FORMATION AND ADSORPTION OF INTERMOLECULAR COMPLEX BETWEEN ALKYL PHOSPHORIC ACID AND NONIONIC SURFACTANT ON THE SURFACE OF HYDROXYAPATITE IN AN AQUEOUS PHASE

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Abstract Dicetyl phosphoric acid (DCP) and monoalkyl phosphoric acid (MAP, carbon number = 12, 14, and 16) are almost insoluble in water, while nonionic surfactants such as Triton X-100 (TX) and Triton N (TN) are soluble but non-adsorbable to hydroxyapatite (HAP) in an aqueous phase. Both DCP and the nonionic surfactant became adsorbable to HAP when these were mixed together in an aqueous phase. The mechanism was as follows; DCP was solubilized in a micelle of the nonionic surfactant and the mixed micelle itself was adsorbed to HAP, forming admicelle. Phosphate groups of DCP exposed on the micelle surface played an important role in the adsorption, because these head groups showed high affinity to the surface of HAP, resulting in adsorption of the micelle (*i.e.*, admicelle). When MAP was used instead of DCP, turbidity or stability of the HAP suspension became high, although single shot of MAP or the nonionic surfactant little affected the stability. These results are showing that the non-adsorbable TX and TN became adsorbable while insoluble alkyl phosphoric acid (DCP, MAP) changed to soluble and adsorbable through their mutual interaction in an aqueous phase and on the surface.

INTRODUCTION

Studies so far have been showing that phosphorylated organic compounds are easily adsorbed to hydroxyapatite (HAP) in an aqueous phase by virtue of similarity in chemical structure between orthophosphate ion on HAP and phosphate group of the organic compounds, that is, isomorphous substitution and/or ion exchange between them is expected to occur ^{1,2,3}. Phosphorylated polyvinylalcohol inhibited the crystal growth of HAP after the adsorption to its crystal seed and/or nuclei in an aqueous phase, for example ^{1,3}. Similar phenomena were also observed in a long chain alkyl phosphate, where the acid was solubilized as a salt of arginine ^{2,3}.

On the other hand, it has been assumed until now that organic compounds

having no phosphate groups in their chemical structures, such as polyvinyl alcohol and polyethylene glycol, were little adsorbed to HAP. Nonionic surfactant, which has polyethylene chain as a hydrophilic group, is also non-adsorbable to HAP when it was added into an aqueous solution in a single shot. However, it was adsorbed in the presence of a water insoluble alkyl phosphoric acid. This cooperative binding mechanism will be discussed in the present paper.

EXPERIMENTAL

Monoalkyl phosphoric acids (MAP, carbon number of the alkyl chain = 12, 14, and 16) were gift from Kao Co. (Tokyo, Dr. A.Yamagishi), and dicetyl phosphoric acid (DCP) was a product of Sigma Co. (USA). Triton X-100 (polyethylene glycol mono-*p*-isooctylphenyl ether, $n=10$, critical micellization concentration (cmc) = 0.275 mM, TX) and Triton N (polyethylene glycol mono-*p*-nonylphenyl ether, $n=10$, cmc = 0.08 mM, TN) were products of Nacalai Tesque Co. Other chemicals used in the present paper were purchased from Nacalai Tesque Co. or Wako Pure Chemicals Co. and used without further purification. Chemical structures of them are shown in FIGURES 1 and 2.

Adsorption amount of a surfactant was calculated from the difference in concentration of the adsorbate in a mother solution before and after the adsorption at 30 deg, where the HAP concentration was kept constant at 2.5 g/dl. The equilibrium concentrations in the supernatant after centrifugation were determined by an optical absorbancy method at 224 nm for TX and 223 nm for TN, respectively.

Solubility of an alkyl phosphoric acid in an aqueous solution of a given concentration of a nonionic surfactant was graphically determined from the concentration at the break point in the relationship between optical density of a sample solution at 550 nm and a concentration of the added alkyl phosphoric acid. The optical density steeply increased with the concentration after attaining the solubility or the break point, where the concentration of a nonionic surfactant was kept constant while [DCP] and [NaCl] were systematically changed at 30 deg. Solution pH was almost constant between 5-6 irrespective of the concentrations of DCP, NaCl, HAP, and nonionic surfactants.

RESULTS AND DISCUSSION

(1) Determination of solubility of DCP in the presence of nonionic surfactant.

Relationship between optical density of the solution and a concentration of DCP added

into the solution containing 10 mM TN is shown in FIGURE 3. When the amount of added DCP was small, the solution was almost clear, that is, DCP was solubilized by the micelle of TN formed in an aqueous phase. However, when the excess amount of DCP was added, the solution became turbid. The concentration at the break point in the relationship between [DCP] and the optical density, as shown in FIGURE 3, was assumed its solubility. The solubility decreased with a concentration of added NaCl. The reason why the solubility decreases was not clear, but it might be due to the fact that

i) Dicetyl phosphate

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iii) Myristyl phosphate

ii) Cetyl phosphate

iv) Dodecyl phosphate

FIGURE 1 Chemical structures of alkyl phosphoric acids

Triton X-100

Triton N

FIGURE 2 Chemical structures of nonionic surfactants

hydrophilic group of TN was dehydrated by NaCl added and, therefore, the micelle became compact/tight to entrap enough amount of DCP with the concentration of NaCl.

Similar phenomenon was observed in the system of DCP-TX-NaCl. FIGURE 4 shows the relationship between the concentration of NaCl added and solubility of DCP. It is interesting to compare the effect of TX with that of TN. 0.8 mM DCP was solubilized in an aqueous solution of 10 mM TX (FIGURE 4) while 3.8 mM DCP in the presence of 10mM TN (FIGURE 3). TN more easily solubilized DCP than TX. Mixing ratio of DCP to the surfactant in mole/mole unit in their micelle in the absence of NaCl was calculated from the data shown in FIGURES 3 and 4, taking their cmc's into consideration. $DCP/TX = 0.8/9.7 = 1/12$, while $DCP/TN = 3.8/9.92 = 1/2.6$. Thus, TN could more easily mix with DCP than TX in the micelle. This is because TN has a long and straight hydrocarbon chain, while TX a short but branched one (FIGURE 2). DCP could more easily penetrate into the TN micelle than the TX one.

(2) Adsorption of nonadsorbable surfactants, where DCP accelerates the adsorption.

Adsorption amount of TX and TN increased with a concentration of DCP, where they formed mixed micelle, as shown above. TX, for example, was scarcely adsorbed by HAP

FIGURE 3 Relationship between a concentration of added DCP and optical density of the solution at $[TN] = 10$ mM, where $[NaCl]/mM = 0$ ($\diamond$), 20 ($\square$), and 50 ($\times$)

in the absence of DCP. However, its adsorption amount increased almost in proportion to the concentration of solubilized DCP, where the adsorption amount was indifferent to the

FIGURE 4 DCP concentration solubilized into an aqueous solution of 10 mM TX as a function of a concentration of NaCl added.

FIGURE 5 Amount of TX adsorbed on HAP as a function of a concentration of DCP solubilized into the TX micelle, where $[TX]/mM = 5(\diamond)$, $10(\square)$, and $20(\times)$ in the absence of NaCl at 30 deg.

concentration of TX added ($[TX] = 5, 10, 20 \text{ mM}$), as shown in FIGURE 5. These results are showing that DCP in the micelle accelerates the adsorption of TX, that is, the TX is adsorbed together with the DCP-TX mixed micelle. Similar results were also obtained for the DCP-TN-HAP system (data not shown here).

Adsorption amount of both TX and TN decreased with the concentration of NaCl, as shown in FIGURE 6. Similar tendency was observed in FIGURES 3 and 4, where the solubility of DCP decreased with a concentration of NaCl added. Combining the results shown in these figures, it might be concluded that these nonionic surfactants become adsorbable after formation of the mixed micelle with DCP. The DCP, which is captured in the micelle, contacts to the surface of HAP, resulting in the adsorption of the mixed micelle itself. Phosphate group of DCP protruding from the micelle surface would bind to the positively charged site of Ca^{2+} on HAP and attack the orthophosphate ion on the surface of the HAP to exchange between them (*i.e.*, isomorphous substitution). Binding mechanism of phosphate groups to HAP was discussed in detail elsewhere^{4,5}.

FIGURE 6 Adsorption amount of TX and TN on the surface of HAP as a function of a concentration of DCP in the presence or absence of 20 mM NaCl. $\blacklozenge$: 10 mM TX, $\blacksquare$: 10 mM TX + 20 mM NaCl, $\triangle$: 10 mM TN, $\times$: 10 mM TN + 20 mM NaCl

(3) Interaction of MAP with the nonionic surfactant

Solubility of MAP in an aqueous solution of 10 mM nonionic surfactants (TX and TN) was studied. It decreased with a hydrocarbon chain length of MAP (*i.e.*, monododecyl > monomyristyl > monocetyl phosphoric acid), and also decreased with a concentration of added NaCl as the same manner as that shown in FIGURES 3 and 4. Solubility of monocetyl phosphoric acid (C=16) was higher than that of DCP by 12 times in 10 mM TX and by 1.6 times in 10 mM TN, respectively. The difference in solubility was caused due mainly to the facts that monocetyl phosphoric acid has one hydrocarbon chain (C=16) while DCP has two ones and that TX has a branched hydrocarbon chain while TN straight one.

Following the consistency of the logic throughout this paper, it was expected that TX and TN might be adsorbed together with MAP (C=12, 14, 16) to the surface of HAP. However, it was difficult to determine the adsorption amount of TX and TN in the presence of MAP. Stability of the HAP suspension was too high to separate the supernatant from the HAP particles by means of an ordinary centrifuge, from which the equilibrium concentration and adsorption amount of the surfactant should be determined.

In other words, however, these facts are suggesting that TX and TN were adsorbed to the HAP surface in the presence of MAP and that hydrophilic tails of the surfactant (*i.e.*, polyethylene chains) are protruding to an aqueous phase, resulting in exhibiting the dispersing effect. This effect was not observed in a single use of either MAP (water insoluble) or the surfactants (non-adsorbable). The cooperative effect is by virtue of formation of the mixed micelle in water and mixed admicelle on the surface.

(4) Interaction of MAP with sodium dodecyl benzenesulfonate

HAP suspended in an aqueous solution of sodium dodecyl benzenesulfonate (SDBS) was easily separated by means of a simple centrifugation method to obtain the supernatant in order to determine the adsorption amount of SDBS⁴. This fact is suggesting that SDBS was adsorbed to HAP mainly through ion exchange or isomorphous substitution of the sulfonate group with an orthophosphate ion on the surface^{1, 4, 5}, where many of the hydrophobic tails of SDBS might be exposed and protruding into an aqueous phase. The suspension stability, therefore, was relatively low and the separation by the centrifugation was easy.

However, when MAP (C=12, 14, 16) was mixed with an anionic surfactant

SDBS, HAP particles were hardly separated from the suspension solution in a similar manner to that observed in the mixed system of the nonionic surfactants and MAP, mentioned in the previous section. This result is showing again that the mixed micelle formed in the aqueous phase was adsorbed to the surface of HAP as the admicelle, which behaves as a strong and effective dispersing agent for HAP particles by virtue of 2 kinds of negative charges of SDBS/MAP protruding from the admicelle to an aqueous phase.

CONCLUSION

1. Water-insoluble DCP was solubilized by micelles of the nonionic surfactants, forming a mixed micelle. The mixed micelle was adsorbed by HAP after isomorphous substitution of the phosphate group of DCP with the orthophosphate ion on HAP.
2. As a result, non-adsorbable TX and TN were adsorbed together with water-insoluble DCP by virtue of the admicelle formed on the surface of HAP.
3. Similar adsorption mechanism was also assumed in the cases of MAP (carbon numbers of the alkyl chain were 12, 14, and 16).
4. It was strengthened that formation of the mixed micelle and/or intermolecular complex are important to make a non-adsorbable compound adsorbable by HAP.
5. Complex formed on the surface might contribute to formation of mammalian hard tissues, because the hard tissues are a composite of biological HAP and organic compounds such as biopolymers, lipids, and so on. These biological compounds may be adsorbable or non-adsorbable in a single use, while these should become adsorbable after formation of the intermolecular complex, resulting in the composite formation.

REFERENCES

1. S. Shimabayashi, N. Hashimoto, H. Kawamura, and T. Uno, in Mineral Scale Formation and Inhibition, edited by Z. Amjad (Plenum Press, New York, 1995), Chap.14, pp.157-167.
2. S. Shimabayashi, K. Furukawa, and T. Hino, in Advances in Crystal Growth Inhibition Technologies, edited by Z. Amjad (Kluwer Academic/Plenum Publishers, New York, 2000), Chap. 4, pp.43-55.
3. S. Shimabayashi and T. Uno, in Calcium Phosphates in Biological and Industrial Systems, edited by Z. Amjad (Kluwer Academic Publishers, Boston, 1998), Chap.9, pp.193-215.
4. S. Shimabayashi, M. Hoshino, and T. Hino, Phosphorus Research Bulletin, **14**, 39-46(2002).
5. S. Shimabayashi and T. Hino, Phosphorus Research Bulletin, **13**, 21-30(2002).