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Adsorption of Phosphate Ion by Hydroxyapatite in Water

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The amount of phosphate ion, x_p, adsorbed by synthetic hydroxyapatite (HAP) was measured under various conditions in water. A long time was required to reach adsorption equilibrium, and the adsorption process was largely irreversible with respect to concentration. The adsorption isotherm was not of Langmuir type, but was dominated by the ionic product of adsorbate, depending on the species of counter ion. The value of x_p decreased with pH due to mutual electrostatic repulsion between adsorbed phosphate ions, which are deprotonated, and due to competitive adsorption between phosphate ion and OH^- ion. The adsorption process was also found to be endothermic, because the adsorption increased with temperature. The adsorbate ion and adsorbent surface may be dehydrated when they are bound together. From all these results, it was concluded that the adsorbate phosphate ion is oriented and coordinated to calcium ion on the surface of HAP, and this is the reason why the adsorption of phosphate ion is irreversible.

Keywords——hydroxyapatite; adsorption of lattice ion; adsorption of phosphate ion; pH effect; counter ion effect; endothermic adsorption; irreversible adsorption; coordination of absorbate ion; dehydration; entropy effect

The major constituent of biological hard tissue is crystalline hydroxyapatite (HAP), $Ca_{10}(PO_4)_6(OH)_2$. The formation of biological apatite is initiated by capturing and binding of Ca^{2+} to proteins containing covalently bound phosphate groups as well as carboxyl groups. The bound Ca^{2+} ions and the formed protein complexes remain reactive, so they can interact with other inorganic ions to form solid particles of the mineralized phase. These particles are assumed to be amorphous calcium phosphate (ACP). Subsequently, biological HAP grows through heterogeneous nucleation and epitaxy by adsorbing lattice ions, such as inorganic phosphate, calcium and hydroxyl ions, from body fluid onto the formed ACP and/or HAP as a nucleus.[1,2]

Adsorption of phosphate ion is also important in other fields. The adsorbability of phosphate ion by HAP from eluting phophate buffer solution (potassium phosphate, for example) is utilized to fractionate proteins and/or polynucleotides by chromatography on HAP columns. Inorganic phosphate ions compete with carboxyl groups or phosphate groups of the polymer chains, depending on their affinity for HAP, for the adsorption sites on the HAP surfaces.[3] Furthermore, adsorption or isotope-exchange of phosphate ion is applied to the measurement of the surface area of HAP or phosphate rocks.[4] In spite of these applications, little fundamental research on the adsorption of phosphate ion by HAP has been done.[5]

In the previous papers,[6-8] adsorption of two of the lattice ions of HAP, calcium and hydroxyl ions, by synthetic HAP was discussed. In the present study, adsorption of inorganic phosphate ion, the remaining lattice ion of HAP, will be discussed, and the effects of temperature, pH of the medium, and species of counter ions will be considered.

Experimental

Materials——All reagents were purchased from Nakarai Chemicals Ltd. or Wako Pure Chemical Industries Ltd. Water was doubly distilled. HAP was prepared as described elsewhere.[9] The X-ray powder diffraction pattern and infrared spectra were typical of HAP, and chemical analysis showed it to be almsot stoichiometric (Ca/P=10.2/6). It was confirmed that X-ray powder diffraction patterns of HAP were the same before and after soaking it in sample phosphate solutions.

Methods——HAP (2 g) was suspended in 20 ml of a given adsorbate solution of known concentration 4°C, 25°C, or 37°C, and shaken vigorously at frequent intervals. Within one hour after preparation of a sample suspension of HAP in an aqueous solution of phosphate salt, phosphate ions were adsorbed up to about 70% of the equilibrium value. However, it took 4 d to attain adsorption equilibrium, which seems to be rather longer than for Ca^{2+} or OH^-.[6,7)

After 4 d at each temperature, the filtrate through a Millipore filter (0.22 μm pore size) was analyzed by colorimetry for phosphate ion, or by pH titration for hydroxyl ion. The pH was measured with a pH-meter (Toa type HM-5ES). Phosphate concentration was determined according to the method of Gee *et al.*[10) The phosphate ammonium molybdate complex formed was reduced by stannous chloride. The absorbance of the resulting color was determined at 720 nm in a Shimadzu model UV-180 spectrophotometer after 15 min.

It was confirmed that the Millipore filter does not adsorb phosphate ion or hydroxyl ion. Therefore, the amount of adsorbed phosphate ion, x_p, or of adsorbed hydroxyl ion, x_{OH^-}, was calculated from the difference of the adsorbate concentrations before and after addition of HAP particles.[6-8) The adsorption isotherm was determined as a function of free concentration of adsorbate ion, or of the ionic product of free species of adsorbate.

Desorption experiments were performed by decanting the supernatant after equilibrium had been attained and replacing it with an equivalent amount of water. The adsorbate concentrations in solution after equilibrium were measured again. Reversibility of OH^- adsorption was confirmed. The amount of OH^- adsorption, therefore, is independent of the order and speed of mixing of water, adsorbate solution, and HAP powder.

This does not appear to be so for the adsorption of phosphate ion. Desorption studies within 4 d showed that adsorption is largely irreversible with respect to adsorbate concentration, and that the overall adsorption process might be represented by two processes, one irreversible and the other reversible, both competing for the same ions in solution, as mentioned by Loebenstein.[5) Accordingly, HAP particles were finally added to the prepared sample solution of phosphate, after sufficient shaking to avoid local high concentrations of phosphate ion in the solution.

When the pH dependence of the amount of adsorbed phosphate ion was measured at constant concentrations of phosphate ion and counter ion, the initial pH, $(pH)_i$, of the sample solution containing M_2HPO_4 ($M=Na^+$, K^+, or NH_4^+) was adjusted by adding HCl or corresponding MOH before addition of HAP. The total concentration of counter ion, [M] $(=2[M_2HPO_4]+[MOH]+[MCl])$, was adjusted by addition of the corresponding MCl to the above solution. Subsequently, the pH was readjusted by adding a small amount of HCl or MOH, if necessary. The HAP (2 g) was added to the sample solution (20 ml), as mentioned above. After the adsorption equilibrium had been reached, the final pH, $(pH)_f$, of the sample solution was measured again in the supernatant. The amount of adsorbed phosphate ion, x_p, was plotted as a function of $(pH)_i$ or $(pH)_f$.

"Treated HAP" was obtained as follows. HAP (40 g) was suspended for 4 d in 500 ml of 0.5 M $(NH_4)_2HPO_4$, 0.5 M $CaCl_2$, 0.5 M NaOH, or pure water, the last of which was for the reference experiment. The supernatant was decanted, and HAP was washed with 500 ml of water 5 times on a filter funnel. After dehydration with acetone, the "treated HAP" was dried at 110°C *in vacuo.*

In this article, the term phosphate ion will be used throughout to include all kinds of orthophosphate ions ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) found in the experimental pH range.

Results

Adsorption Isotherms for Phosphate Ions

Adsorption isotherms for phosphate ions with different cations and at different temperatures are shown in Fig. 1. Isotherms at 25°C (○) and at 37°C (◑) for each salt are almost the same, but that at 4°C (●) is a little lower. The concentration range for 4°C does not cover the full range because of low solubility of each phosphate salt.

Full lines in Fig. 1 were theoretically calculated with the Langmuir adsorption isotherm of Eq. (1),

$$x=\frac{x_\infty kc}{1+kc} \qquad (1)$$

where x, x_∞, k, and c are the amount of adsorption, the saturated amount of adsorption, the binding constant, and the equilibrium concentration of adsorbate, respectively. The numerical values of x_∞ (mol/g-HAP) and k (M^{-1}) for both 25°C and 37°C are 0.258×10^{-3} and 43.5 for Na_2-

HPO_4 (pH 9.3—9.6), 0.202×10^{-3} and 31.6 for K_2HPO_4 (pH 9.2—9.6), and 0.262×10^{-3} and 30.8 for $(NH_4)_2HPO_4$ (pH 8.2—8.8), respectively. These calculated curves are not in good agreement with the experimental values. When the agreement of the data in the range of lower concentration of free phosphate ion is improved by adjusting the numerical values of x_∞ and k, the agreement in the range of higher concentration of free phosphate ion becomes worse, and *vice versa*. The values given above are, therefore, tentative, and are not entirely satisfactory. This will be discussed later.

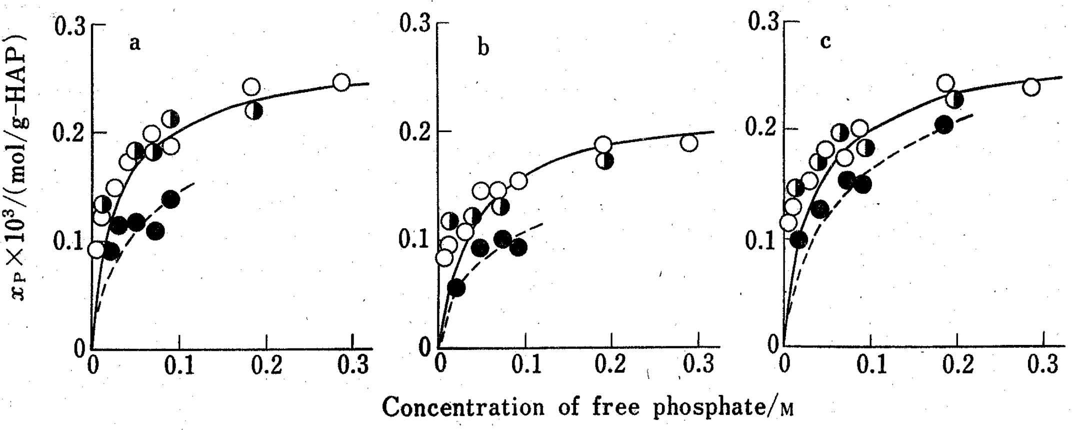

Fig. 1. Adsorption Isotherm for Phosphate Ion as a Function of Concentration of Free Phosphate Ion

Temperature (°C): ● 4, ○ 25, ◑ 37.
a: Na_2HPO_4 (pH 9.3—9.6), b: K_2HPO_4 (pH 9.2—9.6). c: $(NH_4)_2HPO_4$ (pH 8.2—8.8).

Effect of pH on the Amount of Adsorption

The effect of medium pH on the amount of phosphate ion adsorbed from aqueous solution at constant initial concentrations of both phosphate ion and counter ion is shown as a function of equilibrium pH, $(pH)_f$, in Fig. 2. In the case of these systems of HAP-phosphate–water, $(pH)_f$ was almost the same as the initial pH, $(pH)_i$, due to the buffer functions of both phosphate

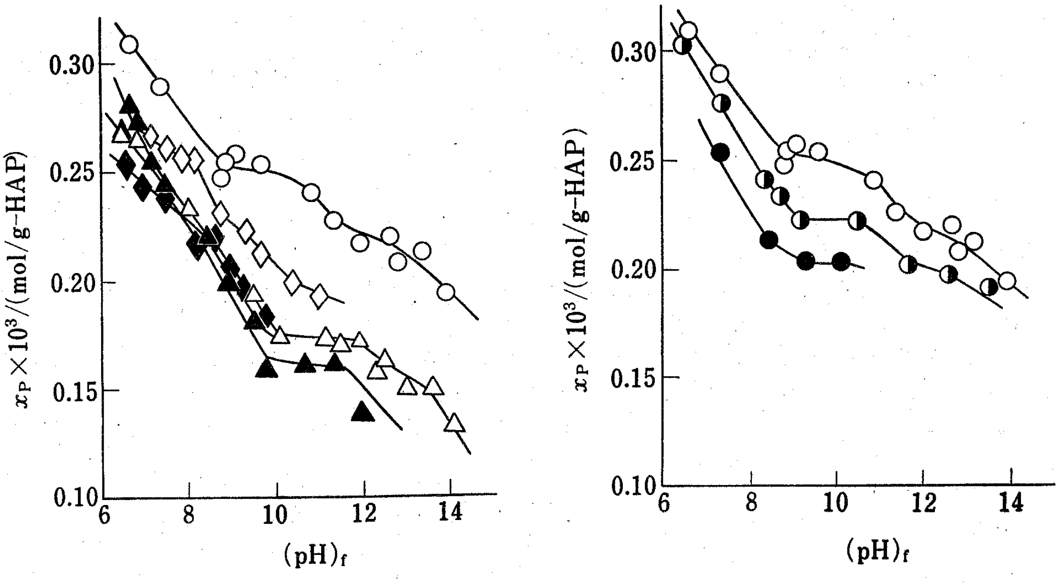

Fig. 2. The Effect of Equilibrium pH on the Amount of Adsorbed Phosphate at Various Concentrations of Counter Ion

phosphate concentration: 0.05 M, Temperature: 25°C, counter ion concentration (M):
Na^+: ○ 1.5, ◑ 0.75, ● 0.15. K^+: △ 1.5, ▲ 0.15. NH_4^+: ◇ 1.5, ◆ 0.15.

ion and the HAP surface in water.[7] Variation of the amount of adsorbed phosphate ion with $(\mathrm{pH})_f$ is, therefore, almost the same as that with $(\mathrm{pH})_i$, which is not shown here, however.

It was found that the amount of adsorbed phosphate ion, x_p, decreases with $(\mathrm{pH})_f$, but increases with the concentration of each counter ion, as shown in Fig. 2. Decrease of the amount of adsorbed phosphate ion with pH has also been found on goethite, gibbsite,[11] pseudoboehmite, and kaolinite.[12] The reason may be that competitive adsorption between OH^- and phosphate ion takes place for the adsorption sites common to both anions, and that mutual electrostatic repulsion between adsorbed phosphate ions and between OH^- and phosphate ion increases with pH due to deprotonation of phosphate ion and due to adsorption of OH^- on the HAP surface. The effect of counter ion concentration will be discussed in the next section.

Adsorption Isotherm as a Function of the Ionic Product

Figure 3 shows the adsorption isotherm for OH^- as a function of the ionic product of $[Na^+][OH^-]$, or $[K^+][OH^-]$, for which appropriate counter ion concentrations were prepared by the use of NaOH and NaCl, or KOH and KCl. All the experimental points are essentially on one curve, irrespective of the concentration of counter ion, as mentioned before.[7,13] This result means that ionic product or thermodynamic activity determines the amount of adsorption.

The adsorption isotherms were confirmed to be of the Langmuir type of Eq. (1), where c should be read as the ionic product for these cases. Numerical values for adsorption parameters, x_∞ (mol/g-HAP) and k (M^{-2}), in Eq. (1) are 0.173×10^{-3} and 518 for NaOH, and 0.164×10^{-3} and 495 for KOH, respectively. Both parameters, x_∞ and k, are larger for NaOH than for KOH. Curves in Fig. 3 are theretically calculated by means of Eq. (1) with parameter given above.

Figures 4-a and -b show the adsorption isotherm for phosphate ion as a function of the ionic product of free concentration of adsorbate, $[Na^+]^2[HPO_4^{2-}]$ or $[K^+]^2[HPO_4^{2-}]$, where the abscissa scale is logarithmic to expand the region of low contration. As the pK values for the dissociation constants of phosphate, pK_1, pK_2, and pK_3, are 2.12, 7.21, and 12.52, respectively, HPO_4^{2-} is assumed to be the dominant ionic species in the solution over the pH range of the measurement (pH 9.2—9.6). The ionic product, $[Na^+]^2[HPO_4^{2-}]$ or $[K^+]^2[HPO_4^{2-}]$, is, therefore, used in Fig. 4 as an approximation for the thermodynamic activity which is controlling the amount of phosphate ion adsorbed.

The adsorption data (○, ◑) are the same as those shown in Fig. 1, and the other data (●) are taken at pH 9.5 from Fig. 2. Full lines in Fig. 4 were calculated by means of Eq. (1),

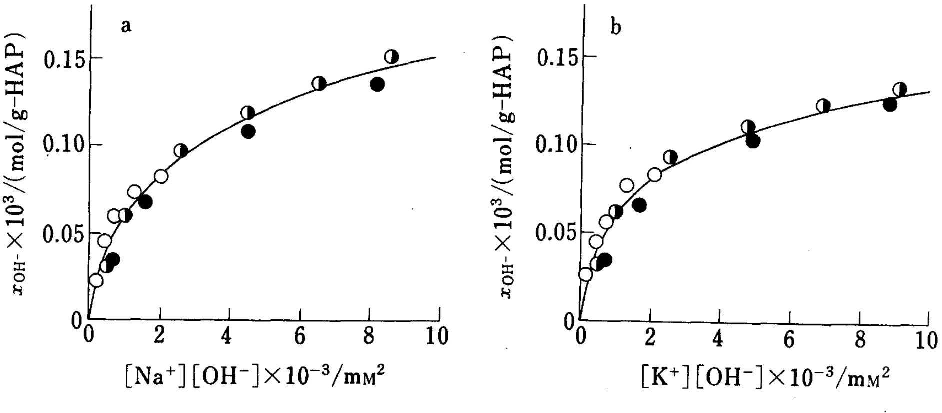

Fig. 3. Adsorption Isotherm for OH^- as a Function of Ionic Product of the Adsorbate, NaOH or KOH

○: MOH, ◑: [MOH]+[MCl]=0.25 M, ●: [MOH]+[MCl]=0.5 M.
M=Na^+ or K^+. Temperature: 25°C. a: NaOH, b: KOH.

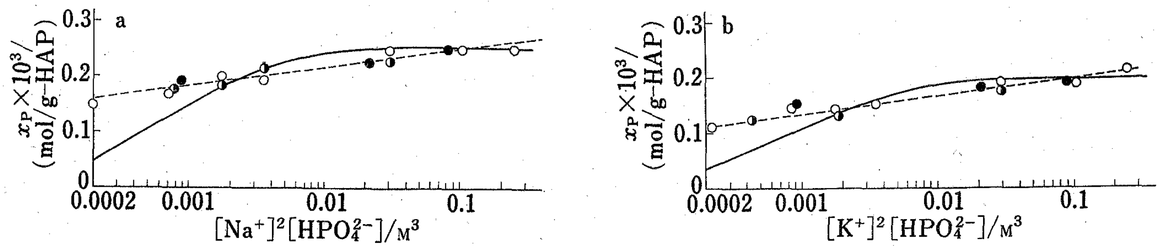

Fig. 4. Adsorption Isotherm for Phosphate Ion as a Function of Ionic Product

a: Na_2HPO_4 ○: 25°C, pH 9.3—9.6 (from Fig. 1), ◐: 37°C, pH 9.3—9.6 (from Fig. 1), ●: 25°C, pH 9.5 (from Fig. 2), ——: Langmuir-type isotherm, ----: experimental curve.

b: K_2HPO_4 ○: 25°C, pH 9.2—9.6 (from Fig. 1), ◐: 37°C, pH 9.2—9.6 (from Fig. 1), ●: 25°C, pH 9.5 (from Fig. 2), ——: Langmuir-type isotherm, ----: experimental curve.

assuming that c in Eq. (1) is the ionic product, and that x_∞ (mol/g-HAP) and k (M^{-3}) are 0.258×10^{-3} and 1.40×10^3 for Na_2HPO_4, and 0.202×10^{-3} and 1.03×10^3 for K_2HPO_4, respectively. These parameters for calculation are temporarily determined to make these curves agree with the experimental values in the region of relatively higher ionic products in Fig. 4. In the region of lower ionic product in Fig. 4, the curves deviate greatly from the experimental values.

On the other hand, the dotted lines drawn in Fig. 4 are in good agreement with the experimental values. The data of the closed circles from Fig. 2 are on the dotted line, as well as the data from Fig. 1. These straight lines could be expressed as follows;

$$x = 0.285+0.0335 \log[Na^+]^2[HPO_4^{2-}] \qquad (2)$$

for Na_2HPO_4, and

$$x = 0.231+0.0325 \log[K^+]^2[HPO_4^{2-}] \qquad (3)$$

for K_2HPO_4. Therefore, it may be concluded that the amount of adsorbed phosphate ion is determined by the ionic product or thermodynamic activity, but does not follow the Langmuir type adsorption isotherm. This is in contrast to the result of OH^- adsorption, where the adsorption isotherm was of Langmuir type as a function of the ionic product of the adsorbate, as shown in Fig. 3 and in the previous paper.[7]

Accordingly the increase in the amount of adsorbed phosphate ion with increasing concentration of counter ion, shown in Fig. 2 or in Fig. 4 by closed circles, is attributed to the increase of the ionic product or activity of the adsorbate in the solution.

The numerical values of the coefficients given in Eq. (2) for Na_2HPO_4 are larger than those in Eq. (3) for K_2HPO_4. In view of the result that the binding parameters for NaOH are larger than those for KOH, as mentioned before in this section, it may be concluded that anion (OH^- or phosphate ion) adsorption depends on the ionic species of cation, and that Na^+ enhances the anion adsorption more than K^+ does.

Adsorption to Treated HAP

The amounts of adsorption of phosphate ion, calcium ion, or hydroxyl ion by four kinds of treated HAP (2 g) were measured from aqueous solution of 100 mM $(NH_4)_2HPO_4$ (20 ml), 27.9 mM $CaCl_2$ (20 ml), or 71.2 mM NaOH (40 ml). The results are given in Table I, where the ionic species underlined in the left-hand column are those of adsorbate, and where electrolytes in the top row are those with which HAP was treated prior to these adsorption experiments.

The amounts of phosphate ion, calcium ion, and hydroxyl ion adsorbed by HAP treated with $CaCl_2$ or NaOH are almost the same as those of the reference HAP, but those by HAP treated with $(NH_4)_2HPO_4$ are remarkably different. The values in parentheses are percentage increases (with a plus sign) or decreases (with a minus sign) from the reference values given in the column "H_2O (reference)" in Table I.

TABLE I. The Amount of Adsorption by "Treated HAP" at 25°C in mmol/g-HAP

Adsorbate (HAP=2.0 g)	Treated with	H_2O (Reference)	$(NH_4)_2HPO_4$	$CaCl_2$	NaOH
$(NH_4)_2\underline{HPO_4}$ 100 mM, 20 ml		0.215	0.0824 (−62%)	0.215 (0%)	0.222 (+3%)
$\underline{Ca}Cl_2$ 27.9 mM, 20 ml		0.0186	0.0303 (+63%)	0.0178 (−4%)	0.0190 (+2%)
$Na\underline{OH}$ 71.2 mM,40 ml		0.0950	0.190 (+100%)	0.0988 (+4%)	0.0983 (+3%)

The surface properties of the HAP treated with $CaCl_2$ or NaOH may become almost the same as those of the reference HAP after water washing, because Ca^{2+} or OH^-, which is adsorbed reversibly by HAP during the treatment, may be desorbed while these treated HAP samples are being washed with water. In contrast, the phosphate ion, left on the surface after washing, may affect the amount of adsorbed ions due to its almost irreversible adsorption.

The amounts of phosphate ion and calcium ion adsorbed by HAP treated with phosphate salt were decreased by 62%, and increased by 63%, respectively. The adsorption sites for phosphate ions are already occupied by the pre-adsorbed phosphate ions when phosphate ions as the adsorbate are added to the HAP. Therefore, the adsorption of phosphate ion is inhibited. On the other hand, the pre-adsorbed phosphate ions form adsorption sites for calcium ion by electrostatic interaction. This co-adsorption of calcium ion with phosphate ion may be similar to what occurs during the crystal growth of HAP.

The increase of OH^- adsorption by up to 100% by pre-adsorbed phosphate ion seems strange in view of the electrostatic repulsion between OH^- anion as the adsorbate and phosphate anion on the surface. However, it can be explained by considering that, instead of direct adsorption of OH^-, consumption of OH^- occurs by protons dissociated from protonated phosphate ions which have been pre-adsorbed on the surface of HAP.

In summary, the characteristic features of phosphate ion adsorption by HAP are as follows: adsorption is rather slow (at least 4 d), and is neither of simple Langmuir type nor completely reversible; the amount of adsorption is determined by the thermodynamic activity but depends on the ionic species of counter ion; the amount of adsorption decreases with decreasing temperature and/or with increasing pH. These obtservaions may be related to each other.

Discussion

Counter Ion Effect

Phosphate ion, one of the lattice ions of HAP, may be adsorbed electrostatically at the site of phosphate-defects and/or in the neighorphood of Ca^{2+} on the HAP surfaces, where positive charge is localized. A cations on the HAP surface may form an adsorption site due to its positive charge, like Ca^{2+} on the surface. Na^+, the crystal ion radius (0.97 Å) of which is almost the same as that of Ca^{2+} (0.99 Å), is more easily adsorbed on the HAP surface than K^+ (1.33 Å).[6] Na^+, therefore, enhances the anion adsorption more than K^+, as shown in Figs. 3 and 4. This counter ion effect cannot be explained by the thermodynamic activity, even though it determines the adsorption isothern for each counter ion, irrespective of Langmuir type or non-Langmuir type adsorption, as shown in Figs. 3 and 4.

On the other hand, the chemical potential of the adsorbed species on the adsorbent surface, μ_{surf}, is equal to the chemical potential of the adsorbate in the solution, when adsorption equilibrium is attained. Therefore, the following equation is obtained,

$$\mu_{surf} = \mu^o + RT \ln a \tag{4}$$

where μ° and a are the standard chemical potential and thermodynamic activity, respectively, of the adsorbate in the solution. Equation (4) shows that not only a but also μ° affects the adsorption equilibrium. Now a is a function of concentration, which is approximated by the ionic product in this article, and μ°, which is independent of the concentration, is assumed to be affected by the species and the crystal ion radius of counter ion. Therefore, differences in the binding parameters, or in the adsorbed amounts of anion at a given activity can be attributed to the difference in the standard chemical potential, μ°. However, quantitative relations between μ° and crystal ion radius were not obtained here.

Temperature Effect and Dehydration

Adsorption is usually exothermic, leading to a decrease in adsorption with temperature. This dose not appear to be so for the adsorption of phosphate ion by HAP. The amount of phosphate adsorbed increases with temperature, as shown in Fig. 1. Similar temperature effect phenomena have frequently been observed in other systems, such as human plasma albumin and polystyrene latex,[14] dextran and silver iodide,[15] and phosphate ion and kaolinite or gibbsite,[16] and so on. The increase in adsorption with temperature implies that adsorption of phosphate ion by HAP seems to be an endothermic process. Consequently a substantial contribution of entropy to the free energy of adsorption must be involved, which may be due to the solvent entropy gain resulting from dehydration of the water around the adsorbate ion of phosphate and/or the adsorbing site on HAP. Water of hydration is certainly dehydrated with increasing temperature even though the hydration is ionic.[17] Dehydrated phosphate ion is assumed to fit the (dehydrated) adsorption site formed by dislocation or defect of phosphate ion on the HAP surface, just as dehydrated Ca^{2+} fits its own adsorption site on HAP, as mentioned in the previous paper.[6] When these lattice ions are adsorbed on the surface of HAP, the sites on the surface of HAP may also be dehydrated, and these lattice ions adsorbed may be included in HAP as intrinsic components. This may be assumed to correspond to partial "crystal growth" of HAP

Orientation of Phosphate Ion and Irreversibility of Adsorption

Brown[18] showed that oxygens of the PO_4^{3-} unit are quite strongly coordinated to the adjacent calcium ions in the crystal of HAP. This results in chains of atoms, ---Ca–O–P–O–Ca---, parallel to the c-axis for 2 oxygenes of PO_4^{3-}, and perpendicular to the c-axis for the remaining 2 oxygenes of PO_4^{3-}. This coordination serves to tie the HAP structure tightly together.

It is natural to assume that oxygens of the adsorbed phosphate ion are also coordinated to the surface calcium ion. This coordination and consequent preferred orientation of adsorbed phosphate ion seem to be responsible for irreversible adsorption. This process could be rather slow. Consumption of phosphate ion by its penetration into cracks of HAP or by its diffusion inside HAP is probably not rate-determining because the time required for the other lattice ions of HAP, Ca^{2+} and OH^-, to attain adsorption equilibrium is not as long as that for phosphate, and because the HAP surface was suggested to be nonporous by Fuerstenau *et al.*[19]

The facts that the adsorption process is of high affinity and is irreversible would lead one to expect that the experimental adsorption data might not follow the Langmuir adsorption isotherm, and that the initial slope of the adsorption plot might be greater than that for Langmuir-type adsorption. This was found to be the case (Fig. 1).

If Eq. (1) is modified to a multisite Langmuir type adsorption isotherm, the calculated curves might then be in agreement with the experimental values shown in Fig. 1, but it would be difficult to relate these sites with the locations of the high-affinity (with large k values) or low affinity (with small k values) sites on the HAP surface, on the basis of the simple coordination theory mentioned above. Therefore, analysis of the experimental data in Fig. 1 in terms of the improved Langmuir-type adsorption isotherm was not attempted here.

High Affinity of Phosphate Ion to Calcium Ion

The high affinity of phosphate ion to calcium ion is reflected in the low solubility of various kinds of inorganic calcium phosphate and the calcium salt of dental phosphoprotein,$^{20)}$ and in the preferential adsorption of phosphate ion rather than biological polymers such as salivary protein to calcium ion on HAP.$^{3,21)}$ Synthetic HAP with a known content of HPO_4^{2-} can be prepared by treating HAP with $(NH_4)_2HPO_4$ under various conditions because of the high affinity adsorption of phosphate ion.$^{22)}$

On the other hand, ethane-1-hydroxy-1,1-diphosphonate$^{23)}$ and monofluorophosphate$^{24)}$ markedly inhibit the rate of crystal growth of HAP and the dissolution rate of dental enamel, respectively. The mechanical properties of dental cements are substantially improved by mixing them with poly (HEMA-phosphate), which is a polymer of phosphorylated 2-hydroxyethyl methacrylate.$^{25)}$ These effects may originate from the high affinity of phosphate or phosphonate groups for calcium ions on HAP.

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