Adsorption of Cetylpyridinium and Cetyltrimethylammonium Ions on Hydroxyapatite and Concurrent Release of Phosphate and Calcium Ions from the Surface of Hydroxyapatite

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Concentrations of phosphate ion ([Pi]f) and calcium ion ([Ca2+]f), liberated from the surface of synthetic hydroxyapatite (Ca10(PO4)6(OH)2; HAP) during the adsorption of cetylpyridinium ion (CP+) or cetyltrimethylammonium ion (CTA+), were determined as a function of the equilibrium concentrations of these surface active ions ([CP+]f or [CTA+]f). A part of CP+ or CTA+ was adsorbed by ion-exchange with calcium ion (Ca2+) on the surface of HAP, and the rest was adsorbed together with its counterion (Cl− or Br−). In the region below the critical micelle concentration (cmc), [Ca2+]f increased with an increase in the adsorbed amount of the surface active ion. On the other hand, [Pi]f decreased with an increase in [Ca2+]f to keep the solubility product of HAP (Ksp) constant. However, in the region above the cmc, [Pi]f increased with [CP+]f or [CTA+]f through the binding of phosphate ion (Pi) to the cationic micelles. The electroneutrality on the surface phase of HAP, the solubility product of HAP in the solution phase, and the counterion binding by micelles determined the relationship between [Pi]f and [Ca2+]f. The species of counterion of the surfactant (Cl− or Br−) affected the adsorbing and releasing behaviors: the release of Ca2+ due to the adsorption of CP+ and the release of Pi through its binding to CP+—micelles are more pronounced with cetylpyridinium chloride than with cetylpyridinium bromide. This result can be explained in terms of the affinity of the halide ion for CP+ in the micellar and adsorbed phases (Br− > Cl−).

Keywords—hydroxyapatite; cetylpyridinium ion; cetyltrimethylammonium ion; adsorption; phosphate ion release; calcium ion release; ion-exchange; micelle; solubility product; counterion

Introduction

Hydroxyapatite (Ca10(PO4)6(OH)2; HAP) is a major inorganic constituent of mammalian hard tissues (teeth and bones) and a major or minor component of renal calculi. In the previous investigations, the influence of surface active ions (dodecyl sulfate and dodecylammonium ions) on the surface properties of HAP was studied. It was found that calcium ion (Ca2+) and/or phosphate ion (Pi) were released from the surface of HAP during the adsorption of the surface active ions. The ratio of these constituent ions released was nonstoichiometric (i.e., Ca2+/Pi ≠ 10/6), and depended on various factors, such as the adsorbed amount and the concentration of the surface active ion, and the solubility product.
The releasing tendencies of Ca$^{2+}$ and Pi were found to be mutually interchanged when the sign of the electric charge of the surface active ion was reversed. However, as regards the competitive adsorption between Ca$^{2+}$ and dodecylammonium ion (DA$^+$), the addition of the former caused a slight decrease of the adsorbed amount of the latter. This fact is in contrast to the case of cetylpyridinium ion (CP$^+$). That is, the adsorbed amount of CP$^+$ was greatly decreased by the added Ca$^{2+}$ and the preferential adsorption of Ca$^{2+}$ occurred. Roseman et al. found, in their kinetic study on the dissolution of HAP in the presence of cationic surfactant, that DA$^+$ was adsorbed more strongly than CP$^+$ on HAP. The hemimicelle formation of DA$^+$ at the surface of HAP may be responsible for the high affinity of DA$^+$ to HAP.

In the present work, the influence of CP$^+$ and cetyltrimethylammonium ion (CTA$^+$) on the surface properties of HAP was studied in order to obtain a clearer view of the influence of surface active cations. The amounts of Ca$^{2+}$ and Pi liberated from the surface of HAP at a constant mixing ratio of HAP to the solution were determined as a function of the concentrations of these surface active ions in order to examine the relationships among them. Furthermore, the effect of the counterion (Cl$^-$ and Br$^-$) on them was studied by comparing the results for cetylpyridinium chloride (CPC) with those for cetylpyridinium bromide (CPB).

**Experimental**

Materials —— HAP was the same sample as that used in the previous studies. CPC, CPB, and cetyltrimethylammonium chloride (CTAC), purchased from Tokyo Chemical Industry Co., Ltd., were of extra pure reagent grade. They were recrystallized twice from acetone for CPC and CPB, or from ethanol-acetone mixture (1 : 14) for CTAC.

Methods —— HAP was suspended in a solution of a surfactant (CPC, CPB, or CTAC) of known concentration at 30 °C, and the suspension was shaken vigorously at frequent intervals. No buffer solutions were used in order to avoid the effects of buffering agents on the properties of the HAP surface and cationic micelles. After at least 14 d, which was sufficient to attain adsorption and dissolution equilibria, the suspension was filtered through a Millipore filter with a pore size of 0.1 μm, and the filtrate was used for chemical analysis. Prior to the filtration, the optical density of the suspension at 850 nm (OD$_{850}$) in the upper part of the test tube was measured on a Shimadzu model UV-180 spectrophotometer at 24 h after the last shaking. This is a convenient index of the stability of HAP suspension, as a portion of the added HAP had already sedimented to the bottom and the rest remained in the aqueous phase. The higher the OD$_{850}$, the more stable the suspension.

The concentrations of CP$^+$ and CTA$^+$ were determined by the methylene blue diphasic titration method (Epton method) using a standard solution of sodium dodecyl sulfate (SDS). The concentrations of Cl$^-$ and Br$^-$ were determined by the mercuric thiocyanate method. Halide ion (Cl$^-$ or Br$^-$) was allowed to react with mercuric thiocyanate in the presence of ferric nitrate to form mercuric chloride and thiocyanate ion. The latter product immediately combined with ferric ion to form the red ferric thiocyanate complex. Its absorbance was measured at 458 nm. It was confirmed that CP$^+$ and CTA$^+$ do not interfere with the determination. Calibration curves were obtained using aqueous solutions of NaCl and KBr. The adsorbed amounts of CP$^+$, CTA$^+$, Cl$^-$ and Br$^-$ were calculated from the differences of the concentrations before and after addition of HAP.

The concentration of Pi released from the surface of HAP was determined by the molybdenum blue method of Gee et al. The phosphate ammonium molybdate complex formed was reduced with stannous chloride. The absorbance of the resulting color was measured at 720 nm after 15 min. Prior to the determination, a slight excess of SDS was added to the sample solutions in order to precipitate CP$^+$ or CTA$^+$. The precipitate was then removed with a Millipore filter (0.1 μm pore size), and the filtrate was used for the determination. A calibration curve was obtained by subjecting aqueous solutions of K$_2$HPO$_4$ to the same treatment as that for the sample solutions. The determination of Ca$^{2+}$ released was determined by ethylenediaminetetraacetic acid (EDTA) chelatometry with 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid (BT indicator) at pH 10. Since CP$^+$ and CTA$^+$ interfere with the color change of the indicator, a slight excess of SDS was added to the sample solutions prior to the determination (it was not necessary to remove the precipitate). The pH of the filtrate was measured on a Toa HM-5ES pH meter.

It was confirmed that the adsorbed amounts of the surface active ion and its counterions, and the equilibrium concentrations of Pi and Ca$^{2+}$, were independent of the mixing order of water, adsorbate solution, and the HAP powder. A dilution experiment was carried out by adding water to the suspension after equilibria had been attained. The adsorbed amounts and the concentrations of the ions after re-equilibration were determined. These experiments showed that the adsorptions and liberations of the ions are reversible.
Henceforth, the symbol $X_i$ represents the increment of species $i$ at the surface of HAP, and was calculated by means of the following equation:

$$X_i = \frac{\text{(initial concentration of species } i \text{) - equilibrium concentration of species } i)}{\text{(weight of HAP added)}}$$

Therefore, $X_{\text{CP}^+}$, $X_{\text{CTA}^+}$, $X_{\text{Cl}^-}$, and $X_{\text{Br}^-}$ take zero or positive values (= the adsorbed amounts). On the other hand, the numerical values of $X_{\text{Pi}}$ and $X_{\text{Ca}^{2+}}$ are negative, because the concentrations of Pi and Ca$^{2+}$ were initially zero.

## Results

### Adsorption Isotherms of CP$^+$ and CTA$^+$ on HAP

Figure 1 shows the adsorption isotherms of CP$^+$ and CTA$^+$ on HAP from aqueous solutions of CPC, CPB and CTAC at a constant mixing ratio of HAP to the solution (solid/solution ratio). The adsorbed amount of CP$^+$ ($X_{\text{CP}^+}$) increased to a maximum, and then decreased slightly with an increase in the concentration of free CP$^+$ ([CP$^+$]$_f$]. This result is in contrast to that with CTA$^+$: the adsorbed amount of CTA$^+$ ($X_{\text{CTA}^+}$) increased monotonously with an increase in the concentration of free CTA$^+$ ([CTA$^+$]$_f$]. It was found that $X_{\text{CP}^+}$ decreased with an increase in the solid/solution ratio (○, ● and ▲) and that $X_{\text{CP}^+}$ from the CPC solution was smaller than that from the CPB solution (compare ● and ▲).

### Adsorption Isotherms of Cl$^-$ and Br$^-$ on HAP

The full lines in Fig. 2 show the adsorption isotherms of Cl$^-$ and Br$^-$ on HAP from aqueous solutions of CPC, CTAC and CPB. The adsorbed amounts of these ions ($X_{\text{Cl}^-}$ and $X_{\text{Br}^-}$) increased monotonously with their concentrations ([Cl$^-$]$_f$ and [Br$^-$]$_f$). The dotted line on open squares shows the adsorption isotherm of Cl$^-$ from an aqueous solution of KCl, an indifferent salt towards the HAP surface. In this case, Cl$^-$ was scarcely adsorbed on HAP. The adsorbed amount of Br$^-$ from an aqueous solution of KBr is also plotted in this figure (indicated by open triangles); adsorption of Br$^-$ was not detectable within the experimental error of the present work. These results lead to the conclusion that Cl$^-$ and Br$^-$ are adsorbed...
not by direct interaction with the HAP surface but by counterion binding with the surface active cation adsorbed on HAP.

**Release of Calcium Ion from the Surface of HAP**

It was found that Ca$^{2+}$ was released from the surface of HAP during the adsorption of CP$^+$ and CTA$^+$ on HAP. The concentration of Ca$^{2+}$ released, $[\text{Ca}^{2+}]_f$, is plotted against $[\text{CP}^+]_f$ or $[\text{CTA}^+]_f$ in Fig. 3. It shows that $[\text{Ca}^{2+}]_f$ increases with $[\text{CP}^+]_f$ or $[\text{CTA}^+]_f$, and with the surface area of HAP added, i.e., with the solid/solution ratio (see ○, ● and ○).

**Release of Phosphate Ion and the Equilibrium pH of the Solution**

The concentration of Pi released ($[\text{Pi}]_f$) and the equilibrium pH of the solution $(\text{pH})_f$ are
plotted as a function of \([CP^+]_f\) or \([CTA^+]_f\) in Figs. 4A and B. It shows that both \([Pi]_f\) and \((pH)_f\) decrease to a minimum, and then increase with an increase in the concentration of the surface active ion. The concentrations of \(CP^+\) and \(CTA^+\) at which \([Pi]_f\) has a minimum value are 0.58, 0.34 and 0.91 mm for CPC, CPB and CTAC, respectively.

Optical Density of the HAP Suspension

It was found that the HAP powder sedimented rapidly and a clear interface between the sediment phase and the liquid phase was observed when the surfactant concentration was low. However, when the concentration became high enough, the suspension remained turbid even though 24h had passed, due to the powder that had not sedimented. Figure 5 shows the relationships between \(OD_{850}\) and \([CP^+]_f\) or \([CTA^+]_f\). The curves increased sigmoidally in the same manner as those shown in Figs. 1 to 3. Therefore, it is reasonable to consider that the adsorption of the surface active ion is very closely related to the stability of the HAP suspension.

Discussion

The curves shown in Fig. 4A reverse their slopes from negative to positive at some concentration of surface active ions. This result suggests the existence of two distinct releasing mechanisms for \(Pi\) below and above this concentration. The values of 

\[-\log(Ca^{2+})^{10}(PO_4^{3-})^{6}(OH^-)^{2}\]

were obtained according to the method mentioned previously\(^2,13,14\) in the region of \([CP^+]_f\) and \([CTA^+]_f\) below the break points. The numerical values obtained were 113.1—116.0, 113.3—114.8, and 113.3—114.9 for CPC, CPB and CTAC, respectively. These values are within the range of the literature values of \(-\log K_{sp}\) for HAP (108—125).\(^{15}\) This result suggests that the decrease of \([Pi]_f\) in the region of concentration of \(CP^+\) or \(CTA^+\) lower than that at the break point was caused by the increase of \([Ca^{2+}]_f\) (Fig. 3) to maintain the solubility product for HAP, \(K_{sp}\), constant. On the other hand, in the region of higher concentration of \(CP^+\) or \(CTA^+\), \([Pi]_f\) increased despite the increase in \([Ca^{2+}]_f\). As \(K_{sp}\) for HAP should be kept constant even in this region, it was concluded that the activity coefficient of \(PO_4^{3-}\) decreased with an increase in \([CP^+]_f\) or \([CTA^+]_f\) owing to the binding of \(Pi\) to the surface of micelles. Accordingly, the concentration of the surface active cation at which \([Pi]_f\) has a minimum value (0.58, 0.34 and 0.91 mm for CPC, CPB and CTAC, respectively) was identified as the critical micelle concentration (cmc) of the system.\(^2-4\) This effect of micelles is more pronounced for CPC than for CPB (compare ■ with ▲). This can be explained by the difference of the binding affinity of \(Cl^-\) and \(Br^-\) to cationic micelles. That is, as \(Cl^-\) is more weakly bound to cationic micelles than \(Br^-\),\(^2,16-19\) \(Pi\) was exchanged more easily with \(Cl^-\) than with \(Br^-\) at the micelle surface. Marra reported that \(Cl^-\) also has a lower affinity than \(Br^-\) to cationic monolayers.\(^20\)

Figure 6 shows the relationship between the adsorbed amount of the surface active cation (\(X_{CP^+}\) or \(X_{CTA^+}\), quoted from Fig. 1) and the increment of the released amount of \(Ca^{2+}\) (\(-\Delta X_{Ca^{2+}}\)) due to the addition of the surfactant in the region below the cmc. The latter values were obtained from the data shown in Fig. 3, according to the following equation;

\[
-\Delta X_{Ca^{2+}} = (\frac{[Ca^{2+}]_f}{\text{weight of HAP added}}) - (\frac{[Ca^{2+}]_f}{\text{weight of HAP added}})
\]

Although \(X_{CP^+}\) and \([Ca^{2+}]_f\) depended on the solid/solution ratio as shown in Figs. 1 and 3, all the experimental points for CPC thus plotted are roughly on one curve irrespective of the
ratio. This result suggests that the increase of [Ca\(^{2+}\)]\(_t\) with an increase in the concentration of the surface active ion in this region was caused by the mechanism of ion-exchange between Ca\(^{2+}\) on the surface of HAP and the surface active cation adsorbed on HAP. However, the size of the surfactant polar group is larger than that of Ca\(^{2+}\).\(^{21}\) The exchange, therefore, may occur owing to electrostatic repulsion between the adsorbed cationic head group of the surfactant and Ca\(^{2+}\) adjacent to the adsorption site. On the other hand, a part of CP\(^+\) or CTA\(^+\) was adsorbed on HAP together with Cl\(^-\) or Br\(^-\) as a counterion, as mentioned in Results. The ratio of the adsorbed amount of Cl\(^-\) to that of CP\(^+\) (X\(_{\text{Cl}^-}/X_{\text{CP}^+}\)) is plotted against [CP\(^+\)]\(_t\) in Fig. 7. The data for CPB (X\(_{\text{Br}^-}/X_{\text{CP}^+}\)) and CTAC (X\(_{\text{Cl}^-}/X_{\text{CTA}^+}\)) are also plotted in this figure as a function of [CP\(^+\)]\(_t\) or [CTA\(^+\)]\(_t\). It shows that the ratio increases with the concentration of the surface active ion. Therefore, the decrease of the Pi release in the region below the cmc (Fig. 4) and the increase of the simultaneous binding of Cl\(^-\) or Br\(^-\) as a counterion to the surface active cation (Fig. 2) depress the accumulation of formal positive charge on the HAP surface. These facts are responsible for the downward deviation of the curves from the dotted line (which shows the relationship of \(-\Delta X_{\text{Ca}^{2+}}/X_{\text{CP}^+}\) and \(-\Delta X_{\text{Ca}^{2+}}/X_{\text{CTA}^+} = 1/2\)) in Fig. 6. The facts that the amount of Ca\(^{2+}\) expelled by CPB was smaller than that by CPC, and that X\(_{\text{Br}^-}/X_{\text{CP}^+}\) was larger than X\(_{\text{Cl}^-}/X_{\text{CP}^+}\) (compare ▲ with ● in Figs. 6 and 7), show that Br\(^-\) is more strongly bound to CP\(^+\) in the adsorbed phase than Cl\(^-\), in a similar manner to their binding to CP\(^+\) micelles, as mentioned previously.

When the concentration of the surface active ion becomes higher than the cmc, Pi is captured by the cationic micelles as a counterion. Therefore, Ca\(^{2+}\) release still continues in this region to compensate for the positive charge remaining on the surface of HAP through the release of Pi. The decrease of X\(_{\text{CP}^+}\) in Fig. 1 may take part in this compensation in order to maintain the electroneutrality of the surface phase.

Figure 8 shows the relationship between the increase of positive charge and that of negative charge at the surface of HAP. The former values were obtained from the amounts of CP\(^+\) or CTA\(^+\) adsorption and Ca\(^{2+}\) release, and the latter ones from those of Cl\(^-\) or Br\(^-\)
adsorption and Pi release. When the valency of Pi on the surface of HAP was considered to be 3 (A), all the experimental points deviated downwards from the dotted line which indicates electroneutrality with respect to adsorption and desorption of the ions. On the other hand, when a value of 1.6 was adopted for the valency of Pi (B), they were almost on the dotted line. These results suggest that Pi on the surface of HAP is protonated and acts as an anion with a valency of 1.6. This protonated phosphate may be more easily liberated from the surface of HAP than PO$_4^{3-}$. Another constituent anion, i.e., OH$^-$, should be released from HAP. Therefore, the value of 1.6 is an apparent valency involving the contributions of H$^+$ bound to released Pi and OH$^-$ released simultaneously.

Hanna and Saleeb reported that the zeta-potential of HAP powder is reversed from negative to positive as the adsorbed amount of CP$^+$ or CTA$^+$ increased. The contact angle and the flotation recovery increased as the zeta-potential increased. Therefore, the increase of OD$_{550}$ shown in Fig. 5 can be attributed to the increase of positive charge at the surface of HAP through the adsorption of surface active cations. This conclusion does not conflict with the requirement for electroneutrality of the surface phase of HAP discussed above for the following reason. That is, the obtained amounts of adsorption of Cl$^-$ and Br$^-$ after filtration are the sum of these ions located at the Stern layer and at the diffuse layer. Consequently, the potential at just outside of the HAP surface is positive and the electrostatic repulsion between positive charges on the HAP powders make the suspension stable.

In conclusion, the amounts of Ca$^{2+}$ and Pi released from the surface of HAP depended on the equilibrium concentrations of CP$^+$ and CTA$^+$, on the adsorbed amounts of these surface active ions, and on the species and affinity of the counterion for the surfactant. Moreover, the requirement of electroneutrality of the surface phase of HAP and the restriction of $K_{sp}$ for HAP in the solution phase also affect the relationships among them.

References and Notes

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