Some features of thermo-activated structural transformation of biogenic and synthetic Mg-containing apatite with β-tricalcium-magnesium phosphate formation

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Temperature transformation of biogenic and synthetic Mg-containing apatite with β-tricalcium-magnesium phosphate (β-TCMP) formation was investigated by X-ray diffraction. Samples were annealed in air at the temperature range from 600 to 1200 °C at intervals 100 °C and cooled down to room temperature. The appearance of β-TCMP was ascertained in samples annealed at 800 °C. As revealed, the relative amount of β-TCMP increases and Mg concentration in this phase decreases as the annealing temperature is raised. While this, the replacement degree of Ca by Mg in the β-TCMP lattice is the annealing temperature function and does not depend either on sample origin (biogenic/synthetic) or on initial Mg concentration. The results of present work together with other investigation data testify to a high thermally activated mobility of Mg both in structure of Mg-containing apatites and in the structure of β-TCMP formed after thermal decomposition. Obtained data can be used for new biomaterials design with varied prolongation of Mg released into the living biological tissue.

1 Introduction

For a long time the calcium phosphate materials attract attention of experts in the field of materials and solid state sciences due to their unique properties and big variety of promising and already realized applications [1-3]. In particular, biocompatible materials on a basis of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) are widely spread in surgical orthopaedics and dentistry. Synthetically prepared HA is studied intensively as the chemical and crystal-chemical analogue of the basic mineral compound of the bone tissue – nanocrystalline bioapatite (BA) [2-4].

The variety of cases in orthopaedic practice demands materials with a wide range of mechanical and physicochemical properties. For instance, necessary rate of biomaterial resorption and replacement by living tissue is determined by character and sizes of filling bone defect, expected mechanical loading, clinical indices, used therapy and many other factors. Presently producing technologies of biomaterials based on HA and TCP with various porosity, crystallinity, size and shape of granules and blocks continue to develop [2, 3, 5, 6]. One of the most promising way of biomaterial science is the HA modification by doping with the elements substituting Ca in cation sublattice leading to the directed change of the crystal-chemical characteristics. Insertion of Mg into HA structure is proved by the known facts concerning the activation of enzyme by Mg^{2+}

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ions in osteogenic cells [7]. In a skeleton of animals and human there is about half of total Mg of an organism, and in a bone mineral Mg is found at the limits of 0.2-0.6 wt. % [8-10]. Presence of Mg in apatite inhibits the growth of crystals and accelerates its solubility [2,3,8]. It should be mentioned that Mg in synthetic as well as biogenic apatites can be incorporated into the mineral lattice, and simply adsorbed on the surface of crystallites. The structural and functional role of Mg in biological minerals requires further consideration [2,4].

The production of biocompatible HA-based ceramics includes high-temperature processing as an important stage of the preparation technique, since the mechanical properties increase significantly with increasing of annealing temperature, while the solubility, i.e. biodegradation ability is reduced [4-6]. The temperature and character of thermal decomposition of both biological and synthetic calcium apatites with formation of non-apatitic phases are significantly determined by imperfections of structure and can be used as the indicator of degree of deviation from the stoichiometry caused by vacancies, impurity and other defects of a crystal [11]. It is well illustrated by temperature unstability of Mg-containing apatite [10,12]. The increase of Mg content in synthetic HA reduces temperature of phase decomposition with formation of magnesium substituted β-TCP (or β-TCMP) $(Ca,Mg)_3(PO_4)_2$, and the extent of HA conversion into β-TCMP on heat treatment appears strongly related to Mg amount of the apatitic solid phase [12-14]. In work [14] the conclusion is made, that whole Mg from nonequilibrium apatitic phase of the different HA samples enters (at T=900 °C) the crystal structure of β-TCMP. It is essential, that β-TCMP in comparison with pure β-TCP have smaller size of unit cell being in agreement with the difference in ion radius of Mg^{2+} and Ca^{2+} [2, 15].

The aim of this work is comparative research of thermo-induced phase transformation of synthetic and biogenic Mg-containing apatite with formation and development of β-TCMP. Identification of crystalline β-TCMP in various pathological calcifications [2, 16] shows high competitiveness of this structure to apatitic one. Although the Mg metabolism in biological environment is very complicated for modeling in laboratory systems, it is impossible to gain understanding of Mg functional role in conversion and development of calcium phosphates without detail information on adequate structural changes.

2   Materials and methods

Sample preparation   Synthetic HA was produced by a standard chemical-precipitation method [15] using aqueous solutions of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ brought up to pH 12 by adding ammonia. To prepare Mg-containing apatite, a solution of magnesium nitrate in a needed concentration was added to an original solution of calcium nitrate. The obtained products were kept in air for 24 h, then filtered, washed with distilled water, dried, and crushed. Thereafter, the samples were annealed in an electric furnace in air at temperatures of 600 °C, 700 °C, 800 °C, 900 °C, 1000 °C, 1100 °C and 1200 °C. The heating rate was about 0.05 K s^{-1} and the heating time at each fixed temperature was about 50 min. After the annealing the samples were slowly cooled down to room temperature in air. Finely divided powders were obtained by mechanical grinding in an agate mortar.

As BA samples we utilized the leg bones of laboratory rats that received Mg-containing preparations with eating for parallel experiments. In preparing the fragments of bone were boiled in water, mechanically cleaned and dried in air. The organic components were removed from the bone tissue by slow heating it on air up to 550 °C and holding it at this temperature for 40-45 minutes until the gas release ceased. Then the samples of biogenic apatite were subjected to a similar heat treatment and grinding as the synthetic samples.

Because of a small amount of powdered sample from the rat bone, we used as a sample holder an aluminium plate with the finely scratched surface. The sample powders were planished and pressed into the Al plate by a massive glass disk. The Al diffraction lines do not overlap with HA and β-TCMP lines and thus can be excluded easily.

Data acquisition and processing   X-ray diffraction data were collected by a Siemens D 5000 diffractometer using Ni-filtered CuK_α radiation and a conventional Bragg-Brentano geometry [17]. The details of the apparatus are described in earlier publications [18,19]. The specimens were first scanned in continuous mode over the range of diffraction angles (2ϑ) from 10 to 60 ° and then in stepwise mode (step size of 0.01°

held at 1.0 second/step) to have accurate fragment of pattern from 30 to 37 ° (2ϑ) containing the most intense picks of HA and β-TCMP.

    All procedures of profile processing, i.e. background separation, smoothing, Kα2 stripping, selection of the profile shape function and determination of the integrated intensity and 2ϑ-position of the diffraction lines were carried out using the X-ray diffraction software DIFFRAC^plus (Bruker AXS, Karlsruhe, Germany). All diffraction profiles were approximated by the modified pseudo-Voigt profile function. The total error in the peak fitting and calculation of the integrated intensity did not exceed 15 %. The crystalline phase identification was also done using the system software with reference to standard JCPDS cards. Quantitative evaluation of crystalline phase composition of heat-treated materials was performed using a preliminary constructed experimental dependence of the integrated intensity of basic diffraction lines from the content of HA and β-TCP in the calibrating biphasic mixtures. Here we assume, that β-TCMP is Mg-substituted β-TCP with the same structure (whitlockite) and at some limited range it is possible to replace the Ca^{2+} by Mg^{2+} ions without essentially changing the β-TCP structure [15].

3   Results and discussion

The data on thermal conversion of biogenic and synthetic Mg-containing apatite (at 900 °C) [20] and proof of surface localization of Mg in BA at temperatures preceding β-TCMP appearance (680-720 °C) [21] allow us to deduce the following regularities. At 680-720 °C the structurally bound Mg of bone apatite (approximately from one-half up to two-thirds of all Mg) passes into a labile state, being located on surfaces of crystals. The annealing at 900 °C leads to formation of β-TCMP in biogenic as well as in synthetic samples. If the whole of Mg takes part in the development of β-TCMP phase, then the quantitative estimation of Mg content in the forming two-phase system can be made using the XRD data only, i.e. β-TCMP-to-HA ratio and the amount of Mg replaced for Ca in the β-TCMP structure resulting from the shift of diffraction lines. However, it seems that the formation of β-TCMP is restricted by both insufficient content of Mg and small dimensions of the apatite crystals. It appears that at 900 °C the whole of Mg passes into the β-TCMP either at a big size of the BA crystals (at 700-900 °C the BA crystals are bigger than crystals of synthetic HA [19]) or at the high initial concentration of Mg in synthetic apatite. Table 1 contains the data illustrating noted features of β-TCMP formation at the conversion of biogenic and synthetic Mg-containing apatites under the heat treatment.

Table 1   Chemical and phase composition of biogenic and synthetic Mg-containing apatite samples annealed at 900 °C, (data from the earlier works [20, 21]).

Type of analytical data	Samples				
	Rats bone	Synthetic Mg-containing apatite			
	(10 samples)	MgHA-0	MgHA-4	MgHA-8	MgHA-16
Mg, wt. % (atomic-absorption spectr. data)	0.25-0.65	0.01	0.90	1.24	1.54
β-TCMP content, wt. % (XRD data)	7-28	not detected	8	37	78

In the present study for comparative examination we selected (i) the typical sample of Mg-containing BA and (ii) the sample of synthetic Mg-containing apatite with a maximum content of Mg entered at synthesis (MgHA-16). Thus, it is expected, that phase transformation of Mg-containing apatites will be restricted to the simplest case when Mg from nonequilibrium apatite phase has to be completely incorporated into the β-TCMP structure, and Mg located outside of β-TCMP lattice can be ignored.

    In this work the XRD characteristics of the products of thermal decomposition of Mg-containing synthetic and biogenic apatite were investigated at a wide annealing range from 600 to 1200 °C. At 600 and 700 °C both type of samples (biogenic and synthetic) give a smeared X-ray diffraction pattern of poorly crystalline HA (ref.: JCPDS no.9-432). Under the further annealing the diffraction patterns transformed demonstrating the β-TCMP emergence (at 800 °C) and development. There are no crystalline phases except of HA and β-TCMP in

the temperature range of interest. The β-TCMP-to-HA ratio at 800-1200 °C can be determined from intensity of strongest diffraction lines: (211) for apatite and (02$\underline{10}$) for β-TCMP (Fig. 1). A high accuracy of this technique for the determination of phase proportions in biphasic system (HA and β-TCP) was proved in several reports (e.g. [22]).
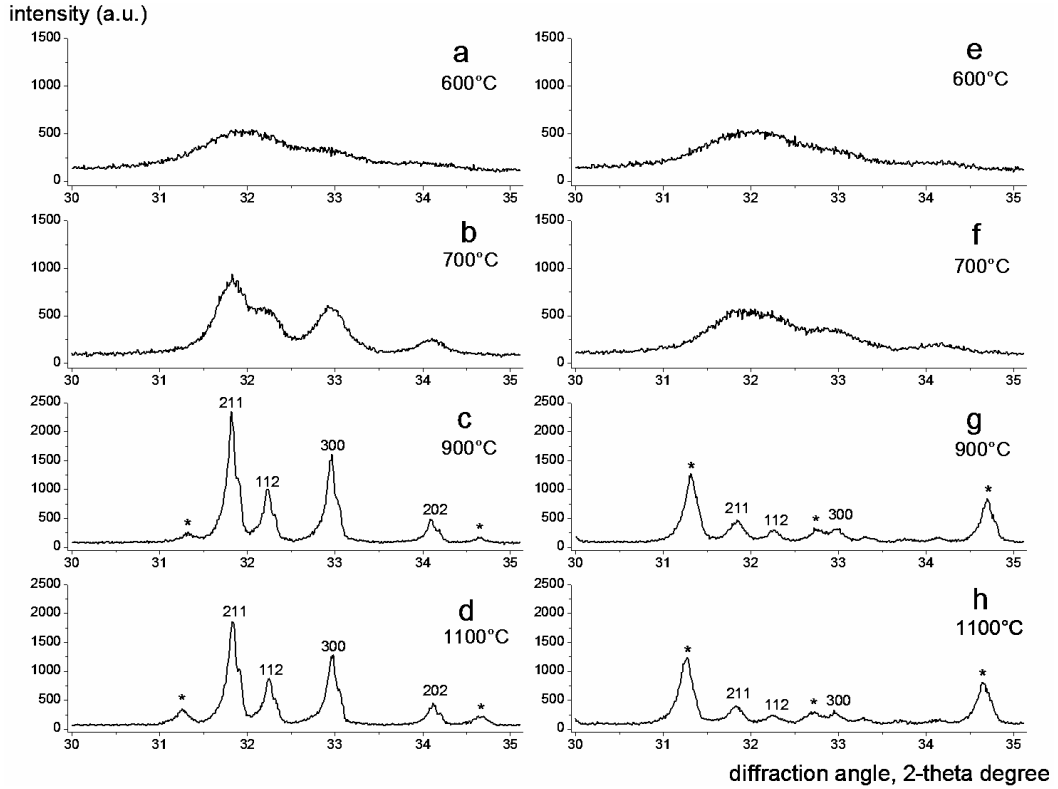

Fig. 1 Fragments of X-ray diffraction patterns of biogenic (a,b,c,d) and synthetic (e,f.g,h) apatites after heating to different temperatures; (the lines marked with Miller indices belong to HA, and ones marked with asterisk belong to β-TCMP, the strongest diffraction line (02$\underline{10}$) for β-TCMP is seat near 31.2 ° 2θ).
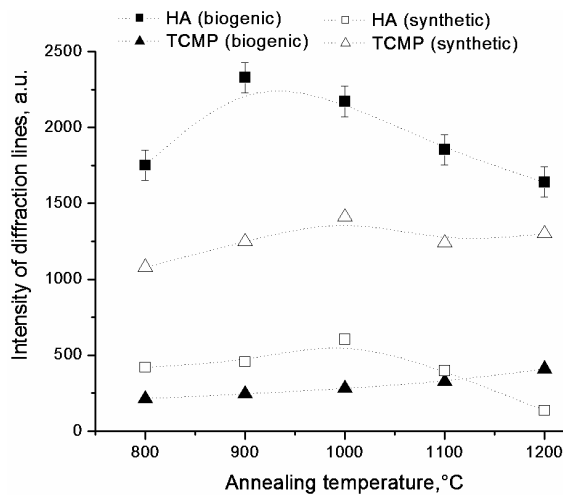

Fig. 2 Intensity of the strongest diffraction lines: (211) for apatite and (02$\underline{10}$) for β-TCMP versus annealing temperature.
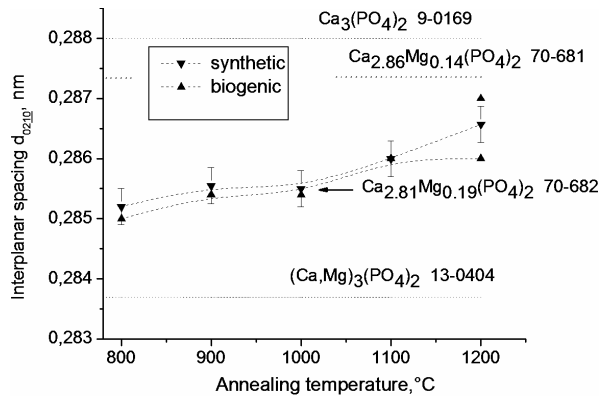

Fig. 3 Experimental interplanar spacing d$_{02\underline{10}}$ versus annealing temperature.

It is obvious (Fig. 2), that the expand of β-TCMP at 1100-1200 °C is due to consumption of apatite phase, but at lower temperatures apatite line intensity change is significantly determined by recrystallization which proceeds faster in biogenic samples [19].

It is essential, that the rise of annealing temperature causes the increase of interplanar spacing d_{0210} of β-TCMP and this change independently of formed β-TCMP amount, viz. Mg concentration in a mix of phases or in an initial sample (Fig. 3), is almost the same for all samples of biogenic and synthetic apatite. From the obtained data it follows, that the composition of forming β-TCMP changes during the temperature evolution, thus the amount of Mg substituting for Ca decreases, as the β-TCMP percentage is raised.

From the reference data of Joint Committee on Powder Diffraction Standards (JCPDS) the several compounds with composition of $Ca_{3-x}Mg_x(PO_4)_2$ are known (Table 2, Fig. 3). At the same time, there are data [15, 23-26] suggesting the continuous character of Mg for Ca substitution in $Ca_{3-x}Mg_x(PO_4)_2$ (up to 14 % cationic positions, total amount of Ca atoms + Mg atoms = 100 %).

Table 2 Referenced interplanar spacing d_{0210}, calculated from equivalent formula percentage of Mg substituted for Ca (Ca atoms + Mg atoms = 100 %) and Mg content (at. % and wt. %) in $Ca_{3-x}Mg_x(PO_4)_2$.

References (JCPDS no.)	Chemical formula (idealized composition)	d_{0210}, nm	Percentage of Mg substituted for Ca in β-TCMP, at. %	Content of Mg in β-TCMP	
				at. %	wt. %
9-0169	$Ca_3(PO_4)_2$	2.2880	0	0	0
70-681	$Ca_{2.86}Mg_{0.14}(PO_4)_2$	2.8734	4.67	1.08	1,09
70-682	$Ca_{2.81}Mg_{0.19}(PO_4)_2$	2.8549	6.33	1.46	1.48

Also it is known [15, 27], that the rhombohedral unit cell of β-TCMP as well as β-TCP corresponds to structure $7[Ca_{3-x}Mg_x(PO_4)_2]$, i.e. consists of 21 cations and 14 PO_4 groups. This structure can be shown as two types of columns running parallel to the **c**-axis («A» and «B») and filled with phosphate tetrahedra PO_4^{3-} and Ca^{2+} ions in a different way. In the «B» columns there are three equivalent structural positions of cations occupied by Ca^{2+} ions only (Ca1, Ca2 and Ca3). The «A» columns have two various Ca^{2+} positions accessible for Mg^{2+} (Ca4 and Ca5). At the gradual substitution of Ca^{2+} by Mg^{2+} on Ca5 sites (up to 9.5 % positions) the lattice constant along the **a**-axis and the **c**-axis decreases monotonously. These linear dependencies are broken due to the filling of the Ca4 sites by Mg^{2+} while Mg content in β-TCMP increases still further [15]. Thus, at some limited range of Mg concentration in β-TCMP (at least over the range 5-7 % of cation sites), the Vegard's law is valid and it is possible to estimate the degree of Mg^{2+} replacement for Ca^{2+} using the structural parameters, such as d_{0210} (Fig. 4).

Table 3 Changes in degree of Mg substituted for Ca in β-TCMP, β-TCMP content and calculated Mg concentration (in HA + β-TCMP phases mixture) during the thermal transformation of biogenic and synthetic Mg-containing apatite.

Samples	Type of data	Temperature of annealing, °C		
		800	900	1100
	Degree of Mg substituted for Ca in β-TCMP (S), at. % ($Ca^{2+}+Mg^{2+}$=100 %)	6.7	6.3	5.8
BA from rat bone (typical sample)	β-TCMP content (N), wt. %	14.2	15.2	18.4
	C_{Mg}, wt. % (calculated from XRD data)	0.22	0.23	0.25
Synthetic Mg-containing apatite (MgHA-16)	β-TCMP content (N), wt. %	74.4	78.0	78.5
	C_{Mg}, wt. % (calculated from XRD data)	1.18	1.16	1.07

For all samples calcinated at 900 and 1000 °C the experimental value of d_{0210} almost coincides with the reference data for $Ca_{2.81}Mg_{0.19}(PO_4)_2$ (JCPDS № 70-682) (Fig. 3). Extrapolation and interpolation of d_{0210} value for samples calcinated at 800 and 1100 °C (Fig. 4) give us the appropriate amount of Mg replaced for Ca (Table 3). As we can see, obtained data do not fall outside the validity limits of Vegard's law (< 9.5 %). The

content of Mg in calcinated samples (C_{Mg}) listed in table 3 was calculated by the procedure described earlier [20], using empirical formula included experimental XRD data, i.e. the degree of Mg replacement for Ca in the β-TCMP (S) and the percentage of β-TCMP in HA + β-TCMP system (N):

$$C_{Mg} = 2,36 \cdot 10^{-3} \cdot N \cdot S.$$

The values of C_{Mg} for biogenic samples change scarcely with annealing temperature and remain in a concentration range known from the elemental analysis [9,21] (0,2-0,6 wt. %), while for the synthetic samples the values of C_{Mg} revealed some decrease with increasing annealing temperature. Furthermore, the value of C_{Mg} at 900 °C calculated from the structural data (1.16 wt. %) (Table 3) is less than that determined by atomic-absorption spectroscopy (1.54 wt. %) (Table 1). These facts indicate that, in the synthetic apatite the formed β-TCMP does not involve all Mg of initial sample, but part of Mg is simply adsorbed on the surface of crystals. Moreover, it seems that Mg bounded in a lattice of β-TCMP can migrate onto the surface as the annealing temperature increases. The partial surface location of Mg in synthetic samples was confirmed by the atomic-absorption spectroscopy showing the temperature loss of Mg [20]. On the contrary, as seem for the biogenic sample the whole of Mg retained in lattice of β-TCMP.
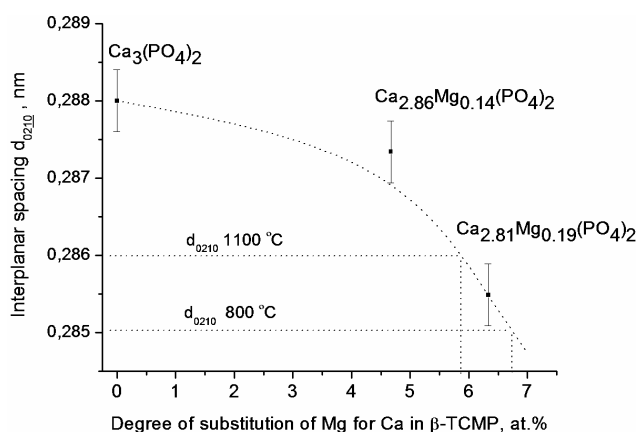

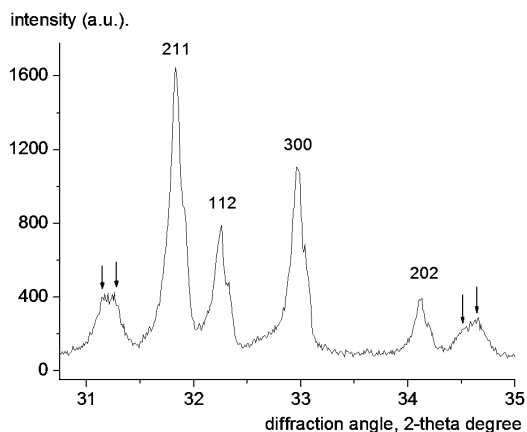**Fig. 4** Interplanar spacing d_{0210} versus degree of substitution of Mg for Ca in β-TCMP (at.%).

Fig. 5 Fragment of X-ray diffraction pattern of biogenic sample annealed at 1200 °C; (Miller indices mark HA lines, arrows mark broad lines which belong to β-TCMP with different degree of Mg substituted for Ca).

According to series of work [10,13,14], for the different biogenic samples the extent of Mg substituted for Ca in the formed β-TCMP is almost the same and accounts for about 7-8 at. %, while for synthetic samples the authors point out that this rate is about 12-13 at. %. From our data it follows, that degree of Mg substituted for Ca in β-TCMP is a bit lower (6.7-5.8 % at 800-1100 °C), is believed to decrease with increasing of annealing temperature and does not depend either on the origin of samples (biogenic/synthetic) or on initial concentration of Mg.

The existence of phases $Ca_{3-x}Mg_x(PO_4)_2$ with some fixed value of **x** can suggests stabilization of the structural states corresponding to these concentration ratio, but do not contradict the existence of continuous line of structures with a changing degree of Mg substituted for Ca.

It is worth mentioning that the formation of β-TCMP from the biogenic sample has another feature, namely, at 1200 °C the β-TCMP formed in BA split onto two components possessing different degree of Mg substituted for Ca (Fig. 5). It is shown in figure 3 by two different values of interplanar spacing for biogenic sample at the 1200 °C.

For better understanding of crystallographic features of β-TCMP formation, other techniques such as XRD line broadening analysis and elemental analysis are required to clarify the effects of crystallite size and

chemical composition on the process. Furthermore, the complete data on Mg concentration will be useful to estimate accuracy and restrictions of Mg percentage determination in initial Mg-containing apatite by means of XRD investigation of phase mixture (HA + β-TCMP) formed under annealing.

4   Conclusions

Temperature evolution of HA and β-TCMP mix formed as the result of the phase decomposition of Mg-containing biogenic and synthetic apatites was investigated by X-ray diffraction. The interplanar spacing d_{0210} and relative amount of the β-TCMP phase were determined in the annealing temperatures interval from 800 to 1200 °C. Obtained data shows that the β-TCMP amount increases owing to HA decreasing and the percentage of Mg in β-TCMP lattice decreases as the annealing temperature is raised. The main interest is the fact that the amount of replacement of Ca by Mg in β-TCMP lattice does not depend either on initial Mg concentration or on the sample origin (biogenic/synthetic) but is the monotonously decreasing function of annealing temperature.

The percentage of Mg in HA + β-TCMP phase mix was estimated by the XRD data and compared to the elemental analysis data of the initial samples. These findings indicate that β-TCMP forms with whole initial Mg rather at smaller specific surface of the apatite crystals that is typically for BA at 700-900 °C.

The present results together with our previous data [20, 21] and the findings in the literature give us the evidence of high thermally activated mobility of Mg in structure of biogenic or synthetic Mg-containing apatites and formed β-TCMP. Apparently, such mobility of Mg has the important physiological significance, while the similar processes *in vivo* at body temperature have different nature and require special research. As seen, Mg mobility in thermal treated products of Mg-containing apatites can be adjusted via annealing. This feature can be used in design of new biomaterials with a varied prolongation of Mg release into the biological environment.

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