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Analysis of Apatite in Phyma Calcified *in Vivo*

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The inorganic matter of calcinoma deposited morbidly in the human muscle was characterized by XRD, FT-IR, TG-DTA, optical microscope and SEM. The chemical analysis of it in calcinoma was precisely carried out by AAS, ICP-AES and ICP-MS. The calcinoma contained inorganic matter of about 40 % and organic matter of about 60 %. The amount of inorganic matter was less than that of natural bones. According to XRD and FT-IR, the inorganic matter was carbonateapatite. After heating of the sample up to 1200 ℃, the line of XRD was shifted by heating, but there were no decomposition. Carbonate ion partially existed in PO_4 site and fully existed in OH site without OH ion of the apatite structure. Elements other than Ca and P were detected from the chemical analysis, and Ca/P molar ratio of it was 1.98. It was suggested that the chemical composition of the apatite in calcinoma was $[Ca_{8.65}Mg_{0.18}Na_{0.33}X_n][(PO_4)_{4.36}(CO_3)_{1.63}Y_m][CO_3]$, where X=Al, K, Zn, Fe, Sr with n<0.01, and Y=SiO_4, SO_4 with m<0.01.
Key words; apatite, calcinoma, phyma, chemical analysis

1. INTRODUCTION

The pathological inorganic crystal deposits are sometimes observed in muscles as the ectopic calcification. The normal calcification in bones and teeth is well known as calcium hydroxyapatite formation, however, substances deposited in muscles are not precisely investigated in detail.

In this study, the substance deposited pathologically in muscles was focused only on inorganic matters and then it was analyzed precisely. The analysis is not only for elucidation of some disease but also for designing and preparation of advanced biomaterials like a scaffold of tissue engineering for cultured bones [1, 2].

2. EXPERIMENTAL PROCEDURE

The sample as phyma in the muscle of buttocks was removed from 68-year-old female who was diagnosed as a CREST (calcinosis, Raynavd's phenemenon, esophageal dysfuncton, sclerodactyly and telangiectasia) syndrome. In order to eliminate organic substance, the sample was ground and then soaked in aqueous ammonia with pH=10 at the room temperature for 12 h. After this treatment, it was dried at 100 ℃ for 24 h in air.

The sample was characterized by optical microscopy, scanning electron microscopy (SEM; JEOL JSM-25S, Japan), X-ray diffractometry (XRD; Rigaku Geiger flex, Japan), Fourier transform infrared spectroscopy (FT-IR; Perkin Elmer Spectrum 2000, USA), and thermal analysis (TG-DTA; Seiko Instruments Inc. TG/DTA32, Japan). The crystallinity of apatite was estimated by measuring FT-IR splitting bands of PO_4 at 600 cm^{-1} [3] with using Origin 6.1 as the peak fitting module (Microcal Software Inc., USA). Chemical composition was analyzed for the sample after heating at 900 ℃ by atomic absorption spectrometry (AAS; Hitachi Z-5310, Japan), inductively coupled plasma atomic emission spectrometry (ICP-AES; SPS7800 Seiko Instruments, Japan) and inductively coupled plasma mass spectrometry (ICP-MS; SPQ9000 Seiko Instruments, Japan).

3. RESULTS AND DISCUSSION

The removed phyma is shown in Fig. 1. The texture of this sample was non-homogeneous, because there were some highly calcified parts and lower calcified parts. The subject in the present investigation was the highly calcified parts.

The content of organic substances in the sample was measured by thermal analysis before elimination of them from the sample. According to TG-DTA (Fig. 2), there was large weight loss at the temperatures from 250 ℃ to 400 ℃ due to burning of organic substances. The weight loss means the loss of organic substances, therefore there were about 60 % organic and about 40 % inorganic substances in this sample. The content of organic matter of this sample was quite larger than that of bones [4].

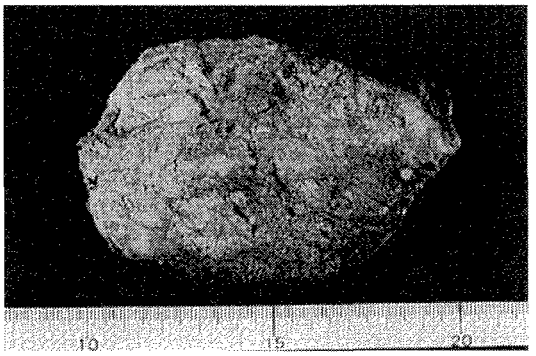

Fig. 1 The removed phyma from the muscle of buttocks.

The pattern of XRD for the sample after drying at 100 ℃ showed that no phases other than apatite were detected in any products studied (Fig. 3). This sample was incompletely crystallized apatite. After heating up to 1200 ℃ in air, the apatite did not decompose, but the line of XRD shifted by heating because of the compositional change. According to IR spectra of the sample after drying at 100 ℃, C-O, C=O and O-H as characteristic bands of organic substances were recognized (Fig. 4). The sample had CO_3^{2-} and little OH^- in the apatite structure. After heating at the temperatures above 500 ℃, OH^- was clearly found in apatite structure because of de-carbonation and substitution of OH^- for CO_3^{2-} in OH site of apatite. The crystallinity of apatite dried at 100 ℃ in air was about 25 % by the estimation method by measuring the spliting of PO_4 bands at 600 cm^{-1} [3]. The sample after heating at 800 ℃ had the crystallinity of apatite of over 80 %.

After heating at 900 ℃, the chemical analysis of the sample was carried out. The elements in this sample were shown in Table I. The Ca/P molar ratio of apatite in calcinoma was 1.98, which was quite larger than the value of stoichiometic apatite of Ca/P=1.67. The reason that Ca/P molar ratio of this sample was larger than that of stoichiometric value was substitution of CO_3^{2-} for the PO_4 site in apatite, therefore the amount of P was smaller than that of stoichiometric apatite. The amount of Mg and K was less than that in natural bones [5]. It seems that used aluminum hydroxide gel as a drug for medical treatment effected on the composition of apatite in calcinoma.

Table I Chemical composition of apatite in calcinoma analyzed by AAS, ICP-AES and ICP-MS.

Ca	Mg	Na	K	Sr	Zn	Fe	Al	P	Si	S
mass %										
40.8	0.52	0.90	0.02	0.01	0.02	0.01	0.02	15.9	0.03	0.40

In the past reports, there are some kinds of chemical formula for carbonate apatite and carbonate haydroxyapatite [6]. The formula of apatite on the present study was decided based on the below formula [7]. This one was characterized by the replacement of CO_3^{2-} on the both sites of PO_4 and OH.

$$Ca_{(10-x/2)}[(PO_4)_{6-x}(CO_3)_x][(OH)_{2-2y}(CO_3)_y]$$

According to FT-IR, there were little OH^- in the apatite structure, therefore y in above formula was regarded as 0. In general, various apatite compounds different in composition can be prepared by the replacement of elements for Ca site and PO_4 site [8-10], therefore the structural formula could be supposed as follows.

$$[Ca_{8.65}Mg_{0.18}Na_{0.33}X_n][(PO_4)_{4.36}(CO_3)_{1.63}Y_m][CO_3]$$

where, X=Al, K, Zn, Fe, Sr

Y=SiO_4, SO_4

n<0.01

m<0.01

The amount of CO_3^{2-} in this apatite was much more than that of natural bones.

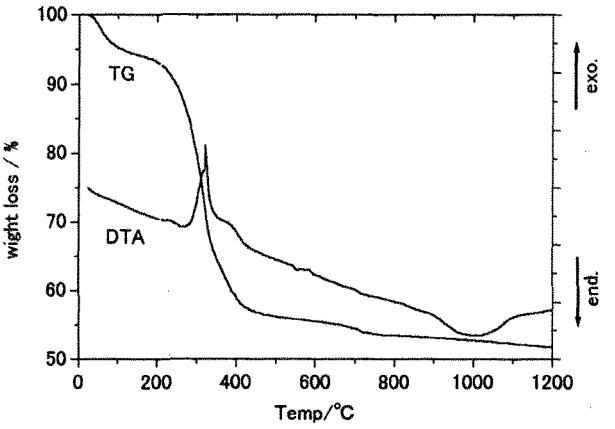

Fig. 2 TG and DTA curves of calcinoma.

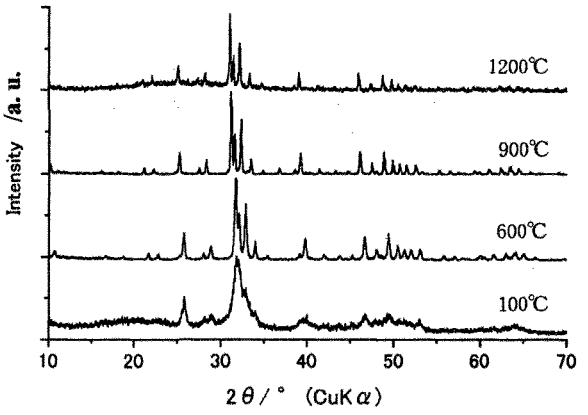

Fig. 3 Patterns of XRD of calcinoma after heating.

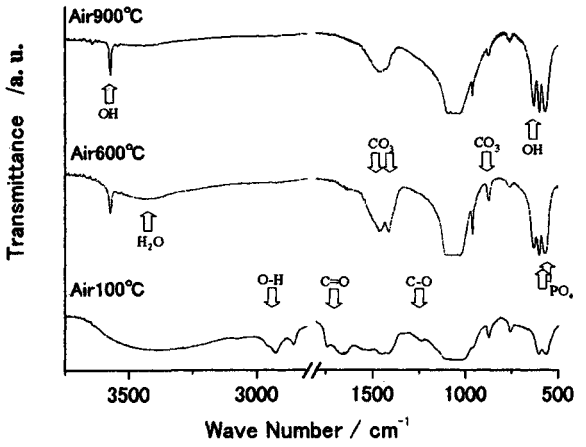

Fig.4 IR spectra of calcinoma after heating.

4. CONCLUSION

The inorganic substance in phyma retrieved from the buttocks muscle was carbonate apatite. According to the chemical analysis and solid state characterization, its structural formula was shown as follows.

$$[Ca_{8.65}Mg_{0.18}Na_{0.33}X_n][(PO_4)_{4.36}(CO_3)_{1.63}Y_m][CO_3]$$

where, X=Al, K, Zn, Fe, Sr, with n<0.01

Y=SiO_4, SO_4, with m<0.01

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REFERENCES

[1] R. Langer and J. P. Vacanti, *Science*, **260**, 920-926 (1993).

[2] K. Ioku, *Chem. Ind.*, **52** [5], 360-365 (2001).

[3] J. D. Termine and A. S. Posner, *Nature*, **211**, 268-270 (1966).

[4] B. K. Hall, "Bone Vol.1", Telford Press, Caldwell, New Jersey (1990).

[5] H. Aoki, "Medical Applications of Hydroxyapatite", Ishiyaku EuroAmerica, Tokyo, St. Louis (1994) pp.1-5.

[6] Y. Suetsugu , *J. Soc. Inorg. Mater. Japan*, **3**, 48-54 (1996).

[7] Y. Suetsugu, *Rigaku-Dennki J.*, **27** [2], 12 (1966).

[8] K. Ioku, M. Fujiki, K. Yanagisawa, N. Yamasaki, *Phosphorus Res. Bull.*, **2**, 27-32 (1992).

[9] K. Ioku, K. Masuda, J-W. Cho, S. Goto, "Apatite Vol.2", Jpn. Soc. Apatite Science, Tokyo (1997) pp.85-88.

[10] K. Byrappa, M. Yoshimura, "Handbook of Hydrothermal Technology", Noyes Publications, Park Ridge, New Jersey (2001) pp.287-314.

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