Structural Phase Transition in Lysozyme Single Crystals

I.Teslyuk, Yu. Nastishin and R.Vlokh

Institute of Physical Optics, 23 Dragomanov str., 79005 Lviv, Ukraine
e-mail: vlokh@ifo.lviv.ua

Received: 20.12.2004

Abstract

Polarization microscopy observations of the lysozyme single crystals in the 4°C–40°C region are represented. The interference colours of the crystal texture observed between crossed polarizers drastically change at T_C=11 °C unambiguously displaying a phase transition. The temperature dependence of the birefringence deduced from these observations indicates that the phase transition is of strongly first order. The low-temperature (T<11 °C) phase intensively scatters light. The phase transition is accompanied by the deformation (about 5%) of the crystal. The observed textural changes are reversible in temperature. We argue that the phase transition is of a ferroelastic type and the low-temperature (T<11 °C) phase might be of a ferroelastic type with symmetry 222 or one of two ferroelectric-ferroelastic types of symmetries 2 or 1.

PACS: 78.20.Fm, 77.84, 64.70.Kb

Key words: biocrystals, lysozyme, phase transition, ferroelasticity, optical birefringence

Introduction

Experimental characterization of biological single crystals is one of the most powerful applications of modern physical approaches in biology. A single crystal composed of periodically ordered biological molecules represents a unique possibility for their structural characterization. Instead of probing disordered molecules in a solvent or in a powdered amorphous sample, a signal detected from single crystal is significantly amplified, due to a periodical molecular arrangement. Having a single crystal at disposal, one can apply numerous modern crystallophysical characterization techniques to advance these studies. However, despite a bulk of research reports claming successful growth of well-faceted single biological crystals, we have not found relevant experimental results in the current literature. The scarcity of experimental physical investigations on biocrystals may be explained by their small size and extreme fragility: in most cases, the biocrystals coexist with their saturated solvent and are easily destroyed even by a slight deviation from the conditions of their stability, *e.g.*, with temperature or concentration changes. A temperature controlled optical characterization is especially difficult in this regard.

In this paper we represent our successful attempt to attack the mentioned difficulties and proceed with our previous investigations of lysozyme crystals (see [1]). We describe a technically simple and reliable solution for the temperature controlled optical studies of biocrystals and report a first estimation of the temperature behaviour of optical birefringence in a biocrystal. The material under study is a lysozyme, which is well known from the current literature. Moreover, we have been lucky enough to document a temperature phase transition in this single crystal.

The paper is organized as follows. The first subsection of experimental section specifies the

growth conditions. The second one analyses experimental difficulties of the temperature controlled optical investigations in application to biocrystals and suggests a temperature controlled set-up based on the polarization microscope, which allows one to measure temperature dependence of the birefringence. The experimental results are presented in the last subsection. The following section discusses obtained results. In particular, we argue that the registered phase transition might be of ferroelastic or ferroelectric-ferroelastic. Finally, the conclusions are summarized in the last section.

Experiment

Crystal Growth. The crystals were grown from the prepared solution, which contained lysozyme, Na:Ac buffer and an aqueous solution of NaCl. 0.12g of lysozyme was dissolved in 2ml of the 50mM Na:Ac buffer. After the full dissolving of lysozyme, 2ml of 10% NaCl solution was added. Lysozyme crystals were grown at the temperature 18^0C. The seed crystals appeared after 10 days of growing process. Small single lysozyme crystals with the average sizes of $0.42\times0.2\times0.35 mm^3$ were obtained in the capillary after 15 days.

Experimental technique. Although there are numerous optical techniques to characterize single crystals, most of them cannot be applied directly for the case of biocrystals, because they are based on the analysis of an integrated (transmitted or reflected) light signal passed not only through a specimen under test but also through other elements such as container walls (usually glass capillary), solvent, solid inclusions suspended in the solvent, *etc*. Moreover, even if an isolated well-faceted single biocrystal is available, it is usually a tiny object with several different facets across the probing light beam. Therefore, the only optical techniques allowing optical characterization of biocrystals are those based on the polarization microscopy control.

In the most cases reported in the literature, like in our case, single crystals grown from the saturated solution in a circular glass capillary are dealt with. This particular geometry is very convenient for our purpose, because it enables one to observe the specimen from different sides, while rotating continuously the capillary around its axis. However, a cylindrical shape of the capillary brings large optical distortions of the microscopy image, due to a difference in the refraction indices inside and outside the capillary. To exclude this inconvenience, we have fixed the sealed capillary in a Petri dish and filled the dish with water. The resulting image appears to be free of curvature distortions and has much higher contrast and resolution.

A more serious experimental difficulty is that of temperature control. Traditional microscope hot-stages cannot be used here. First, most of the hot-stages are not transparent and have a hole (an optical window) for the light. Such a construction produces temperature gradient across the window. It is known that even small temperature gradient along a capillary induces flow of the liquid along the capillary axis, due to temperature dependence of the surface tension. The flow of the solution can be fatal, since the crystal may appear out of the solution or it is rolled over. Another problem of the hot-stage with the window is a difficulty with sample manipulation. In our case, water playing a role of immersing liquid in the Petri dish, can be simultaneously used as a heat-transfer agent. In order to change the temperature, we have placed ice pieces into the Petri dish, cooling the sample close to 0^oC and then letting ice to melt. The temperature is measured with a thermocouple thermometer, which is in contact with the capillary. As a result, the accuracy of the temperature control has been not worse than 0.5^oC. It is acceptable in our case, when the temperature slowly (on average, with the rate of about 0.5^oC per minute) and continuously increases in the range between 0 and 20^oC. To obtain higher temperatures, we have added hot water drop by

drop, preserving the same level of water in the Petri dish. The sample can be also cooled down with placing the ice pieces in water and, again, preserving its level. The objective of the microscope (Karl Zeiss, magnification 4×12.5) has been kept well above the water surface, while the capillary has been deep in water.

Results. After rotating the capillary around its axis and around the microscope axis, we have found that the optic axis is tilted by 25° with respect to the normal of the capillary wall. When the sample is observed between the crossed polarizers and the optic axis is parallel to the plane of the microscope stage and, at the same time, parallel either to the polarizer or the analyser transmission axis, the crystal texture is totally optically extinguished. On the contrary, it displays bright interference colours in the "diagonal" position (the corresponding angle being 45°).

The observed changes in the colours under the rotation of capillary around its axis has allowed us to conclude that the yellowish and red colours in Fig. 1a are of the first order and correspond approximately to the optical path difference of about 550 and 250 nm between the ordinary and extraordinary waves. The microphotographs in Fig. 1a correspond to the light pass geometry: the capillary axis and the optic axis are inclined by 25° with respect to the microscope axis and the projection of the optic axis on the microscope stage is in the 45° position with respect to the crossed polarizers.

While observing modifications of the interference colours on heating (see Fig. 1a), one finds that, in the temperature range between 3 and 11°C, the observed interference colour changes very slightly (not more than 25-30nm) so as the path difference decreases. When the temperature approaches approximately 11°C, the texture of the crystal changes drastically and acquires a bright yellowish colour. This change of the interference colour implies in fact a notable change in the birefringence and so the point $T_c \approx 11^{\circ}$C corresponds definitely to a phase transition.

Figure 2 unambiguously reveals a presence of phase transition of first order at $T=T_c$, which separates two different structural phases. At T<11°C the crystal intensively scatters light. At T_c the crystal texture brightens so much that we have had to introduce a grey filter to resolve its peculiar details. We suppose that the brightening of the crystal texture at T>11°C is due to the disappearance of numerous scattering centres, existing below T_c. It is natural to suggest that the intensive light scattering results from the spatial in-homogeneity of the refractive index, which can be considered as a manifestation of the domain structure of the low-temperature phase, although we do not resolve it on the texture. If the domains exist, their size is smaller than the resolution of our observations, *i.e.* smaller that few microns.

When the grey filter is inserted, one can clearly see the appearance of new defect planes in the crystal texture at the temperatures T>11°C. However, these defects are absent in the temperature range T<11°C. The new defects existing above T_c might be those appeared due to the phase transition. The phases separated by the point T_c are thermodynamically stable and they exist on both heating (Fig. 3a) and cooling (Fig. 3b).

We have detected a change in the size of the crystal at T_c. The length of the long diagonal in Fig.1 increases on about 5% above the phase transition. The observed deformation is only slightly larger than the limit of our microscopy resolution. The detailed quantitative study of this phenomenon requires larger crystal pieces and special long-focus objectives for higher magnification. At the time being our preliminary results allow to speculate that the observed size change corresponds to spontaneous ferroelastic deformation of $e \approx 5\times10^{-2}$.

Discussion

The presence of spontaneous deformation suggests that the phase transition in lysozyme crystals is ferroelastic. It is accompanied by the

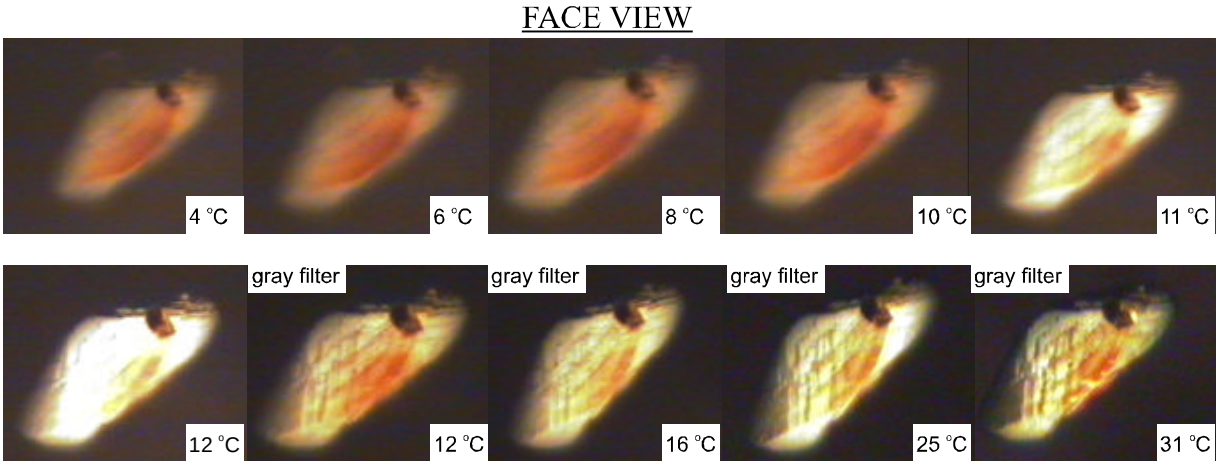

Fig. 1. Textures of the lysozyme crystal at different temperatures on heating.

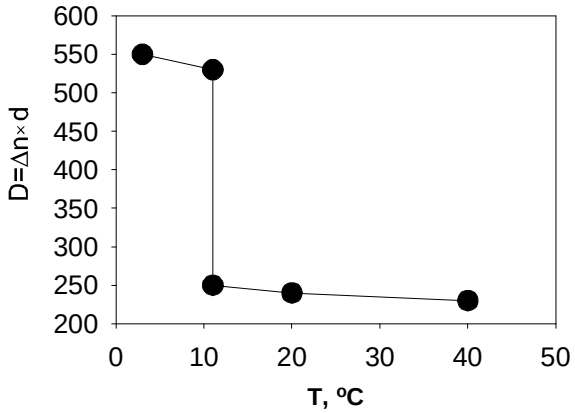

Fig. 2. Temperature dependences of the birefringence (D is the optical path difference).

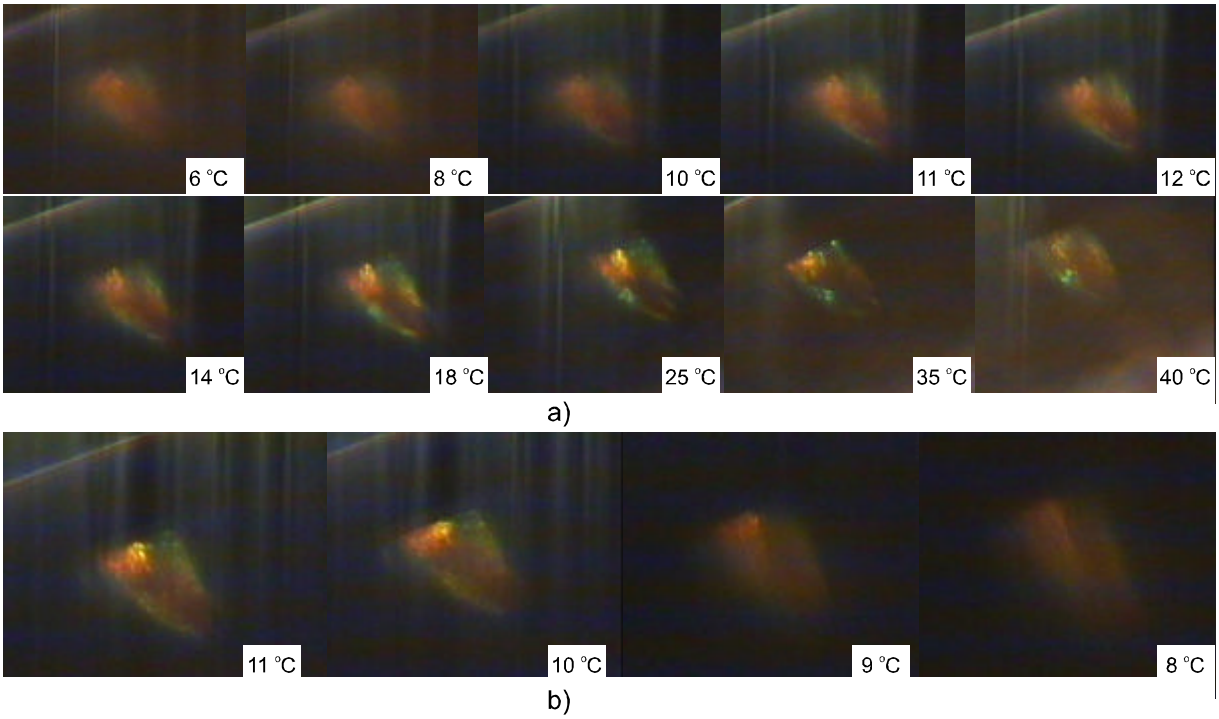

Fig. 3. Side-view textures of the crystal (the same that shown in Figure 1) corresponding to the observation geometry: a) heating, b) cooling.

appearance of the spontaneous deformation . As it follows from the symmetry classification of ferroelastic phase transitions by *K.Aizy* [2], three kinds of phase transitions can occur from the initial phase with the point symmetry group 422, namely: 422F2, 422F1 and 422F222 and two phase transitions for the scenario 222F2, 222F1. The two former ones are ferroelectric-ferroelastic. The shear spontaneous deformations should appear at the phase transitions 422F2, 422F1, 222F2 and 222F1. Finally, the domains that appear in the phases 2 and 1 should differ by the birefringence and, subsequently, can lead to intensive light scattering . It is also interesting to remind that the lysozyme crystals may be grown in the two modifications, characterised with the point groups of symmetry 422 and 222 [3]. These two latter might correspond to the low- and high-temperature phases, which are separated by the ferroelastic phase transition temperature, alluded to above. The fact of approximate constancy of the birefringence and spontaneous deformation in the low-temperature phase, as well as the jump-like appearance of these parameters at T_c, suggest that the phase transition in lysozyme crystals is of strongly pronounced first-order, with a relatively large value of spontaneous deformations. Here we do not specify the type of the phase transition but leave it for further detailed studies.

Conclusions

We have suggested a simple reliable set-up for temperature study of the birefringence in biocrystalas based on the polarization microscopy observations. A phase transition at T_c=11^{o}C accompanied by the drastic changes in the interference colors observed at crossed polarizers and in the linear size of the crystal, has been documented. The temperature dependence of optical birefringence in the lysozyme crystal is represented in the temperature range of 4–40^{o}C. We propose that the observed phase transition in the lysozyme crystal is a first-order ferroelastic phase transition.

Acknowledgement

The authors are grateful to the Ministry of Education and Science of Ukraine (the Project N0103U000698) for financial support of this study.

References

1. Vlokh R., Marsel L., Teslyuk I., Vlokh O.G. Ukr. J. Phys. Opt. **2** (2001) 84.
2. Aizu K. J. Phys. Soc. Japan **28** (1970) 717.
3. Ries-Kaunt M. and Ducruix A. Crystallization of Nucleic Acids and Proteins (IRL, Oxford, 1995), p. 195.