Effect of L-Arginine, and L-Glutamic Acid in Solution on the Crystallization of Hydroxyapatite

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Statement of Purpose: Polar and charged amino acids (AAs) are main components of non-collagenous proteins, and are involved in hydroxyapatite (HA) mineralization in bone. These AAs are able to either promote HA mineralization by attracting calcium and phosphate ions in body fluids and increasing the local supersaturation or inhibit HA formation by binding to nuclei of calcium phosphate and preventing its further growth. They are also effective in modifying the morphology and crystalline structure of mineralized HA due to the electrostatic and stereochemical effects of their charged residues, especially carboxyl and phosphate groups. In this work, we investigate the effect of different AAs (L-arginine and L-glutamic acid) on the morphology and crystalline structure of HA and other calcium phosphates synthesized at room temperature and pH of 7.4. The AAs will be either in an aqueous solution or bound to a gold-coated silicon surface. In the latter case, we will produce highly controlled self-assembled monolayers (SAMs) to analyse the effect of AA density and ordering on HA mineralization. The purpose of this work is to determine the HA mineralization inhibiting or promoting effect of the investigated AAs, and explore the conditions that favour the synthesis of HA similar to the mineral phase of natural bone.

Method: The aqueous solutions of $CaCl_2$ and NaH_2PO_4 were prepared and the pH of the solutions was adjusted at 7.4 using the NaOH solution of 1 normality and the temperature of solutions was adjusted at 37°C. Both of these reactant solutions were mixed simultaneously and the pH of the reaction solution was kept constant using the 100 mM buffer solution of tris(hydroxymethyl)aminomethane. The summary of experimental conditions are shown in Table 1. The Ca/P ratio of samples was measured using the ion-coupled plasma spectroscopy (ICP) technique and the morphology of dried powder was investigated under scanning electron microscopy (SEM).

Table 1. Experimental conditions

Sample	$CaCl_2$ Conc. (mM)	NaH_2PO_4 Conc. (mM)	AAs Conc. (mM)	pH	Temp. °C	Time (hr)
A	3.33	2	0, 10	7.4	37	18, 24, 48
B	5	3	0, 10	7.4	37	0, 0.5, 1, 2, 24, 48

Results: Our results so far indicate that the effect of L-arginine and L-glutamic acid on the synthesis of HA is more significant when the reactants' concentrations are around the threshold required for HA precipitation at 37°C and pH of 7.4, i.e. 3.33 mM and 2 mM for $CaCl_2$ and NaH_2PO_4 solutions, respectively. In these conditions, the formation of a precipitate is significantly delayed from 1 hr to 2 hr and 1 day in the presence of 10 mM L-arginine and L-glutamic acid respectively. The ICP results show that the synthesized samples have calcium to phosphorous ratios very close to that of HA (1.67); therefore, it can be concluded that the powders produced here have structures very similar to hydroxyapatite irrespective of the experimental conditions are applied. However, the calcium to phosphorous ratios (Ca/P) for both A-Control and B-control series (samples without AAs) increase with increase of reaction time, and the Ca/P deviation from 1.67 (1.67-Ca/P) decreases for these samples. This may indicate that increases in reaction time enhance the formation of hydroxyapatite. However, neither L-Arg nor L-Glu show significant effect on formation of hydroxyapatite. Scanning electron microscopy (SEM) results (Fig. 1) show that both L-Glu and L-Arg amino acids resulted in the formation of nicely shaped micro size spherical particle consisting of plate like nano crystallites. However, the sample without AAs shows the randomly oriented crystallites in the mass of agglomerated particles.

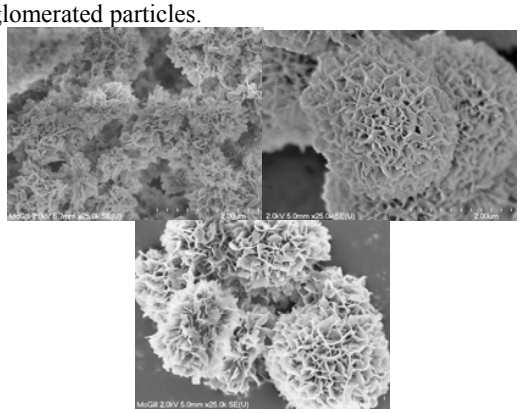

Fig. 1. The SEM images of sample A-Control (a), A-LGlu (b), and A-LArg (c) at the reaction [time of 2 days.

Conclusions:

The results show the significant effect of L-arginine and L-glutamic acid on the morphology and precipitation time of hydroxyapatite. The precipitation time was delayed in the presence of both L-Arg and L-Glu indicating the inhibitory effect of these AAs on the nucleation of HA crystals. Nicely shaped spherical particles consisting of oriented plate like nano crystallites were obtained in the presence of both L-Arg and L-Glu. The particles produced in the absence of AAs were the aggregates of randomly oriented crystals. For the future study, we will use a varieties of characterization techniques to investigate the different phases of calcium phosphate formed during the reaction and determine the time required for the HA to precipitate. Our objective is to investigate the kinetics of HA mineralization in the presence of AAs. Moreover, we will also investigate the effect of AAs bound to a surface on HA crystallization.