

Transmission Electron Microscopic Study on the Migration of Ca Ions in Ca-Deficient Hydroxyapatite during Thermal Decomposition

Masato TAMAI, Kiyoko SAKAMOTO,* Shunro YAMAGUCHI** and Atsushi NAKAHIRA

Kyoto Institute of Technology, Goshō-kaido-cho, Matsugasaki, Sakyo-ku, Kyoto-shi 606-8585

*Osaka Sangyo University, Nakagaito, Daito-shi, Osaka 574-8530

**ISIR, Osaka University, 8-1, Mihogaoka, Ibaraki-shi 576-0047

カルシウム欠損型アパタイトの熱分解過程におけるカルシウムイオンの移動に関する電子顕微鏡学的研究

玉井将人・坂本清子*・山口俊郎**・中平 敦

京都工芸繊維大学工学部物質工学科, 606-8585 京都市左京区松ヶ崎御所海道町

*大阪産業大学教養部化学, 574-8530 大東市中垣内 3-1-1

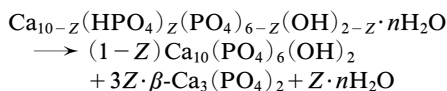
**大阪大学産業科学研究所, 576-0047 茨木市美穂ヶ丘 8-1

The migration of Ca ions during thermal decomposition of Ca-deficient hydroxyapatite (Ca-def HAp) involving the formation of β -TCP was discussed in the present paper. The migration of Ca ions at high temperature was examined by studying the thermal behavior of Sr ions in Sr-substituted Ca-def HAp using transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (TEM-EDX). Substitutions of Ca ions in Ca-def HAp by Sr ion were carried out by hydrothermal treatment in $\text{Sr}(\text{NO}_3)_2$ solution. The composition of samples obtained by hydrothermal treatment was identified as $\text{Ca}_{9.27}\text{Sr}_{0.27}(\text{HPO}_4)_{0.46}(\text{PO}_4)_{5.54}(\text{OH})_{1.54}$. In addition, it was considered that Sr ions would be in Ca(II) site in Sr-substituted Ca-def HAp lattice. Since the thermal behavior of Sr-substituted Ca-def HAp was almost the same as that of Ca-def HAp, the migration of Sr ions in Sr-substituted Ca-def HAp during thermal decomposition could be regarded as the same as that of Ca(II) ions in Ca-def HAp. By TEM and TEM-EDS analysis, it was revealed that Sr ions were detected only in β -TCP phase after thermal decomposition. Therefore, these results suggested that β -TCP phase would be formed by the priority migration of Ca(II) ions in Ca-def HAp. [Received July 23, 2004; Accepted October 26, 2004]

Key-words: Ca-deficient hydroxyapatite, Thermal decomposition, Migration, Transmission electron microscopy, Strontium ion, Substitution, Hydrothermal treatment

1. Introduction

Calcium-deficient hydroxyapatite (Ca-def HAp, $\text{Ca}_{10-Z}(\text{HPO}_4)_Z(\text{PO}_4)_{6-Z}(\text{OH})_{2-Z}$, $Z=0-1$) has lower Ca/P molar ratio than that of stoichiometric HAp (s -HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, hexagonal, $a=0.943$ nm, $c=0.688$ nm, space group; $\text{P6}_3/\text{m}$). Ca-def HAp is of greater biological interest than s -HAp, since the Ca/P molar ratio in bone is lower than 1.67. It has been suggested that Ca-def HAp plays important roles in several processes such as bone remodeling and bone formation.¹⁾ On the other hand, Ca-def HAp decomposes to s -HAp and β -tricalcium phosphate (β -TCP, $\beta\text{-Ca}_3(\text{PO}_4)_2$ /rhombohedral, $a=1.044$ nm, $c=3.738$ nm (hexagonal setting), space group; $R3c$) at more than 600°C as following reaction.^{2),3)}



The understanding of thermal decomposition is also very important for the preparation of biomaterials, since HAp/ β -TCP biphasic composites with high biocompatibility can be easily fabricated by sintering process.⁴⁾⁻⁶⁾ Various studies for thermal decomposition of Ca-def HAp have been carried out.⁷⁾⁻¹²⁾ However, atomic rearrangement or migration involving formation of β -TCP phase is not clarified, because the crystal structures of HAp and β -TCP are not closely related and are very complex.

Ca ions in HAp occupy two non-equivalent positions, which are named as Ca(I) and Ca(II). Ca(I) ions are on the

threefold axis at 1/3, 2/3, z (where z is usually <0.01) and three other equivalent site. Ca(II) ions are on the mirror plane at 0.01, 0.26, 1/4 and five other equivalent site. In addition, each Ca(I) ions and Ca(II) ions are known as screw-axis Ca and columnar axis Ca, respectively. s -HAp, therefore, can be written as $[\text{Ca}(\text{I})_4\text{Ca}(\text{II})_6](\text{PO}_4)_6(\text{OH})_2$. From intensity measurement of powder X-ray diffraction of solid solution of Sr ions substituted in HAp and also from analysis of ordering energies, Khudolozkin et al.^{13),14)} have shown that Sr ions in Sr-substituted Ca-def HAp occupy mainly Ca(II) and the degree of ordering energy decrease almost linearly with an increase in Sr content. Furthermore, Sudarsanan and Young^{15),16)} investigated the structural location of Sr ions partially substituting for Ca ions in HAp and determined from crystal structure refinements with X-ray data from synthetic single crystals having Sr/Ca ratio from 0.02 to 1.0. They revealed that Sr ions substituted only for Ca(II) on mirror plane but at a slightly different position.

The purpose of this study is to elucidate the migration of Ca ions in HAp during thermal decomposition of Ca-def HAp involving formation of β -TCP phase. Especially, we focused on the Sr ions having site selectivity for Ca sites in HAp and investigated local structure of Sr ions in order to clarify the behavior of Ca ions in Ca(II) site. Namely, the migration of Ca ions at high temperature was examined by studies of the thermal behavior of Sr ions in Sr-substituted Ca-def HAp using transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (TEM-EDX).

2. Experimental procedures

Ca-def HAp was prepared by hydrolysis of α -tricalcium phosphate (α -TCP Taihei Chemical Industries, Co., Ltd.). 0.01 mol of α -TCP were stirred for 48 h at 70°C in 1-octanol/ H_2O binary solvent (100 cm³/60 cm³). Then, the pH of binary solvent adjusted to 11.0 by ammonia aqueous solution. The details for the synthesis of the Ca-def HAp was referred to our previous paper.¹⁰⁾

Substitutions of Ca ions in Ca-def HAp by Sr ion were carried out by hydrothermal treatment. 0.5 mmol of Ca-def HAp and 50 cm³ of 0.5 mol/dm³-Sr(NO₃)₂ were mixed. The suspension was placed in double-walled vessel and the vessel was introduced into a drying oven and heated at 150°C for 1–24 h. All of Ca-def HAp and Sr-substituted Ca-def HAp after hydrothermal treatment were annealed at 600–850°C in air (heating rate: 5°C/min) for 6 h.

Quantitative analysis for the element in Sr-substituted Ca-def HAp was performed by X-ray fluorescence spectrometer/XRF (Shimadzu, XRF-1700, Rh K α radiation, 40 kV, 95 mA). Sr-substituted Ca-def HAp before and after annealing were identified by powder X-ray diffraction analysis (XRD: Cu K α radiation, 40 kV, 50 mA: RINT 2000/Rigaku Co., Ltd.). The XRD profiles were collected between 20–60° of 2θ angles with a step interval of 0.01° and scanning rate of 4°/min.

The Ca-def HAp and Sr-substituted Ca-def HAp before and after annealing were mounted on a Cu grid with a carbon coated holey film for transmission electron microscopy (TEM) by dripping the ethanol suspension of the sample. TEM observation and energy dispersive X-ray spectroscopy (EDS: Vantage/Noran Instrument) were carried out with JEOL JEM-2010SP (accelerating voltage: 200 kV).

3. Results and discussion

From results of XRD, IR and XRF analysis, the Ca-def HAp prepared by hydrolysis of α -TCP was identified as well-crystallized Ca-def HAp with Ca/P molar ratio was 1.58 (Table 1). Figures 1(a)–(f) show XRD patterns of Ca-def HAp annealed in the temperature range between 700–750°C. XRD patterns of Ca-def HAp annealed below 720°C showed only HAp phase. However, β -TCP phase was confirmed in the sample annealed at 720°C. In temperature range between 710–750°C, β -TCP phase and HAp phase are observed. The peak intensities from β -TCP become to strong with increasing annealing temperature. The XRD profiles of Ca-def HAp annealed above 740°C were almost the same as the sample annealed at 750°C. According to the results of XRD analysis, it can be concluded that thermal decomposition of Ca-def HAp occurred in narrow temperature range from 720 to 740°C.

TEM observation showed that the Ca-def HAp has a whisker-like morphology with elongated along *c*-axis with 2–5 μ m in length and 100 nm in diameter. Figures 2(a) and (b)

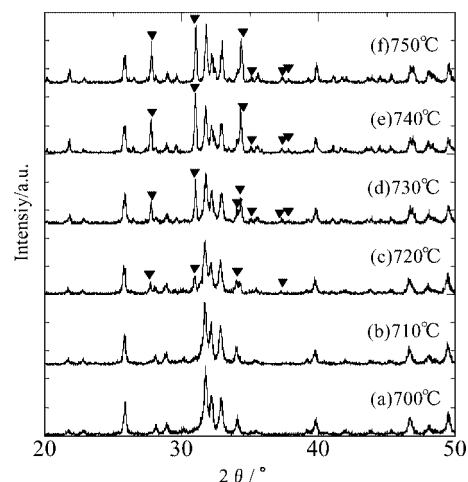


Fig. 1. X-ray diffraction (XRD) patterns of Ca-def HAp annealed at 700–750°C. No marked XRD peaks represent HAp and several peaks marked by solid triangles represent β -TCP.

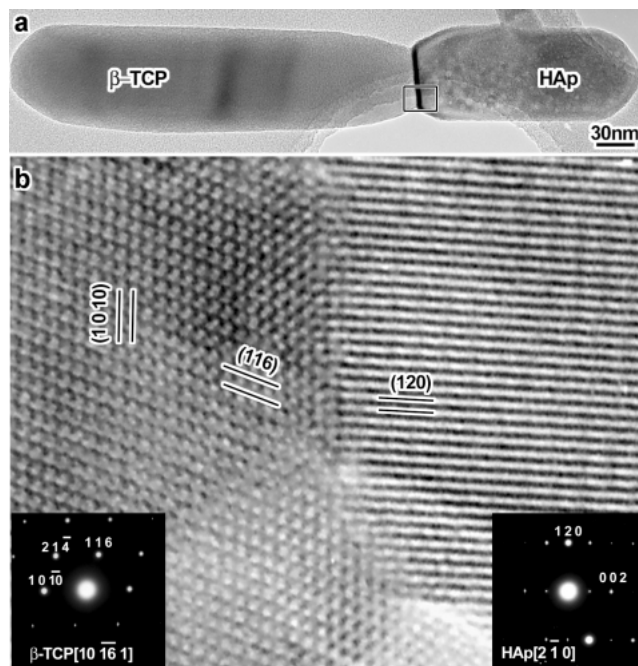


Fig. 2. TEM images of Ca-def HAp annealed at 750°C for 6 h. Low-magnified TEM image (a) and HRTEM image (b) of the area enclosed by a rectangle in (a), respectively.

Table 1. Compositions and Lattice Parameters of Ca-def HAp and Sr-Substituted Ca-def HAp in This Study

Sample	Ca/P	(Ca+Sr)/P ¹⁾	Sr/Ca ¹⁾	Chemical formula	<i>a</i> -axis ²⁾	<i>c</i> -axis ²⁾
Ca-def HAp	1.58	—	—	Ca _{9.48} (HPO ₄) _{0.52} (PO ₄) _{5.48} (OH) _{1.48}	0.944	0.689
Sr substituted Ca-def HAp	—	1.59	0.030	Ca _{9.27} Sr _{0.27} (HPO ₄) _{0.46} (PO ₄) _{5.54} (OH) _{1.54}	0.945	0.690

1) molar ratio, 2) nm.

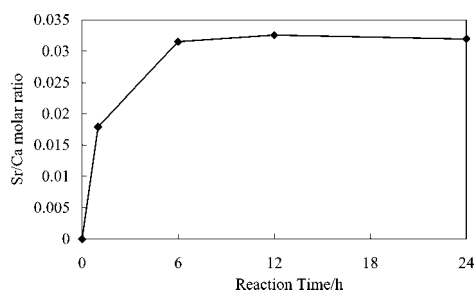


Fig. 3. Sr/Ca molar ratio in Ca-def HAp prepared by hydrothermal treatment in $\text{Sr}(\text{NO}_3)_2$ solution.

show a whole image of Ca-def HAp annealed at 750°C for 6 h and an HRTEM image of the area enclosed by a rectangle in (a), respectively. β -TCP phase was observed in a Ca-def HAp whisker. From an HRTEM image of the boundaries, there is no amorphous layer and distinct epitaxial relation between HAp phase and β -TCP phase (Fig. 2(b)). Here, the Ca/P molar ratio in s-HAp and β -TCP is 1.67 and 1.50, respectively. Therefore, it is obvious that the formation of β -TCP phase at high temperature was caused by the migration of Ca ions within single Ca-def HAp crystal. So, the investigation of the migration of Sr ion is expected to bring important information about the migration of Ca ions.

All XRD peaks of the samples obtained by hydrothermal treatment in $\text{Sr}(\text{NO}_3)_2$ solution were in agreement with those of the crystal structure of hydroxyapatite. The Sr/Ca atomic ratio in the sample as a function of the reaction time is shown in Fig. 3. Amount of Sr ions in Sr-substituted Ca-def HAp prepared by hydrothermal treatment increased with reaction time and the Sr/Ca atomic ratio was saturated to the maximum value of 0.03 in the sample treated for 24 h. The compositions and lattice parameters of Ca-def HAp prepared in the present study are summarized in Table 1. Ca/P molar ratio in Ca-def HAp was 1.58, while the $(\text{Sr} + \text{Ca})/\text{P}$ molar ratio in Sr-substituted Ca-def HAp prepared by a hydrothermal treatment for 24 h was 1.59. Lattice parameters in a -axis and c -axis of Sr-substituted Ca-def HAp slightly increase compared with Ca-def HAp. According to those results, Sr ions in solutions thought to be taken into HAp lattice during hydrothermal treatment and some of Ca vacancies in Ca-def HAp would be occupied by Sr ions. Therefore, the sample obtained by hydrothermal treatment could be identified as Sr-substituted Ca-def HAp, which the composition could be written as $\text{Ca}_{9.27}\text{Sr}_{0.27}(\text{HPO}_4)_{0.46}(\text{PO}_4)_{5.54}(\text{OH})_{1.54}$.

Sudarsanan and Young^{15),16)} reported that Sr ions was substituted only for Ca(II) site in the sample having Sr/Ca ratio from 0.02 to 1.0. Amount of Sr ion in Sr-substituted Ca-def HAp prepared in the present study are 0.03. So, it is considered that Sr ions will be in Ca(II) site in Sr-substituted Ca-def HAp lattice.

Amount of β -TCP phase in the samples annealed at various temperature, which calculated from integral intensities of X-ray diffraction peaks due to $(0\ 2\ 10)$ plane of β -TCP and $(2\ 1\ 1)$ plane,¹²⁾ are shown in Fig. 4. As shown in Fig. 4, Sr-substituted Ca-def HAp decompose to HAp and β -TCP, although final amount of β -TCP decrease with decreasing of Ca-vacancies. Since the thermal behavior of Sr-substituted Ca-def HAp was almost the same as that of Ca-def HAp, Sr ions in Sr substituted Ca-def HAp could be regarded as Ca(II) ions in Ca-def HAp for the migration during the thermal decomposition.

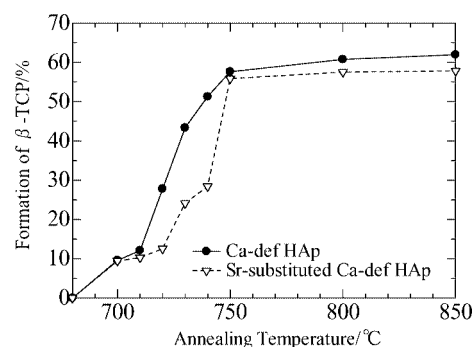


Fig. 4. Amount of β -TCP phase in the samples annealed at various temperature, which calculated from integral intensities of X-ray diffraction peaks due to $(0\ 2\ 10)$ plane of β -TCP and $(2\ 1\ 1)$ plane. Amount of β -TCP in Ca-def HAp and Sr-substituted Ca-def HAp are represent by solid line and broken line, respectively.

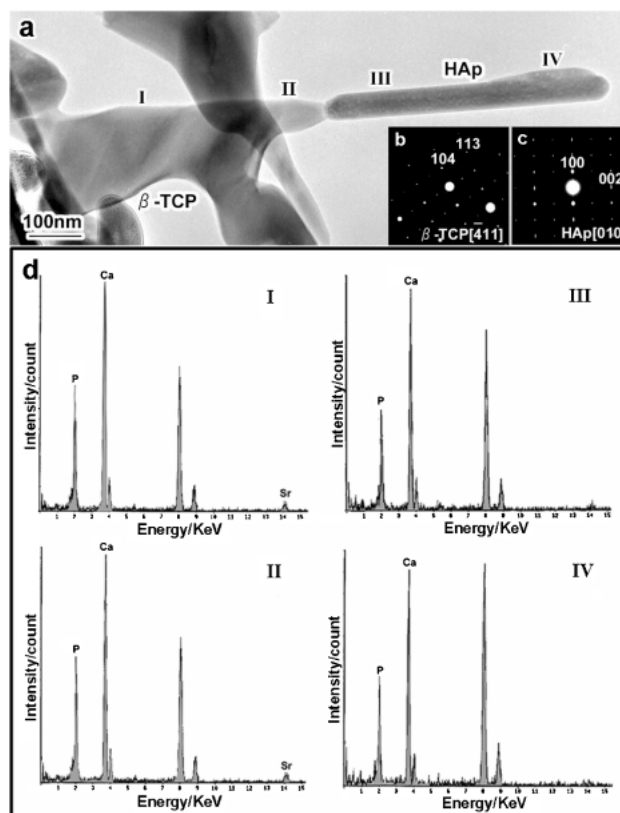


Fig. 5. TEM-EDS analysis of Sr-substituted Ca-def HAp (I) annealed at 750°C for 6 h. Low-magnified TEM image (a), SAED pattern of the region in right-hand (b) and left-hand (c) of the grain and TEM-EDS spectra (d) at the region of I, II, III and IV.

Figure 5 shows typical results of TEM-EDS analysis for Sr-substituted Ca-def HAp annealed at 750°C for 6 h. Figure 5 shows whole images of Sr-substituted Ca-def HAp (a) and selected area electron diffraction (SAED) pattern of the region in right-hand (b) and left-hand (c) of the grain and TEM-EDS spectra at the region of I, II, III and IV in Fig. 5 (a) are also shown in Fig. 5(d). From SAED patterns in Figs. 5(b) and (c), the region of I and II can be regarded as β -TCP, while III and IV are HAp. The result of TEM-EDS analysis show that Sr ions localized in β -TCP phase and HAp have little Sr ions. The fact that Sr ions detected only in β -TCP

phase suggest that β -TCP would be formed by the migration of Ca(II) ions in Ca-def HAp. In other words, Ca(II) ions in Ca-def HAp would contribute to the formation of β -TCP phase. The reason would be considered as following. Ca(I) ions and Ca(II) ions in HAp are in different energy state, which Ca(I) ions are more stable than Ca(II) ions.¹⁷⁾ Therefore, it is speculated that the migration of Ca ions in HAp at high temperature begin to occur in Ca(II) ions. It is considered that β -TCP phase would be related with the priority migration of Ca(II) ions. The detailed understanding in this study would bring to elucidation of atomic rearrangements during thermal decomposition process of Ca-def HAp.

4. Conclusion

The migration of Ca ions during thermal decomposition of Ca-def HAp involving the formation of β -TCP was discussed in the present paper. The migration of Ca ions at high temperature was examined by study of the thermal behavior of Sr ions in Sr-substituted Ca-def HAp using TEM and TEM-EDX. By TEM observation and TEM-EDS analysis, it is revealed that Sr ions are localized in β -TCP phase in annealed Sr-substituted Ca-def HAp. In addition, it is speculated that β -TCP phase would be formed by the priority migration of Ca(II) ions.

References

- 1) Posner, A. S., *Physiol. Rev.*, Vol. 49, pp. 760–772 (1969).
- 2) Monma, H., Ueno, S., Tsutsumi, Y. and Kanazawa, K., *J. Ceram. Soc. Japan (Yogyo-Kyokai-Shi)*, Vol. 86, pp. 590–596 (1978).
- 3) Gibson, I. R., Rehaman, I., Best, S. M. and Bonifield, W., *J. Mater. Sci. in Med.*, Vol. 12, pp. 799–804 (2000).
- 4) Hench, L. L., *J. Am. Ceram. Soc.*, Vol. 74, pp. 1487–1493 (1991).
- 5) Nakahira, A., Tamai, M., Sakamoto, K. and Yamaguchi, S., *J. Ceram. Soc. Japan*, Vol. 108, pp. 99–104 (2000).
- 6) Tamai, M., Miki, S., Pezzotti, G. and Nakahira, A., *J. Ceram. Soc. Japan*, Vol. 108, pp. 915–920 (2000).
- 7) Yubao, L., Klein, C. P. A. T., Vandemeer, S. and Degroot, K., *J. Mater. Sci. in Med.*, Vol. 5, pp. 263–268 (1994).
- 8) Monma, H., Ueno, S. and Kanazawa, K., *J. Chem. Tech. Biotechnol.*, Vol. 31, pp. 15–21 (1981).
- 9) Valdes, J. J. P., Lopez, J. O., Morales, G. R., Malagon, G. P. and Gortcheva, V. P., *J. Mater. Sci. in Med.*, Vol. 8, pp. 297–302 (1997).
- 10) Nakahira, A., Sakamoto, K., Yamaguchi, S., Kaneno, M., Takeda, S. and Okazaki, M., *J. Am. Ceram. Soc.*, Vol. 82, pp. 2029–2032 (1999).
- 11) Sakamoto, K., Okazaki, M., Nakahira, A. and Yamaguchi, S., *Bioceramics*, Vol. 10, pp. 241–245 (1997).
- 12) Ishikawa, K., Ducheyne, P. and Radin, S., *J. Mater. Sci. Mater. in Med.*, Vol. 4, pp. 165–169 (1993).
- 13) Khudolozkin, V. O., Urusov, V. S. and Tobelko, K. I., *Geochem. Int.*, Vol. 9, pp. 827–831 (1972).
- 14) Khudolozkin, V. O., Urusov, V. S. and Tobelko, K. I., *Geochem. Int.*, Vol. 10, pp. 266–270 (1973).
- 15) Sudarsanan, K. and Young, R. A., *Acta Cryst.*, Vol. B36, pp. 1525–1529 (1980).
- 16) Sudarsanan, K. and Young, R. A., *Acta Cryst.*, Vol. B30, pp. 1381–1386 (1974).
- 17) Terra, J., Jiang, M. and Ellis, D. E., *Philo. Mag. A*, Vol. 82, pp. 2357–2377 (2002).