SCIENTIFIC PAPERS

Effects of collagen types II and X on the kinetics of crystallization of calcium phosphate in biomineralization*

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Received March 12, 2001; revised April 10, 2001

Abstract The effects of the components of cartilages matrix on the process of endochondral ossification and the kinetics of crystal growth of calcium phosphate have been studied in the presence of type II or X collagen. During the experiments, type I collagen was added as the seed material. FT-IR analysis shows that calcium phosphate crystallized on the surface of type I collagen was mainly hydroxyapatite. Both type II and X collagens could reduce the growth rate of calcium phosphate crystals, and the effect of type X collagen is more obvious. The reaction was in the fourth order in the presence of type II collagen. The results showed that type II or X collagen had the ability to make Ca^{2+} accumulate in the process of endochondral ossification, but has little effect on crystal growth and the product of biomineralization.

Keywords: type II collagen, type X collagen, biomineralization, kinetics.

Type II collagen is mainly secreted from chondrocytes in proliferation phase. The chondrocytes in hypertrophic phase synthesize collagen types X and II[1~3]. While type I collagen is secreted from chondroblast, and the matrix of bone containing type I collagen turns to ossification[4]. It has been reported that type II and X collagens are involved in biomineralization. The amount of C-propeptide of collagens II and X increases with the accumulation of Ca^{2+} on cartilage matrix[5]. However, the effect of type II or X collagen on the biomineralization remains obscure. In this study, a mimesis biomineralization experiment *in vitro* was designed and the kinetics of crystallization of calcium phosphate on the surface of type I collagen was investigated using the constant solution composition method[6] in the presence of type II or X collagen, and the effects of collagen types II and X on the biomineralization were evaluated.

1 Materials and methods

1.1 Materials

Type Icollagen was purchased from Sigma. Other reagents were of analytical grade (Beijing Laboratories). Type II collagen was extracted from chicken articular cartilage[7] according to Ref. [8].

1.2 Kinetics experiments

A constant solution composition method was used in this work. All experiments were performed at the temperature of 37℃, pH of 7.4, the ionic strength of 0.10 $mol \cdot L^{-1}$, and with bubbled nitrogen through the solution. Concentration of the seeds was chosen at 1 $mg \cdot mL^{-1}$. Preliminary experiment had determined that the stability of the supersaturated solution is over 6h. Based on this, metastable supersaturated solution of calcium phosphate was stabilized for 6h, followed by addition of certain amount of dry type I collagen to this solution as the seed material. When investigating the effect of type II or X collagen on the reaction, the supersaturated solution was stabilized for 6h with type II or X collagen added. The kinetics of calcium phosphate crystal growth was recorded automatically under conditions as listed in Table 1.

* Project supported by the National Natural Science Foundation of China (Grant No. 29871007)

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Table 1. The conditions for kinetics experiments of crystal growth

Sample	$T_{Ca^{2+}}$ (mmol·L^{-1})	T_P (mmol·L^{-1})	σ_{DCPD}	σ_{OCP}	σ_{HAP}	ΔG_{DCPD} (KJ·mol^{-1})	ΔG_{OCP} (KJ·mol^{-1})	ΔG_{HAP} (KJ·mol^{-1})
1	0.8	0.48	-0.56	0.62	8.18	1.84	-4.32	-22.34
2	1.0	0.60	-0.45	0.95	10.09	1.37	-5.99	-24.05
3	1.1	0.66	-0.40	1.11	4.02	1.17	-6.70	-25.07
4	1.4	0.84	-0.26	1.57	13.70	0.66	-8.47	-27.09

T, Total concentration; σ, supersaturation degree; ΔG, Jibbs function.

1.3 Analysis of IR spectra

The product was collected by centrifugation, washed with distilled water and then dried in air. Hydroxyapatite, type I collagen and the product were characterized by their IR spectra in the range of 4000~400 cm^{-1}, which were recorded by a Magana 750 FT-IR spectrometer using KBr pellets.

2 Result and discussion

2.1 Effect of the concentration of type I collagen seed on crystal growth rate

The crystal growth rate to the concentration of type I collagen seed material is plotted in Fig. 1. It can be seen that the rate of crystallization is proportional to the concentration of inoculating seed. When the seed concentration is below a critical value the growth rate is very slow. Once this concentration reaches above 0.1 g·L^{-1}, the growth rate increases rapidly.

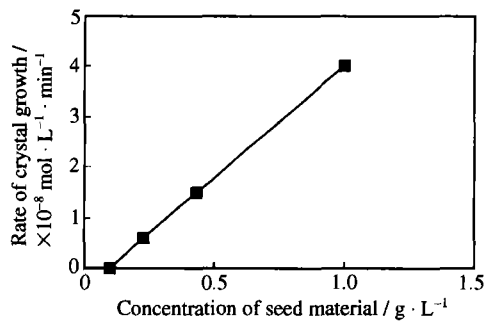

Fig. 1. Plot of the crystal growth rate to the concentration of seed material.

The crystal growth rate for insoluble salt system can be expressed as

$$R = kS\sigma^n, \quad (1)$$

and it is more common to express this by

$$\lg R = \lg kS + n\lg\sigma, \quad (2)$$

where R represents the crystal growth rate, k is the rate constant, S the effective surface area for crystal growth, n the reaction order and σ the supersaturation degree. Due to k is a constant, R is proportional to S when σ is fixed, and S is proportional to the concentration of seed material; therefore the reaction rate is proportional to the concentration of seed material.

2.2 Kinetics of the crystal growth of calcium phosphates in the presence of type II or X collagen

Fig. 2 shows that with the growth of calcium phosphate crystals in the presence of type II or X collagen, it is necessary to add Ca^{2+} and other components into the system to maintain the constant concentrations of each component in the system. Both type II and X collagens could reduce the growth rate of calcium phosphate crystals on the surface of type I collagen, and the effect of type X collagen is more obvious.

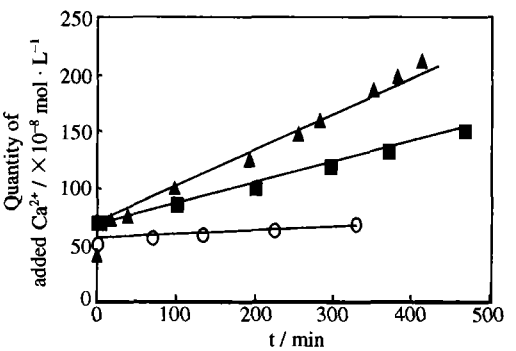

Fig. 2. Kinetics of crystal growth in the presence of collagens. $T_{Ca^{2+}}$ = 1.0 mmol·L^{-1}; T_P = 0.6 mmol·L^{-1}; ▲ control; ■ containing 0.04 g·L^{-1} type II collagen; ○ containing 0.06 g·L^{-1} type X collagen.

Fig. 3 illustrates that the rate of crystallization depends on the content of type II or X collagen, the more the collagen added, the slower the rate is. This is because both the collagens type II and X can bind strongly to Ca^{2+} which results in a reduced content of Ca^{2+} in the solution and a decreased supersaturation degree σ, thus in turn slowing the growth rate. And again, the binding of type X collagen to Ca^{2+} is stronger than that of type II.

2.3 Reaction order of calcium phosphate in the presence of type II collagen

Plots of kinetics of calcium phosphate crystal

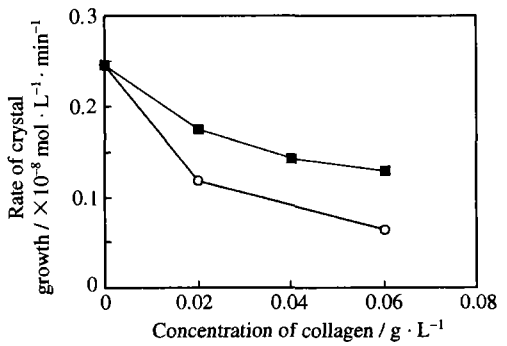

Fig.3. Relationship between content of collagen and crystallization rate.
$T_{Ca^{2+}} = 1.0$ mmol·L^{-1}; $T_P = 0.6$ mmol·L^{-1}; ■ type II collagen; ○ type X collagen.

growth in the presence of type II collagen for different supersaturation degrees are shown in Fig. 4. The experiment was conducted with 0.04 g·L^{-1} type II collagen . From the plots, the crystal growth rate R can be calculated.

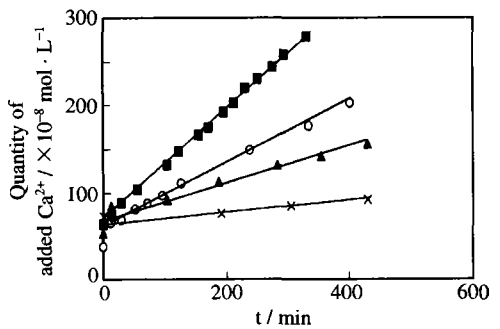

Fig.4. Plots of kinetics of crystal growth at different supersaturation degrees(σ) when containing 0.04 g·L^{-1} type II collagen . ■ $T_{Ca^{2+}} = 0.8$ mmol·L^{-1}, $T_P = 0.48$ mmol·L^{-1}; ○ $T_{Ca^{2+}} = 1.0$ mmol·L^{-1}, $T_P = 0.6$ mmol·L^{-1}; ▲ $T_{Ca^{2+}} = 1.1$ mmol·L^{-1}, $T_P = 0.66$ mmol·L^{-1}; × $T_{Ca^{2+}} = 1.4$ mmol·L^{-1}, $T_P = 0.84$ mmol·L^{-1}.

A linear relationship between logarithm R and logarithm of supersaturation is shown in Fig. 5. The

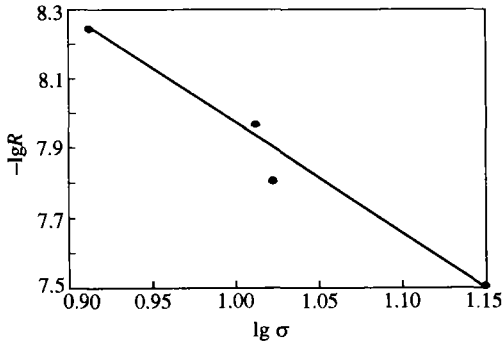

Fig.5. Plot of reaction order.

slope of the straight line is 4, which is close to that obtained in the absence of collagen type II in Ref. [9], suggesting that this process is surface nucleation controlled. The presence of type II collagen does not influence the mechanism of the reaction.

2.4 IR spectra of the synthesized product

The optical absorption frequencies of the synthesized product, hydroxyapatite and type I collagen detected by IR are given in Table 2. The synthesized product has similar absorption in the region above 1100 cm^{-1} to that of hydroxyapatite.

Table 2. IR wave number (cm^{-1}) for hydroxyapatite, type I collagen and the synthesized product

Sample	υ(cm^{-1})								
Hydroxyapatite	571vs	601vs	1041vs	1090vs					1458m
Type I collagen	570m				1164m	1239m	1336m	1404m	1452m
Product	563m	603m	1049s	1080s	1161s	1232s	1336w	1404w	1454m

vs, very strong; s, strong; m, middle, w, weak.

From the above results, we conclude that the binding of collagen types II and X with Ca^{2+} can cause the accumulation of Ca^{2+} in calcification sites in the process of cartilaginous ossification, this favors the biomineralization process, but has little effect on the mechanism of crystallization growth and the composition of the mineral product.

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