Growth and optical microscopy observation of the lysozyme crystals

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Abstract

The little single lysozyme crystals in the capillary after 15 days of growth process with average size $0.1\times0.1\times0.16mm^3$ were obtained. It was shown that lysozyme crystals are optically anisotropical and birefringence along *a* axis is $\Delta n=(2.2\pm0.5)\times10^{-3}$ in visible spectrum region. From the measurements of crystallographic angles follows that on the {001} faces angles equal $\alpha=81^o$, $\beta=99^o$. On the sexangle faces angles equal $\varepsilon=100^o$, $\phi=140^o$ and $\gamma=120^o$. On the base of obtained results the lysozyme crystal habit is constructed. It was shown that this habit corresponds to 222-point group of symmetry. Little birefringence was observed along *c* axis that means that in crystals small strains exist that could be connected as with growth remain strains as well as with existing in the lysozyme crystals ferroelastical phase transition with symmetry lowering 422→222.

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Introduction

Biological crystals belong to the quite new class of materials that is called "soft state materials" [1]. Among them there are lysozyme [2], meoglobin and haemoglobin [3], insulin crystals [4] etc. Unfortunately the physical properties of the biological crystals are not studied yet. This is connected with the fact that it is very difficult to grow large, perfect crystals. A lot of papers are devoted to the growth technology and structural studying of biocrystals (see example [5]). Never the less the investigations [1] show the presence of the ferreoelectricity in the L-alanine crystals. As about optical properties of the biocrystals, we did not find any dates in the scientific literature. From other side these crystals could possess unique optical properties due to the unusually large chiral biological molecules and unusually large lattice parameters.

The structure of biocrystals strongly depends on the growth conditions. The process of the growth of biological crystals from the biological macromolecules depends on different factors such as: the ionic strength, temperature and concentration of acetate buffer. It means that the growth conditions are quite complicated. For example, frequently biocrystals are grown in the condition of the microgravitation on the space-ships [6], by gel method [7] or with the help of centrifuge [8] etc. The aim of present studying is to grow the biocrystals of optical quality by more simplify method and studying these crystals with the help of the optical microscope. Lysozyme was selected for our investigation, as well as these crystals are one of more stable in the surrounding conditions.

Crystal growth

The lysozyme crystals were grown at the temperature T=17^{o}C from 5.5% (w/w) protein solutions at pH 4.7 crystals growth chambers were a glass petri dish (50mm in diameter and solution were 3-5mm in depth) and a thin glass capillary of 1.5mm diameter. The lysozyme polycrystals thick film is obtained at the temperature 25-30^{0}C in a glass petri dish within few hours.

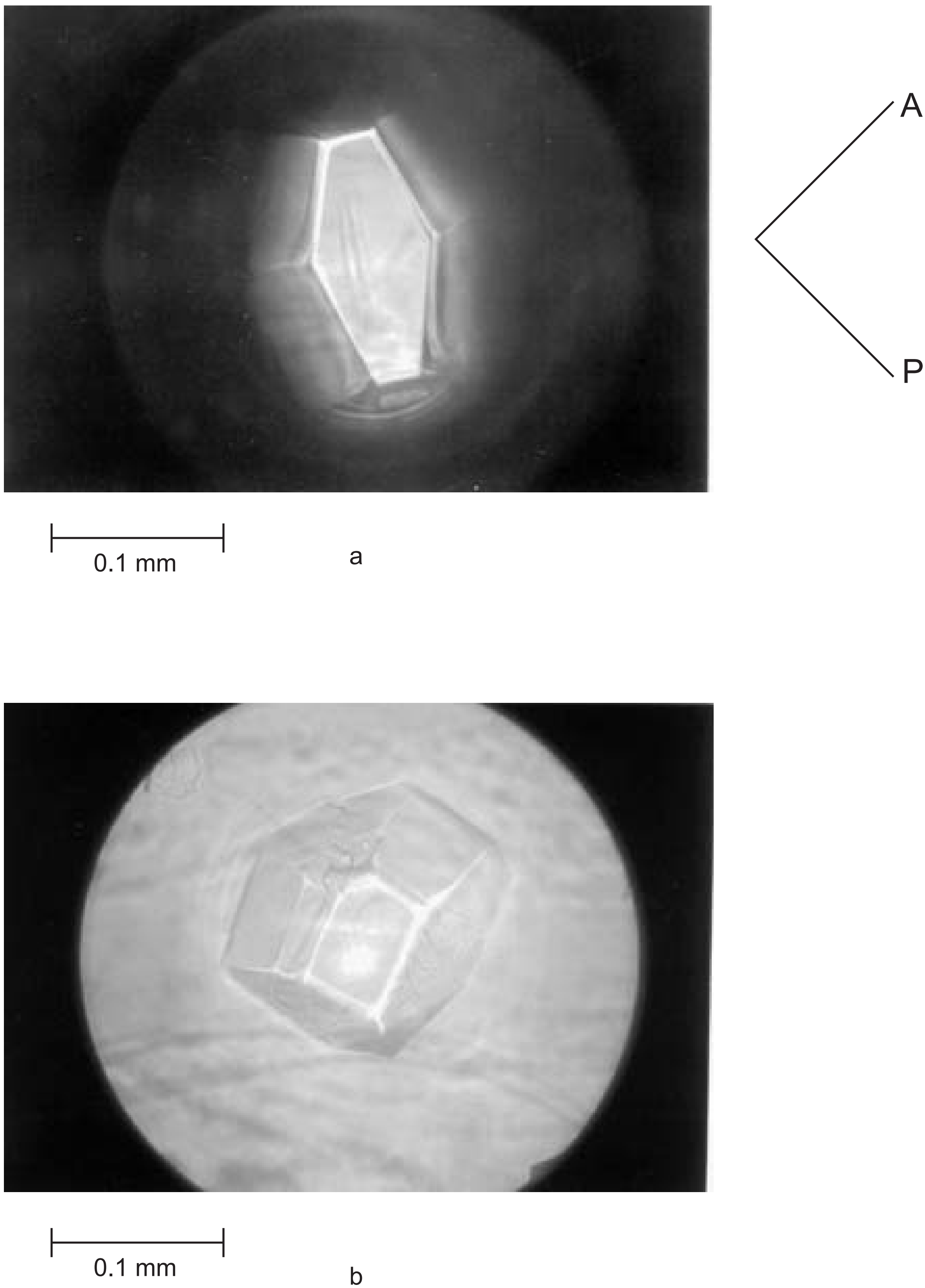

Fig.1 Lysozyme crystals: a)under the crossed polarizes in diagonal position (sexangle face); b)(001)-face

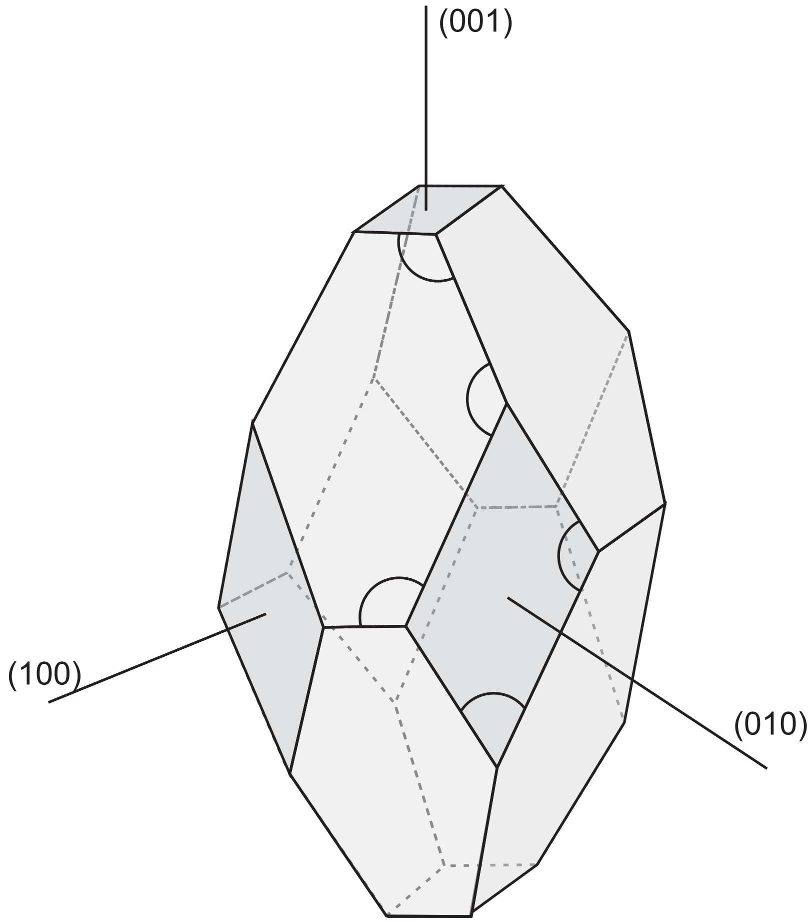

Fig.2.Habit of the lysozyme crystals.

The first seed crystals appear in capillary after five days of the growth process. After nucleation and growth of the crystals at different locations in the capillary after 15 days of growth process the little single crystals with average size $0.1\times0.1\times0.16$ mm^3 were obtained.

Optical microscopy observation of the lysozyme crystals and discussion

As it is visible from Fig.1,a crystals are longer along *c* direction and shouter along *a* direction. Between crossed polarizes lysozyme crystals show the optical anisotropy and extinction position coincide with the principle crystallographical axes *a* and *c* in all samples. On the base of the observation of the interference colours in crossed (lightly gray) and parallel (lightly brown) polarizors one can estimate the value of the birefringence - $\Delta n=(2.2\pm0.5)\times10^{-3}$ in visible spectrum region. More over, lysozyme crystals can not belong to cubic syngony as well as they are optically anisotropical and can not belong to syngonies lower then orthorhombic because in this case the turning of optical indicatrix around crystallographical axes should be observed. From the measurements of crystallographical angles follows that on the {001} faces angles equal $\alpha=81^o$ and $\beta=99^o$ (Fig.1,b). Since this direction could coincide with two fold symmetry axis. There are six such trapezoidal faces - (100), ($\bar{1}00$), (001), ($00\bar{1}$), (010) and ($0\bar{1}0$) that coincides under the rotation of crystal on 180^o. Existing of trapezoidal faces that are perpendicular to <001>, <100> and <010> directions mean that lysozyme crystal do not possess centre of symmetry and the symmetry mirror planes. On the sexangle faces angles equal $\varepsilon=100^o$, $\phi=140^o$ and $\gamma=120^o$ (Fig.1,a)

On the base of obtained results one can construct the lysozyme crystal habit (Fig.2). This habit corresponds to 222-point group of symmetry. Little difference in the light intensity propagated along <001> direction in crystals that are between crossed polarizes at the rotation of samples around *c* direction means that the lysozyme crystals are not far from 422 point group of symmetry and they can possess ferroelastical phase transition with symmetry lowering 422→222.

Conclusions

1.The little single lysozyme crystals in the capillary after 15 days of growth process with average size $0.1\times0.1\times0.16$mm^3 were obtained.

2.It was shown that lysozyme crystals are optically anisotropical and birefringence along *a* axis is $\Delta n=(2.2\pm0.5)\times10^{-3}$ in visible spectrum region.

3.From the measurements of crystallographical angles follows that on the {001} faces angles equal $\alpha=81^o$ and $\beta=99^o$. On the sexangle faces angles equal $\varepsilon=100^o$, $\phi=140^o$ and $\gamma=120^o$.

4.On the base of obtained results the lysozyme crystal habit is constructed. It was shown that this habit corresponds to 222-point group of symmetry. Little difference in the light intensity propagated along <001> direction in crystals that are between crossed polarizes at the rotation of samples around *c* direction. That means, that the lysozyme crystals are not far from 422 point group of symmetry and they can possess ferroelastical phase transition with symmetry lowering 422→222.

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