FORMATION OF INTERMOLECULAR COMPLEX AND POLYMER-DRUG INTERACTION ON THE SURFACE OF HYDROXYAPATITE IN AN AQUEOUS PHASE

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Abstract: Triton X-100, water-soluble but non-adsorbable to HAP, was adsorbed after formation of a mixed hemimicelle together with SDS. Water-insoluble dye, MY, was incorporated into an SDS aggregate formed on the surface in a similar manner to that of ordinary solubilization performed in an aqueous phase. Water-soluble polymer HPC formed a surface complex of SDS-HPC, which bridged among HAP particles, resulting in flocculation of the particles. Water-soluble MO was captured by BSA adsorbed on HAP, resulting in an increase in its amount of adsorption on the surface of HAP. Mono- and dialkylphosphate was adsorbed to HAP in a similar manner to that of SDS, i.e., after isomorphous substitution. Understanding these phenomena is important for understanding mechanisms of the interaction between drugs and hard tissues and the absorption of drugs into hard tissues as well as the formation of animal hard tissues composed of HAP and organic compounds.

INTRODUCTION

Hydroxyapatite (HAP, Ca_{10}(PO_4)_6(OH)_2) is a main inorganic component of mammalian hard tissues, which are composite of HAP and organic compounds such as lipids, proteins, and polysaccharides. These have both hydrophilic and hydrophobic groups in their chemical structures, which capture other organic compounds, externally added as a drug or medicine, through mechanisms such as hydrophobic and/or ionic interactions and hydrogen bonding between them. Taking these facts into consideration, the binding mechanism of amphiphilic compounds to and on the surface of HAP was reviewed from the viewpoints of colloid and surface chemistry in the present paper. The “drugs” used in the present paper are just only those of model compounds, which are used in order to discuss clearly and simply the binding mechanism between HAP and organic compounds and mutual interaction between organic compounds on the surface, i.e., formation of the surface complex.
HAP easily adsorbs sodium dodecylsulfate (SDS, NaC₁₂H₂₅SO₄) mainly through two mechanisms, as shown in FIG. 1: (1) electrostatic attractive force between Ca²⁺ on the surface of HAP and dodecylsulfate ion (DS⁻) in an aqueous phase, and (2) isomorphous substitution of a surface orthophosphate ion (PO₄³⁻) with a sulfate group (-SO₄⁻) of DS⁻. Both are of tetrahedral structure, and the interatomic distances are quite similar; P and O is 0.155 nm while S and O 0.149 nm. The HAP surface becomes hydrophobic to some extent after the adsorption, because the C₁₂ hydrocarbon tails of the adsorbed DS⁻ are protruding into an aqueous phase from the surface. Thus, the adsorption results in surface modification of HAP after implanting the hydrophobic groups on the surface. These tails interact with each other (i.e., lateral interaction), forming hemi- and admicelles or hydrophobic domains on the surface. The process of adsorption and following formation of hemi- and admicelle of SDS on HAP are schematically shown in FIG. 2.

These patches on the surface capture another hydrophobic compound through hydrophobic interaction, which induces formation of mixed hemi- and/or admicelles on the surface. Hydrophobic aggregates on the surface in this way play an important role in binding other organic compounds to the surface of HAP through hydrophobic interaction. Some examples on the topics are discussed here.

**(1) SDS+Triton X-100 (formation of mixed hemimicelle)** Polyethylene-glycol mono-p-isoctylphenyl ether (Triton X-100, degree of polymerization of EG group...
n=10) is scarcely adsorbed on the raw surface of HAP. This is because both hydrophilic and hydrophobic groups of Triton X-100 do not show any affinity for hydrophilic surface of HAP, remaining unadsorbed in an aqueous phase. It was, however, found that this nonionic surfactant is easily adsorbed in the presence of SDS or after the treatment of the surface with SDS. This fact means that adsorbed SDS offers the hydrophobic adsorption sites to Triton X-100. In other words, SDS and Triton X-100 formed mixed hemimicelle or admicelle on the surface. Similar phenomena were observed also in the system of SDS and polyethyleneglycol mono-p-nonylphenylether (Triton N, n=10) on HAP.

The adsorption of Triton X-100 and Triton N is cooperative with SDS, of which fact approves an important result that water-soluble but non-adsorbable compounds such as Triton X-100 and Triton N became adsorbable with the help of adsorbed SDS owing to the interaction or formation of mixed hemimicelle/admicelle on the surface through hydrophobic interaction (see FIG. 2).

(2) SDS+methylyellow(solubilization)\cite{4,5} Methylyellow(MY, see FIG.3), almost water-insoluble, was adsorbed to HAP in the presence of SDS. This is because the array

\[
\begin{align*}
\text{(A)} & \quad \text{C}_{12}\text{H}_{25} \quad \text{SO}_3^- \\
\text{(B)} & \quad \text{C}_{12}\text{H}_{25} \quad \text{SO}_3^- \\
\text{(C)} & \quad \text{C}_{12}\text{H}_{25} \quad \text{SO}_4^- \\
\end{align*}
\]

FIGURE 2. Schematic illustration of formation of hemimicelle and admicelle

\[
\begin{align*}
\text{(D)} & \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{SO}_4^- \\
\text{(E)} & \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \\
\end{align*}
\]

FIGURE 3. Chemical structures. (A) dodecylbenzenesulfonate, (B) dodecylsulfonate, (C) dodecylsulfate, (D) methylorange(MO), (E) methylyellow(MY)
or patch of hydrophobic tails of SDS adsorbed on the surface can trap MY in a similar manner to that an ordinary micelle can solubilize MY into an aqueous phase. The trap for MY on the surface is schematically shown in FIG. 4(A)-(1).

Two ways of the experimental procedure for the adsorption are shown in FIG. 4, where the mixing ratio of HAP to a mother solution was kept constant at 0.5 g / 20 ml in common. In the method (A), chemical potential of MY could be kept constant even after attaining the adsorption equilibrium, because an excess amount of solid MY (powder) still remains in the system and it determines the chemical potential of MY slightly dissolved/ solubilized in the mother solution. On the other hand, in the method (B) a known amount of MY was added to the system, which would be consumed in part during the adsorption and, therefore, the free concentration at the adsorption equilibrium becomes less than that added before the adsorption. That is, the method (B) is an experiment at a constant concentration of the added MY. Needless to say, the concentration of SDS should be higher than its critical micellization concentration in order to solubilize MY added.

The adsorption amount of MY as a function of a concentration of SDS added to the system is shown in FIG. 5. It decreased after attaining a maximum with a concentration of SDS added at a given concentration of NaCl. The affinity of MY for HAP was low in the absence of SDS, while it increased depending on a concentration of added SDS. The adsorption amount of SDS also decreased after attaining a maximum (not shown here), and the increase and decrease in the adsorption amount of MY were synchronized with those of SDS. This result is suggesting that the adsorbed SDS offers the adsorption sites for MY and also showing that water-insoluble organic compounds could become adsorbable to the surface by virtue of hemimicelle and/or admicelle formed on the surface after the solubilization by ordinary micelle.

(3) SDS+hydroxypropylcellulose

Although the adsorption amount of hydroxypropylcellulose (HPC) from an aqueous phase is small, it was much adsorbed in the presence of SDS by virtue of hydrophobic interaction between hydrophobic segments of HPC and SDS adsorbed on the surface. The adsorption amount of SDS also concomitantly increased more than that in the absence of HPC due to the binding to the segments of HPC protruding from the surface after its adsorption. Thus, in fact, one’s adsorption amount increased in the presence of the other (i.e., cooperative adsorption).
Surface complex of SDS-HPC caused dispersion or flocculation of the HAP particles, depending on the concentrations of HPC and SDS. Schematic illustrations of the binding on the surface (A) and of the aggregation/dispersion (B) are shown in FIG. 6.

FIGURE 4. Two experimental procedures are illustrated. (A) Semipermeable membrane is dividing 2 compartments. 500 mg of HAP and 10 ml of an aqueous solution of SDS were placed in one compartment (1), while a small amount of MY (powder) and 10 ml of the SDS in the other compartment (2). (B) 10 ml of an aqueous solution of SDS solubilizing a known amount of MY was added into an aqueous solution SDS (10 ml) suspending 500 mg HAP. The HAP suspension was vigorously shaken after the mixing.

FIGURE 5. Adsorption amount of MY in the presence of NaCl at 30 deg. is shown. Data (A) in (1)-(3) are from the method (A), while data (B) from the method (B). Results obtained from these two methods are fairly in agreement with each other in the tendency of the adsorption. The decrease at a concentration range higher than the maximum is due to the fact that MY is more stable in the ordinary micelle in an aqueous phase than in the hemimicelle/admicelle on the surface. [NaCl]/mM = 0(1), 5(2), and 10(3). MY (powder) was added more than its saturated concentration in (A), while for (B) [MY]/microM = 0.199(1), 0.221(2), and 0.236(3). Total concentration of MY in (B) before the adsorption was kept constant over the range of concentration of SDS studied.

Surface complex of SDS-HPC caused dispersion or flocculation of the HAP particles, depending on the concentrations of HPC and SDS. Schematic illustrations of the binding on the surface (A) and of the aggregation/dispersion (B) are shown in FIG. 6.
Monoalkylphosphate such as C_{12}H_{25}PO_{4}H_{2} was adsorbed together with arginine by virtue of isomorphous substitution between an orthophosphate ion on the surface and a terminal phosphate group of the monoalkylphosphate dissolved in an aqueous phase. Excess amount of arginine was added to the system in order to obtain a clear solution, where arginine behaved as both a counter ion for monalkylphosphoric acid and a pH adjustor. The solution pH was 7-8, depending on the concentration of arginine. The mixing ratio of arginine to monoalkylphosphate was kept constant at 3 to 1 in mole^3.8.9.

Crystal growth of HAP was inhibited in the presence of monoalkylphosphate, that is, the alkylphosphate behaved as a crystal poison after its adsorption on the crystal seed. It is because the binding affinity of monoalkylphosphate for the active site of the crystal growth on the seed is strong and competitive with that of orthophosphate ion. Mono-alkylphosphate also behaved as a dispersing agent for suspension of the matured HAP particles after the adsorption^9.

Dialkylphosphate such as dicetylphosphate (DCP, (C_{16}H_{33})_{2}PO_{4}H ) is practically insoluble in water. It was, however, solubilized in a micelle of Triton X-100. When HAP was added into the solution after the solubilization, Triton X-100 was adsorbed to the HAP surface. The adsorption amount increased with a concentration of DCP added, as shown in FIG. 7. Triton X-100 was non-adsorbable to HAP when it alone was added to the HAP suspension, of which fact is also shown at [DCP] = 0 in FIG. 7. The adsorption amount of Triton X-100 seems to depend mainly on the concentration of DCP while to be almost independent of the concentration of Triton X-100 added to the system^10.
Unfortunately, an adsorption amount of DCP was not determined directly. However, taking into account the fact that Triton X-100 and Triton N were adsorbed in the presence of SDS, it was concluded that Triton X-100 was adsorbed or captured by alkyl groups of the adsorbed DCP through hydrophobic interaction. DCP should be adsorbed in a similar manner to that monoalkylphosphate was adsorbed, although the head group of DCP might be somewhat bulkier than that of monoalkylphosphate due to its two alkyl chains bound to the head group.

This result is quite interesting. Although DCP is insoluble in water, it was adsorbed after solubilization in the micelle of Triton X-100. DCP might be pulled out from the mixed micelle when the micelle happened to contact to HAP surface, and Triton X-100 was concomitantly adsorbed to the surface by virtue of hydrophobic interaction. Another explanation of the adsorption mechanism is possible: the mixed micelle itself might be adsorbed to the surface by an affinity to HAP of the DCP phosphate groups exposed on the micelle surface when the mixed micelle collides against the surface. Thus, formation of the admicelle results in

![FIGURE 7. Adsorption amount of Triton X-100 is shown as a function of a concentration of added DCP at 30 °C. Concentration of Triton X-100 was kept constant at 5 mM(diamond), 10 mM(square), and 20 mM(circle), while that of DCP was increased. [HAP] = 2.5 g/dl.](image-url)
concurrent adsorption of both Triton X-100 and DCP.

In spite of everything, the water-insoluble compounds such as mono- and dialkylphosphate (DCP) were adsorbed after the solubilization into an aqueous phase with the help of a solubilizing agent such as arginine and Triton X-100.

**METHYLOrange+BSA (INTERMOLECULAR COMPLEX FORMATION)**

Water-soluble dye methylorange (MO) has sulfonate group, $-\text{SO}_3^-$, in its chemical structure, as shown in FIG. 3. The size of sulfonate group is almost the same as that of sulfate group of SDS. Therefore, MO was expected that it would be easily adsorbed to the surface of HAP after isomorphous substitution in a similar manner to that observed in a system of SDS and HAP. Although sodium dodecylsulfonate and sodium dodecylbenzenesulfonate, of which chemical structures are also shown in FIG. 3, were adsorbed by HAP, MO was not. This unexpected fact was explained in terms that hydrocarbon moiety of MO is relatively weak in hydrophobicity. It was emphasized from this fact that physicochemical properties of not only hydrophilic groups (e.g., $-\text{SO}_3^-$, $-\text{SO}_4$) but also hydrophobic groups are significant in the adsorption of amphiphilic compounds to HAP. Difference between MO and MY is just presence or absence of sulfonate group (see FIG. 3). One is easily soluble in water but non-adsorbable to HAP, while the other insoluble but adsorbable after hydrophobic interaction.

On the other hand, MO was adsorbed by HAP in the presence of bovine serum albumin (BSA) through formation of the intermolecular complex of MO-BSA. The surface complex might be formed through 2 ways; one is formation of the complex after adsorption of BSA to the surface, while the other is adsorption of the complex after the formation in an aqueous phase. In these ways MO, water-soluble but non-adsorbable, was adsorbed to the surface by virtue of formation of the intermolecular complex with BSA.

FIGURE 8 shows the adsorption amount of MO as a function of a concentration of added BSA, where concentrations of added MO and NaCl were kept constant. The adsorption amount of MO was almost independent of a concentration of added NaCl. However, it decreased after attaining a maximum with a concentration of added BSA, because a concentration of BSA free from HAP increases with that of total BSA added to the system and the free BSA also forms the complex of MO-BSA in an aqueous phase. Therefore, MO is consumed and the amount of MO available to form the complex decreases.
CONCLUSION

Binding mechanisms of amphiphilic compounds to HAP was discussed in the present paper for understanding mechanisms of both formation of mammalian hard tissues and binding of drugs to the surface of hard tissues, where the hard tissues are composed of HAP and organic compounds. This study was done using model compounds such as synthesized surface active agents, dyes, polymers, and so on.

Triton X-100, non-adsorbable but water-soluble, was adsorbed after formation of the mixed hemimicelle or admicelle with SDS. Water-insoluble dye, MY, was incorporated into the aggregate of SDS formed on the surface in a similar manner to that of solubilization in an aqueous phase. Water-soluble polymer HPC, which has hydrophobic groups in its structure, formed a surface complex of SDS-HPC. The polymeric complex bridged among the HAP particles, resulting in an aggregate composed of inorganic HAP and organic compounds. Water-soluble but non-adsorbable dye, MO, was captured by BSA adsorbed on HAP, forming the surface intermolecular complex. Monoalkylphosphate was adsorbed in the same manner as that

FIGURE 8. Adsorption amount of MO at 30 °C was shown as a function of a concentration of added BSA. Concentration of added MO was kept constant at 60 microM, while that of added NaCl was 0 mM (diamond), 50 mM (square), and 100 mM (triangle).
observed for the adsorption of SDS, i.e., isomorphous substitution. Dialkylphosphate, DCP, was also adsorbed to HAP although the size of its head group might be rather bulkier than that of monoalkylphosphate. Its adsorption was indirectly confirmed by the fact that the adsorption amount of Triton X-100 increases in the presence of DCP.

As mentioned above, the methods by which non-adsorbable compounds (Triton X-100 and MO) and practically water-insoluble compounds (DCP and MY) become adsorbable to HAP were mainly discussed in the present paper. The most important factor is formation of the mixed hemimicelle and/or micelle on the surface, that is, the solubilization on the surface. Thus, the contribution of hydrophobic interaction on the surface and, to some extent, in an aqueous phase was emphasized for the adsorption and binding of amphiphilic compounds to the surface of HAP.

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