INHIBITION OF THE AMORPHOUS CALCIUM PHOSPHATE PHASE TRANSFORMATION REACTION BY POLYMERIC AND NON-POLYMERIC INHIBITORS

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Abstract The induction time for the transformation of amorphous calcium phosphate (ACP) to hydroxyapatite (HAP) was monitored at pH 7.40, 37 °C, and calcium = phosphate = 3.20 mM, in the presence of polymeric and non-polymeric inhibitors containing various functional groups. The polymeric inhibitors evaluated include: poly(acrylic acid), molecular weight 850 to 20 000; poly(acrylamide), PAm; poly(diallyldimethylammonium chloride), PDa; copolymers of acrylic acid:hydroxylpropyl acrylate, AA:HPA; acrylic acid:2-acrylamido-2-methylpropane sulfonic acid, AA:SA; acrylic acid:vinyl acetate, AA:VAc; acrylic acid: vinyl alcohol, AA:VOH. The non-polymeric inhibitors investigated include: pyrophosphate, PYP; 1-hydroxyethylidine-1,1-diphosphonic acid, HEDP; and 2-phosphonobutane-1,2,4-tricarboxylic acid, PBTC. It was found that PAc and acrylic acid-based copolymers were effective in delaying ACP transformation reaction. The inhibitors effectiveness was greatly affected by polymer M_w and ionic charge. For homopolymers the order in terms of decreasing effectiveness is: PAc >> PAm > PDa = control (no inhibitor). Among copolymers, a AA:SA copolymer showed the best overall performance in terms of delaying the ACP phase transformation. For non-polymeric inhibitors, it was observed that both polyphosphate and phosphonates are potent inhibitors with HEDP more effective than PYP, as expected. The combination of PAc and PYP or HEDP or PBTC leads to a synergistic delay in the onset of the phase transformation. Reaction solutions containing 30 ppm PAc-2 (M_w 2100) and 17 ppm HEDP resulted in a > 400% increase in induction time over what would be anticipated from an additive effect from these inhibitors.

INTRODUCTION

Dental tartar is a calcareous deposit on teeth consisting primarily of calcium phosphates and other calcium magnesium salts. The various calcium phosphate phases that may be present in the tartar include dicalcium phosphate dihydrate [$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$], tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$, TCP], octacalcium phosphate [$\text{Ca}_8(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, OCP], hydroxyapatite [$\text{Ca}_5(\text{PO}_4)_3\text{OH}$, HAP], and fluorapatite [$\text{Ca}_5(\text{PO}_4)_3\text{F}$, FAP] (1, 2). Tartar formation has been cited as a contributing factor in the

accumulation of plaque and can adversely affect the staining of tooth surfaces by various agents (3)

It is generally agreed that initially precipitated calcium phosphate phase from aqueous solution of high supersaturation and at pH above 7, is an amorphous calcium phosphate, ACP. The formation and stability of ACP phase in aqueous solution has been the subject of numerous investigations. It has been suggested that if ACP is allowed to remain in contact with the solution, it transforms to the thermodynamically stable HAP perhaps via the hydrolysis of OCP. HAP is the main component of teeth and bones.

It is well established that addition of certain compounds to a calcium phosphate supersaturated solution not only influences the kinetics of crystal growth but also affects the stability of precursor phase(s). The investigation of the influence of magnesium ion on the kinetics of crystal growth of calcium phosphates by Bachra et al. (4) and Termine et al. (5) pointed to a stabilization of ACP. Tomazic (6) and Nancollas (7) in their studies on seeded growth at lower supersaturation showed that magnesium ion induces the formation of a precursor phase rather than OCP, perhaps a mixture of OCP and DCPD. Boskey and Posner (8) reported that magnesium ion reduces the solubility of ACP, thus increasing the induction period for the transformation to HAP. Using a mixture of magnesium and Adenosine triphosphate (ATP), Blumenthal et al. showed that in the presence of these compounds the conversion of ACP to HAP is markedly delayed (9). It has also been reported that additives containing hydroxyl, carboxyl, or amino groups such as citric acid, malonic acid, benzene hexacarboxylic acid, polyglycine, etc., markedly affect the kinetics of precipitation of calcium phosphate phases (10, 11).

In previous publications, we showed that polymeric additives such as poly(acrylic acid), poly(maleic acid), and carboxyl containing copolymers are effective precipitation inhibitors for sparingly soluble salts such as calcium fluoride, barium sulfate, calcium sulfate, and calcium phosphates (12 - 15). This paper presents an ongoing effort to investigate the influence of a variety of polymeric inhibitors containing different functional groups such as carboxyl, amido, sulfonic, etc., on the ACP transformation reaction to HAP. Additionally, experiments have been conducted to study the influence of mixed inhibitors for possible synergistic effect on the ACP transformation reaction.

EXPERIMENTAL

All experiments were performed at $37 \pm 0.1^\circ\text{C}$ in a thermostated double-walled water jacketed cell, volume totaling 270 mL. Reagent grade chemicals and carbon dioxide free distilled water were used in the preparation of the solutions. The preparation of the standard solutions of calcium chloride, disodium hydrogen phosphate, and sodium hydroxide was done as described in detail elsewhere (15). Polymeric inhibitors used in this study were selected from commercial and experimental materials supplied by The

BFGoodrich Company. Phosphonates used in the present study were commercial samples received from Bayer and Monsanto Companies.

Subsaturated calcium phosphate solutions were prepared in a thermostated glass cell by adding stock solutions of calcium chloride and disodium hydrogen phosphate to the distilled water to attain initial calcium and phosphate concentrations of 3.20×10^{-3} M. Experiments involving inhibitors were performed by adding the inhibitor to the subsaturated calcium phosphate solution before inducing spontaneous precipitation. Hydrogen ion measurements were made with a combination electrode equilibrated at the required temperature. The electrode was standardized for each experiment using NBS standard buffer solutions. The calcium phosphate solutions were continuously stirred with a Teflon coated stirring bar at ~ 350 revolutions per minute and nitrogen gas, presaturated at 37°C , was bubbled to exclude carbon dioxide from the solution. Spontaneous precipitation was induced by raising the pH of the solution to 7.40 with 0.10 M NaOH solution. The pH of the solution was maintained at 7.40 ± 0.01 using a Metrohm pH-stat instrument (Model 600 series, Brinkmann Instruments, Westbury, New York). The induction time at which the transformation of ACP to HAP process starts was determined as described previously (16).

RESULTS AND DISCUSSION

Table 1 shows the structure of inhibitors used in these experiments.

Inhibitor	structural formula	mol wt	Acronym
poly(acrylic acid)	$\begin{array}{c} \text{---}(\text{CH}_2\text{---CH})\text{---}_n \\ \\ \text{COOH} \end{array}$	850	PAC-1
		2100	PAC-2
		6000	PAC-3
		10 000	PAC-4
		20 000	PAC-5
poly(acrylamide)	$\begin{array}{c} \text{---}(\text{CH}_2\text{---CH})\text{---}_n \\ \\ \text{CO} \\ \\ \text{NH}_2 \end{array}$	6000	PAm
poly(acrylic acid / 2-acrylamido 2-methyl propane sulfonic acid)	$\begin{array}{c} \text{---}(\text{CH}_2\text{---CH})\text{---}_n \text{---}(\text{CH}_2\text{---CH})\text{---}_m \\ \qquad \qquad \\ \text{COOH} \qquad \text{CO} \\ \\ \text{NH} \\ \\ \text{CH}_3\text{---C---CH}_3 \\ \\ \text{CH}_2\text{SO}_3\text{H} \end{array}$	6000	PSa

TABLE 1. Structures of polymeric and non-polymeric inhibitors evaluated

Typical plots of the amount of NaOH added to the calcium phosphate solution to maintain constant pH are given in Figure 1.

Figure 1. NaOH uptake as a function of time for various inhibitors.

The data of Figure 1 was used to determine the induction time, β , for the transformation of ACP to HAP phase. This was as determined by the dotted line in Figure 1. The influence of PAc-2 (poly(acrylic acid), molecular weight 2100) on the transformation reaction of ACP to HAP was investigated by a series of experiments summarized in Table 2. Typical examples of NaOH uptake - time profiles at varying concentrations of PAc-2 are illustrated in Figure 1. Curve (a) corresponds to ACP precipitation in the absence of PAc-2. As shown in curve (b), the addition of 20 ppm (parts per million) of PAc-2 to the calcium phosphate solution delays the onset of the transformation reaction. Increasing the inhibitor concentration from 20 to 55 ppm results in approximately ten fold increase in β and at 70 ppm polymer concentration, ACP phase was stabilized for at least 600 minutes. It is apparent from Figure 1 that β is greatly prolonged by increasing polymer concentration. It is interesting to note that similar observations relating to delay in ACP to HAP phase transformation have been noted in early investigations involving the influence of inhibitors such as polyphosphates, pyrophosphates, citric acid, and magnesium ion on the stabilization of ACP (17,18). In addition, Amjad (19), Lieu and Nancollas (20), and Sheehan and Nancollas (21), in their studies on the precipitation of calcium sulfate, calcium phosphate, calcium oxalate, and barium sulfate, also showed that crystallization of these sparingly soluble salts is preceded by an initial slow reaction.

To determine the effect of M_w (molecular weight) of PAc on the transformation reaction of ACP to HAP, the potency of poly(acrylic acids) of varying M_w (from 850 to 20 000) were compared as shown in Figure 2.

Figure 2. Induction time as a function of molecular weight of poly(acrylic acid)

The β versus PAc M_w profile as shown in Figure 2 clearly demonstrates that, under similar experimental conditions, inhibitory power of PAc increases with decreasing M_w

of PAc. For example, β values obtained for PAc-2 (M_w 2100), PAc-3 (M_w 6000) and PAc-4 (M_w 10 000) are 72, 40, and 3 minutes respectively, compared to 1 minute obtained in the absence of inhibitor. It is worth noting that the effect of PAc M_w on the stabilization of ACP phase observed in the present study is consistent with the observations made for CaF_2 , $CaSO_4 \cdot 2H_2O$, and $BaSO_4$ (13, 14, 22).

In order to investigate the role of ionic charge of the functional group (i.e., - $CONH_2$ (neutral), - SO_3H (anionic), - $\overset{+}{N}$ - (cationic)) present in the polymeric inhibitor, on the precipitation and subsequent transformation into HAP, a series of experiments were carried out under similar experimental conditions. Results summarized in Table 2 and illustrated in Figure 3 clearly show that polymers which are devoid of - $COOH$ group (i.e., poly(acrylamide), PAm; poly(sulfonic acid), PSa; and poly(diallyldimethyl ammonium chloride), PDa), exert poor inhibitory influence, compared to PAc-2, in inhibiting ACP to HAP phase transformation. Based on the β values, the overall ranking in terms of decreasing inhibitory power of homopolymers is: PAc >> PSa > PAm ~ PDa = control (no polymer).

Exp.	Inhibitor	Conc., ppm	β , min
1.	none	---	1
2.	none	---	1
3.	PAc-1	55	10
4.	PAc-2	5	1
5.	PAc-2	10	2
6.	PAc-2	20	7
7.	PAc-2	25	12
8.	PAc-2	30	15
9.	PAc-2	30	17
10.	PAc-2	40	28
11.	PAc-2	55	72
12.	PAc-2	70	600
13.	PAc-3	55	40
14.	PAc-4	55	10
15.	PAc-5	55	3
16.	PAm	55	2
17.	PSa	55	10
18.	PDa	55	3
19.	AA:HPA	55	42
20.	AA:SA	55	62
21.	AA:VAc	55	25
22.	AA:VOH	55	14
23.	PYP	10	7
24.	HEDP	10	17
25.	PBTC	10	13

TABLE 2. Induction times for the transformation reaction of ACP to HAP in the presence of inhibitor.

Figure 3. Induction times of various homopolymers with differing functional groups.

The effect of substituting sterically bulky groups onto PAc was also examined using copolymers of AA:HPA (acrylic acid : hydroxylpropyl acrylate); AA:PSa (acrylic acid: 2-acrylamido-2-methyl propane sulfonic acid); AA:VAc (acrylic acid:vinyl acetate); and AA:VOH (acrylic acid:vinyl alcohol). The results summarized in Table 2 and illustrated in Figure 4, clearly indicate that inhibitory activity of the copolymer increases with the size of the steric groups. It is evident from Figure 4 that the overall ranking of these copolymers in terms of decreasing effectiveness is:
AA:PSa > AA:HPA > AA: VAc > AA:VOH.

Figure 4. Induction times for various acrylic acid copolymers.

Inhibition of crystal growth of a number of sparingly soluble salts by PYP (pyrophosphate) has been the subject of numerous investigations. In biological calcification process, PYP ion has been found to be an effective crystal growth inhibitor for calcium oxalates and calcium phosphates systems (15, 23). Another class of compounds that proved to be effective in inhibiting calcification and mineral scale formation is the organic phosphonates. These inhibitors are characterized by the relatively inert P - C - P and P - C - N - C bonds which substitute for the hydrolyzable P - O - P bond (Table I).

ACP precipitation experiments were carried out in the presence of various phosphorous containing compounds. Results summarized in Table 3 and shown in Figure 5 indicate that the presence of low concentrations of phosphonates lengthen the induction time. For example, the β values obtained at 10 ppm of PYP, HEDP, and PBTC are 7, 13, and 17 minutes, respectively. Among the phosphorous containing inhibitors the order of effectiveness as inhibitors of ACP transformation reaction is: HEDP > PBTC > PYP.

Exp.	Inhibitor-I	Conc., ppm	Inhibitor- II	Conc., ppm	β , min
37.	PAC-2	30	HEDP	0	3
35.	PAC-2	0	HEDP	10	17
38.	PAC-2	30	HEDP	10	89
34.	PAC-2	0	PYP	10	7
39.	PAC-2	30	PYP	10	32
36.	PAC-2	0	PBTC	10	12
40.	PAC-2	30	PBTC	10	47

TABLE 3. Induction time (β) for phase transformation reaction of ACP to HAP in the presence of mixed inhibitors

The results of experiments to test the influence of mixed inhibitors (i.e., PAC and phosphonates) on the ACP to HAP transformation are summarized in Table 3. The data presented in Figure 5 clearly illustrate that the effect of PAC-2 plus phosphorous containing compound is to lengthen the induction time, i.e., large increase in the delay of onset conversion relative to the PAC-2 or phosphonates alone.

Figure 5. Induction times of various phosphorus-containing compounds and blends with poly(acrylic acid).

For example, β values obtained in the presence of 30 ppm Pac-2 and 10 ppm HEDP are 3 and 17 minutes respectively, compared to 89 minutes (> 400% increase in induction time) observed in the presence of 30 ppm PAc-2 and 10 ppm HEDP. It is interesting to note that similar observation have been made in studies involving the inhibition of ACP by mixture of Mg + polyphosphates and Mg + ATP. Amjad also reported similar synergistic performance of PAA and phosphonates in the precipitation of BaSO_4 and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (24).

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