EFFECT OF ADSORPTION OF DODECYL PHOSPHATE ON THE CRYSTAL GROWTH AND SURFACE MODIFICATION OF HYDROXYAPATITE IN AN AQUEOUS PHASE

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ABSTRACT: Fresh hydroxyapatite(HAP) was prepared in an aqueous phase by mixing CaCl_2 with K_2HPO_4 in the presence of dodecyl phosphate(DP) and arginine(Arg), where $\text{DP/Arg} = 1/3$ in molar ratio. Arg was added in order to enhance the solubility of DP. The degree of crystallinity of the HAP decreased with a concentration of DP, because DP was adsorbed to the growth sites on the nuclei through its terminal phosphate group as a crystal poison. The effect of adsorption of DP on a ripened HAP was also studied. Though polyethylene glycol *p*-isooctylphenyl ether (TX-100) was scarcely adsorbed on the raw surface of HAP, it was adsorbed after pretreatment of the surface with DP. This fact was explained in terms of surface modification by the adsorption of DP. Alkyl chains of DP protruding from the surface captured those of TX-100 through hydrophobic interaction on the surface. It was concluded that the phosphate group of DP plays an important role in regulation of the crystal growth and surface modification by virtue of isomorphous substitution of phosphate group of DP for phosphate ion on the surface of HAP.

INTRODUCTION

Effects of dodecyl phosphate(DP, FIG. 1) on the formation of hydroxyapatite(HAP) in an aqueous phase were already discussed by means of various techniques in the presence and absence of arginine(Arg) in the previous paper.¹⁾ Arg was added in order to increase the solubility of dodecyl phosphoric acid. Mean diameter of the particles of HAP prepared in an aqueous phase decreased with a concentration of DP added together with Arg. It was concluded that this dispersing effect is owing mainly to the adsorption of DP on the HAP particles freshly formed. The adsorption occurred through an isomorphous substitution of a phosphate group of DP for a phosphate ion on the surface of HAP and through electrostatic attractive force between a phosphate group of DP and a surface calcium ion, as schematically shown in FIG. 2. Similar substitution was observed²⁾ in the adsorption of sodium dodecyl sulfate (SDS), because the size of sulfate group of SDS (atomic distance of $\text{S-O} = 1.49 \text{ \AA}$) is quite similar to that of phosphate ion($\text{P-O} = 1.55 \text{ \AA}$). The HAP surface was modified to a rather hydrophobic one and easily adsorbed hydrophobic polymers such as hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP) after the adsorption of and pretreatment with SDS,³⁻⁵⁾ forming the surface complex.

In the present paper, the effects of addition of DP (1) on the crystallinity of HAP

freshly prepared and (2) on the surface modification of a commercial ripened HAP in an aqueous phase will be discussed, taking the results mentioned above into consideration. We are expecting that DP might be a crystal poison against the formation of a fresh HAP. An amphiphilic compound such as polyethylene glycol *p*-isooctylphenyl ether(TX-100, nonionic surfactant) might be easily adsorbed by HAP after the pretreatment of a ripened HAP with DP in an aqueous phase, though it is known that a nonionic surfactant is scarcely adsorbed directly to the bare surface of HAP.

FIGURE 1. Chemical Structure of Dodecyl Phosphate(Acid Form and ArginineSalt) and pK Values for Arginine

FIGURE 2. Schematic Illustration for a Mechanism of the Adsorption of DP via Its Terminal Phosphate Group: Isomorphous Substitution of a Phosphate Group of the Long Chain DP for a Surface Inorganic Phosphate Ion, and Electrostatic Attractive Force toward a Calcium Ion on the Surface (left-hand side) Size of Phosphate Ion and the Surface before the Adsorption (right-hand side) Size of Phosphate Group of DP and the Surface after the Adsorption

EXPERIMENTAL

A matured HAP and TX-100 were purchased from Nacalai Tesque (Kyoto). Dodecyl phosphoric acid and Arg were supplied from Kao Inc(Tokyo). The other chemicals used were reagent grade. These were used as received. Dodecyl phosphoric acid was dissolved in water in the presence of Arg to form an Arg salt(FIG.1), where dodecyl phosphoric acid/Arg= 1/3 in molar ratio. The HAP samples for an X-ray diffraction test were prepared by mixing 1.25 mmol/dm³ CaCl₂ with 2.5 mmol/dm³ K₂HPO₄ in an aqueous solution of 0.9 % NaCl containing known concentrations of DP and Arg at 35 °C. The precipitate was filtered off at 120 minutes after the mixing, washed with water and acetone, and dried in vacuo. It was stored in acetone until the measurement in order to prohibit from its further crystal growth. The specific diffraction angles were observed at $2\theta = 25.8$ and 31.8 deg by means of a CuK α radiation. The diffraction angles were almost constant over the concentration range of DP in this study while the intensity changed with a DP concentration.

The adsorption amount of TX-100 on a ripened HAP(Nacalai) was obtained as a function of a concentration of the added DP, where an added concentration of TX-100 was kept constant. A concentration of TX-100 at the adsorption equilibrium was determined by an UV absorptiometry at $\lambda = 276$ nm after obtaining a supernatant by centrifugation. It was confirmed that Arg does not interfere the determination. The adsorption amount was calculated from the difference in concentrations before and after the adsorption.

FIGURE 3. Relative Strength(I/I_0) of Powder X-Ray Diffraction as a Function of a Concentration of an Additive

I and I_0 are the intensities in the presence and absence of an additive. Additives are Dodecyl Phosphoric Acid(open circle at $2\theta = 25.8$ deg and closed circle at 31.8 deg), DP in the presence of Arg(open triangle at 25.8 deg and closed triangle at 31.8 deg), Arginine(shown as $[Arg]/3$, x at 25.8 deg and + at 31.8 deg).

RESULTS AND DISCUSSION

Inhibition of Crystal Growth of HAP: FIGURE 3 shows an X-ray diffraction intensity of the HAP prepared by mixing calcium and phosphate ions in an aqueous phase containing dodecyl phosphoric acid, DP, and/or Arg. The relative intensity(I/I_0) decreased with a concentration of an additive irrespective of its chemical species used here, although the dominant species in a mother solution is 154 mmol/dm^3 NaCl. This fact is suggesting that these additives are easily adsorbed on the surface of the nuclei of HAP and, therefore, these are showing a high affinity to HAP and amorphous calcium phosphate(a precursor of HAP) even at a high ionic strength prepared by NaCl. The adsorption mechanism is so-called isomorphous substitution(see FIG. 2). Phosphate group of DP, instead of inorganic phosphate ion, occupies the growth site during the crystal growth, resulting in inhibition from the crystal growth (that is, crystal poisoning). It is reported elsewhere^{6,7} that phosphate group of phosphorylated polyvinylalcohol(Phos PVA) also inhibited the crystal growth in a similar manner. The inhibiting effect of Phos PVA seems stronger than that of DP because the polymeric adsorption layer is formed on the surface of a crystal seed.

Chemical structure of Arg is quite different from that of each of the lattice ions of HAP. However, Arg inhibited from the crystal growth by a low concentration, as shown in FIG. 3. It might be strongly adsorbed to the growth sites on the nuclei probably through its amino and/or guanidinium groups, in other words, by electrostatic attractive force to interfere in the crystal growth. The reason why Arg shows a high affinity for the nuclei even at a high ionic strength was not clearly explained in this study.

FIGURE 4. Adsorption Amount of TX-100 on the Ripened HAP(Nacalai Tesque) as a Function of a Given Concentration of DP. [TX-100]/(mg/dm³) = 20.1(diamond), 30.1(square), 40.1(triangle), [HAP] = 25 g/dm^3 , Temperature = 30°C

FIGURE 5. Schematic Illustration for Cooperative Adsorption of TX-100 and DP. Monodispersed TX-100 and DP and their mixed micelles are depicted in the aqueous phase. The micelle might be formed if their concentrations are appropriate for the formation.

Cooperative Adsorption of TX-100 and DP: Ripened HAP was pretreated with an aqueous solution of DP(+Arg) of a given concentration immediately before the addition of a known concentration of TX-100. An equilibrium concentration of TX-100 was determined at 24 hours after the mixing and the amount of adsorption of TX-100 was calculated from a difference in concentrations of the initial and equilibrium ones, assuming that its molar mass is 646.8 g/mole and the degree of polymerization of ethylene oxide(EO) is 10.

TX-100 was not adsorbed in the absence of DP, as shown on the ordinate of FIG.4. It was rather negative value (i.e., negative adsorption) beyond the experimental error. This fact was explained as follows. The EO chain does not show any affinity for the HAP surface due to the fact that both of these are hydrated by virtue of hydrophilic hydration. On the other hand, the hydrocarbon chain of TX-100 is hydrophobic and incompatible with the hydrophilic surface of HAP. Therefore, TX-100 is separated off from the surface of HAP.

However, when HAP was pretreated with DP, the adsorption amount levelled off after increasing with a concentration of the added DP. The reason for the levelling off is consumption of the TX-100 from a mother solution. That is, more than 80 % of the added TX-100 was adsorbed in the presence of 5 mmol/dm³ DP irrespective of the initial concentration of TX-100, as shown in FIG.4. This accelerating effect of DP was explained in terms of its adsorption on the surface after an isomorphous substitution of the phosphate group of DP for the surface orthophosphate ion of HAP, as expected from FIG.2. The hydrocarbon chain of DP(C 12) was implanted on the surface and the surface becomes hydrophobic, where the hydrocarbon chain is protruding from the surface. Therefore, that of

TX-100 (*p*-isooctylphenyl group) are easily adsorbed on the surface after a hydrophobic interaction, forming a mixed hemimicelle or admicelle between them. To our regret, it was too difficult to determine the adsorption amount of DP on HAP in this study.

FIGURE 5 illustrates the schematic relationship between HAP, TX-100, and DP at the adsorption equilibrium. TX-100 is captured to the surface by virtue of hydrophobic interaction, forming a mixed hemimicelle (and/or admicelle). Ordinary spherical mixed micelles might be concomitantly formed in the aqueous phase, even though their respective concentrations are less than their critical micellization concentrations(CMCs) in the absence of the other. According to our determination by means of a Du Nouy ring method (surface tensiometry), the CMCs were 0.25 and 10.5 mmol/dm³ for TX-100 and DP,¹⁾ respectively.

On the other hand, according to a measurement by a Coulter counter (type TAIL), the mean diameter of the particles before the pretreatment was the same as that after the pretreatment. Therefore, we can assume that the surface area of the HAP is almost constant before and after the pretreatment with DP and/or the adsorption of TX-100, where the specific surface area of the original HAP was 51.0 m²/g.

Thus, we found that the non-adsorbable molecule, TX-100, becomes an adsorbable one, where the phosphorylated organic compound DP plays an important role as an intermediary for TX-100 to bind to HAP through the hydrophobic interaction. This idea is significant for understanding the mechanism of formation of mammalian hard tissues. These are a composite of organic and inorganic compounds, where both components are tightly bound with each other. It has been reported elsewhere⁸⁾ that dodecyl sulfate also behaves as an anchor for TX-100 to be bound to the HAP surface.

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