged side groups of proteins are attracted by calcium or phosphate ions of the apatite surface, being done competitively with ions of PBS. Therefore, the initial desorption in 20 mM PBS suggests that the phosphate ion of higher concentration in PBS was substituted mainly for the negatively-charged C terminal or carboxyl anion of BSA, because its isoelectric point is 4.7–4.9 and so the C terminal would have been negatively charged in our neutral experimental conditions. The CHAp of 8.6 mass %-content also showed similar adsorption and desorption behavior.

The adsorbed amount of BSA per surface area of CHAp, Q, was estimated and compared with those under a batch system (**Table 1**). Q showed a similar dependency on the carbonate-ion content in both systems; greater content led to a smaller Q. The decrease in the adsorbed amount was ascribed to two possible causes, as discussed in a previous paper.[11] One possible cause is that the carbonate ion might be located on the OH-vacancy of the apatite structure, which is considered to be a location of BSA. The other possible cause is that incorporation of the carbonate ion retards crystal growth along the c-axis. This causes a decrease in the exposed area of the a (b) face (the primary adsorption face of BSA[15),16]), in which the calcium ion is exposed. Although the dependencies on carbonate-ion content were similar, the amounts in the flow system were much smaller than those in the batch system, possibly due to a shorter surface contact time of BSA in the flow system.

The desorption rate, r_{de}, defined as the ratio of the totally desorbed amount to the initially adsorbed amount, was considered an index of binding strength of BSA to CHAp surface, and is shown in Table 1. In 5 mM PBS, r_{de} for the flow system

was larger than that for the batch system. In the batch system, conformational change of BSA on the surface may have occurred during the 72-h adsorption time and greater numbers of charged side groups of BSA may have been involved in adsorption, leading to its stronger binding to the surface and decreased r_{de}.

Under the flow system, the r_{de} values for CHAp of the two different carbonate-ion contents were almost equal in 5 mM PBS. In 20 mM PBS, both r_{de} values became much larger than in 5 mM PBS, which is ascribed to the substitution of greater numbers of phosphate ions for BSA, as mentioned before. r_{de} for the greater content was clearly larger than that for the lower content. This can be explained by the decrease in the exposed area of the a (b) face, i.e., the decrease in the primary adsorption site of BSA, by incorporation of the carbonate ion into the HAp structure, weakening the binding of BSA to the surface.

The r_{de} in 5 mM PBS under the batch system was larger for CHAp with smaller carbonate-ion content, which is in contrast to the tendency in 20 mM PBS under the flow system. This can be explained in the following manner: 1.68 mg BSA/m^2-CHAp for 0.9 mass % corresponded to a surface coverage of 0.86, assuming side-on (multiple site) adsorption, meaning that almost the entire surface was covered with BSA. This may lead to greater surface density of BSA and to more electrostatic repulsion among the molecules than for the lower surface coverage (less than one-third) for 6.3 mass %. Therefore, binding of BSA to the surface for 0.9 mass % can be expected to become weaker.

In conclusion, we were able to follow the dynamic adsorption and desorption behavior of BSA on apatite reproducibly with our flow system. As a next step, more quantitative parameters for estimating drug-release properties will be investigated.

References

1) M. Hashida and Y. Takakura, "Seitainai-Yakubutsu-Sotatsu-Gaku", Sangyo-Tosho, Tokyo, (1994) pp. 10–14 [in Japanese].
2) P. K. Bajpai and H. A. Benghuzzi, *J. Biomed. Mater. Res.*, **61**, 1245–1266 (1988).
3) J. E. Barralet, S. Aldred, A. J. Wright and A. G. A. Coombers, *J. Biomed. Mater. Res., Part A*, **60**, 360–367 (2002).
4) Y. Mizushima, T. Ikoma, J. Tanaka, K. Hoshi, T. Ishihara, Y. Ogawa and A. Ueno, *J. Controlled Release*, **110**, 260–265 (2006).
5) W. Paul, J. Nesamony and C. P. Sharma, *J. Biomed. Mater. Res.*, **61**, 660–662 (2002).
6) H.-T. Shi, T. Gong, S.-H. Liu, S. Chen and Z.-R. Zhang, *J. Ceram. Soc. Japan*, **113**, 579–583 (2005).
7) H. Seshima, M. Yoshinari, S. Takemoto, M. Hattori, E. Kawada, T. Inoue and Y. Oda, *J. Biomed. Mater. Res., Part B*, **78B**, 215–221 (2006).
8) T. Yabutsuka, S. Tsuboi, M. Hibino and T. Yao, *Key Eng. Mater.*, **361–363**, 1199–1202 (2008).
9) M. Itokazu, T. Sugiyama, T. Ohno, E. Wada and Y. Katagiri, *J. Biomed. Mater. Res.*, **39**, 536–538 (1998).
10) T. Kanno, T. Sendai, K. Tada, J. Horiuchi, K. Itabashi and T. Akazawa, *J. Ceram. Soc. Japan*, **117**, 1101–1104 (2009).
11) T. Kanno, T. Sendai, K. Tada, J. Horiuchi and T. Akazawa, *Phosphorus Res. Bull.*, **21**, 25–30 (2007).
12) H. Aoki, "Apatite," Ishiyaku Publishers, Inc., Tokyo (1999) p. 19 [in Japanese].
13) J. R. Cameron, J. G. Skofronick and R. M. Grant, "Physics of the Body," 2nd ed., Medical Physics Pub Corp., Madison, WI (1999).
14) Q. Luo and J. D. Andrade, *J. Colloid Interface Sci.*, **200**, 104–113 (1998).
15) K. Kandori, M. Saito, T. Takebe, A. Yasukawa and T. Ishikawa, *J. Colloid Interface Sci.*, **174**, 124–129 (1995).
16) K. Kandori, M. Mukai, A. Yasukawa and T. Ishikawa, *Langmuir*, **16**, 2301–2305 (2000).