EFFECT OF SULFATE GROUP ON THE FORMATION OF HYDROXYAPATITE
IN AN AQUEOUS PHASE

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Abstract Formation of hydroxyapatite(HAP) via amorphous calcium phosphate(ACP) was studied by mixing CaCl_2 with K_2HPO_4 in an aqueous phase in the presence of various sulfate compounds. Sodium sulfate and sodium chondroitin-6-sulfate did not show any effect on the formation of HAP up to ca. 1 mM. Transformation of ACP to HAP was retarded in the presence of potassium polyvinylsulfate(PVSK), while its mother compound, polyvinylalcohol, showed no specific effect over the same concentration range investigated. This result means that sulfate group on PVSK competes significantly with phosphate ion for the active growth sites on the HAP nuclei. Polyvinylpyrrolidone(PVP) accelerated the formation of HAP by virtue of its depletion effect. The induction time for the transformation was shortened in the presence of micelle of sodium dodecylsulfate(SDS) owing to its Ca^{2+} condensation effect. This micelle effect gradually diminished with the addition of PVP due to the formation of PVP-SDS complex, which behaved like a polyelectrolyte such as PVSK.

INTRODUCTION

The interatomic distance of S-O(= 0.149 nm) of sulfate group is

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quite similar to that of P-O(= 0.154 nm) of phosphate group, where both sulfate and phosphate ions are oxoacid groups(XO_4 , $\text{X} = \text{S}, \text{P}$). Therefore, phosphate ion on the surface of HAP is easily exchanged with inorganic sulfate ion added in a mother solution although their valencies are different each other.¹ On the other hand, phosphorylated organic compounds such as partially phosphorylated polyvinylalcohol(Phos.PVA) and phosphoserine(PSer) retard the transformation of amorphous calcium phosphate (ACP) to hydroxyapatite(HAP) through the competitive adsorption between their phosphate group and inorganic phosphate ion(Pi) for the growth sites on the surface of HAP nucleus.² In the present paper, the effect of sulfate group on the transformation of ACP to HAP was discussed, tak-

FIGURE 1 Relationship between relative induction time and concentration of sulfate ion/group of the additives. Added compounds were Na_2SO_4 (●), SDS(□), Na_2Chs (△), and PVSX(○). Dotted line is for Phos. PVA, quoted from a literature.² Abscissa for Phos. PVA should be read as a concentration of phosphate group of Phos. PVA.

ing into consideration its isomorphous substitution and concurrent inhibition against the Pi binding to the HAP nuclei.

EXPERIMENTAL

Time courses of calcium ion activity, $[Ca^{2+}]$, and pH of the solution containing 1.25 mM $CaCl_2$, 2.50 mM K_2HPO_4 , and an additive in 0.9 % NaCl were obtained. The induction time, T , was determined from the intersection of the tangents drawn to the curves of $[Ca^{2+}]$ and pH just before and after the second steep decrease.^{3,4} The induction time obtained from these two curves agreed reasonably well. Data in the figures are those relative to the induction time without an additive ($T_0=20$ min).

FIGURE 2 Schematic illustration for the competition of sulfate group with inorganic phosphate ion for the active growth sites on the surface of HAP nucleus. (A) Na_2SO_4 , (B) SDS, (C) Na_2Chs , (D) PVSK

RESULTS

Na_2SO_4 and sodium dodecylsulfate(SDS) of low concentration range did not show any specific effect on T/T_0 (Fig. 1). This is probably because the size of sulfate group is smaller than that of Pi and, therefore, desorption of sulfate group and selective binding of Pi to the nuclei easily occur after the competition between them(Fig.2(A) and (B)).

However, SDS of higher concentration than its critical micellization concentration(c.m.c. = 8.2 mM) shortened the induction time(Fig.3). Calcium ion in the solution was condensed on the negatively charged micelle as a counter ion. Therefore, the Ca^{2+} , Na^+ -micelle behaves as a Ca^{2+} -reservoir and plays an important role in the nucleation of HAP.⁵

Sodium chondroitin-6-sulfate(Na_2Chs) did not affect the T/T_0 also, as shown in Fig. 1. This fact is explained as follows: Na_2Chs is an acidic polysaccharide having hydrophilic sulfate, carboxylate, and hydroxyl groups. The size of carboxylate group is far smaller than that of sulfate and phosphate groups, which means the low affinity of car-

FIGURE 3 Effect of SDS on the relative induction time in the presence or absence of PVP K-90. [PVP K-90]/ % = 0($\circ$), 0.1($\bullet$), and 0.2($\square$)

boxylate group to HAP.¹ In addition to these factors, charge density of $\text{Na}_2\text{C}_2\text{O}_4$ is low; one negative charge per 0.49 nm along a polymer chain. Therefore, Pi , one of the lattice ion, easily penetrates into the adsorbed layer of $\text{Na}_2\text{C}_2\text{O}_4$ on the HAP nuclei and electrostatically peels it off from the surface(see Fig.2(C)).⁶

Potassium polyvinylsulfate(PVSK), on the other hand, showed a remarkable retardation effect although it was weaker than that of Phos.PVA (Fig. 1). Mechanism of the retardation seems similar to that of Phos.-PVA.² That is, some of sulfate groups of PVSK occupy the Pi sites for the crystal growth of HAP through isomorphous substitution, and the others of the same polymer chain are unattached but close to the surface, forming an adsorption layer(see Fig.2(D)). Negative charges of the adsorbed layer of PVSK(degree of esterification of PVSK = ca. 92 %, i.e., in average, one negative charge per 0.27 nm along polymer chain) repel the Pi approaching to its site on the surface, resulting in the inhibition of the crystal growth and the retardation of the transformation.

The induction time decreased with an increase in the concentration of polyvinylpyrrolidone(PVP) K-90, as shown in Fig. 3, although this

FIGURE 4 Schematic illustration of the depletion effect of PVP K-90 on HAP particles.

polymer is little adsorbed by HAP/ACP.⁷ This phenomenon is explained in terms of the depletion effect: PVP polymer coils occupy considerable volume in the solution and exclude HAP/ACP fine particles/nuclei. Subsequently, the nuclei are concentrated in a segregated area(Fig.4). This effect accelerates the transformation to HAP due to the high concentration of the nucleus.

It has been known that PVP and SDS, the latter of which is adsorbed by HAP, form a surface complex through hydrophobic interaction on HAP.⁷ Some of free SDS are concurrently bound to the PVP segments protruding into the solution. Property of this surface complex is quite like that of a polyelectrolyte adsorbed on HAP. In fact, it suppressed the transformation in a similar manner to that of PVSK. The acceleration effect of SDS micelle was weakened and, instead, the induction time increased with an increase in a concentration of PVP at a give concentration of SDS when $[SDS] \geq c.m.c.$ (Fig. 3). The effects of monodispersed SDS, SDS micelle, PVP alone, and PVP-SDS complex are, thus, different each other.

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