Growth of Lysozyme Crystals in Flat Cells Between Specially Treated Substrates

[1]Teslyuk I.M., [1]Nastishin Yu.A., [2]Boiko O. P, [2]Nazarenko V.G. and [1]Vlokh R.O.

[1]Institute of Physical Optics, 23 Dragomanov St., 79005 Lviv, Ukraine
[2]Institute of Physics of the National Academy of Sciences of Ukraine, Nauky Ave., Kyiv, Ukraine

Received: 28.04.2005

Abstract

It has been shown that electrical properties of flat glass substrates affect the conditions of growth of biological lysozyme (LZ) crystals. It follows from our results that negatively charged substrates, including the untreated precleaned glasses, absorb selectively the ionic LZ residues and so they are applicable for the growth of biocrystals in flat cells. The largest LZ crystals obtained by us have the size of 0.7 mm.

Key words: biological crystals, lysozyme

PACS: 81.10.Dn

Introduction

Biological crystals are extremely intriguing objects that come nowadays to the interest of experimentalists. The interest in characterization of biocrystals (BCs) is multiple. First, being representatives of a crystalline medium, BC bear all the resemblance with their inorganic counterparts and, thus, exhibit a rich variety of phenomena, which have been deeply studied for years and well understood at present. At the same time, in contrast to the simple inorganic crystals, composed of atoms or simple complexes, biomolecules themselves have a complicated structure. They exhibit different levels of molecular architecture and this implies multiple ways for their packing into a crystalline lattice. In principle, any two crystals belonging to the same symmetry group should carry qualitatively similar physical properties, independent of their elementary building units (either atoms or large biopolymeric molecules). However, due to their giant elementary cells, the BCs are expected to be a sort of unique objects designed by nature specially in order to study the optical phenomena of spatial dispersion, while in the conventional solids these effects remain negligible in the most of known cases. Numerous optical effects of spatial dispersion predicted by the theory are still not discovered or debated because of their small size. Although the expectances from the BCs in this respect are indeed challenging, the experimental works dealing with the studies of their optical properties are almost absent in the current literature. The data for the physical and, in particular, the optical properties are limited to the elastic modules studied with the Brillouin scattering method for the lysozyme (LZ) crystals [1,2]. Scarcity of the optical investigations on the BCs is explained by difficulties concerned with the growth of single crystals. First, single BC samples reported in the literature are typically not larger than a few hundreds of microns [2,3]. Second, the BCs are extremely fragile and sensitive to external conditions and the environment. As a result, they are usually grown in circular capillaries. Such the circular containers preserve the samples from drying but, at the same time, this geometry is quite inconvenient for optical studies, because of the curvature refraction of the probing light.

Recently, we have performed a polarization-microscopy study for the temperature transformations in LZ crystals grown in circular capillary [4] and observed clearly the phase transitions between the two crystal modifications [5]. This finding encourages us for deeper investigations, implying examinations of the other physical properties. The circular geometry of the capillary represents an obstacle for the most of characterization techniques. It would be more convenient to get crystalline specimens in a flat cell, where the sample is sandwiched between two solid transparent substrates and sealed from the sides. This cell geometry is of the type traditionally used for preparation of lyotropic liquid crystals. The advantages of the flat cell geometry are numerous and evident.

Remembering of the liquid crystal sample preparation, when the surfaces of the cell substrates are covered with special layers, we have decided to test the influence of the layers of different nature, deposited on the glass substrates, on the process of nucleation and further growth of the LZ BCs. Among the principal possibilities, the surface can be hydrophilic or hydrophobic and, moreover, it can be electrically neutral or charged (positively or negatively, in its turn). In this paper we report the results of nucleation of the LZ crystals on the substrates that are specially treated or covered by different layers.

Experimental

Substrates treating. We cut the glass substrates with the dimensions 1×2 cm^2. All the cells were assembled, using the spacers (0.8 mm thick plastic strips) between the substrates. Then the substrates were fixed together and sealed with 5-min epoxy along the spacers, which were placed parallel to the longer side of the substrates.

Pre-washed, Pw-cells. The glass substrates were consequently washed in acetone, isopropanol and distilled water and then dried.

Hydrophylic, HPl-cells. To obtain the surface with hydrophilic properties, we spin-coated a polyimide polymer (polyvinylacetate, PVA) layer on the glass substrates, which is used for the planar orientation of thermotropic nematic liquid crystals. We refer to these cells as *HPl-cells*.

Hydrophobic, HPb-cells. The substrates conventionally used for homeotropic alignment of thermotropic nematics (spin-coated with the polymer SE-204 from *Nissan*) were used to prepare the substrates with hydrophobic properties.

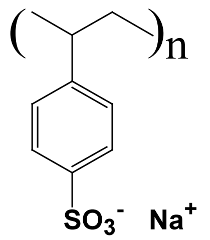
The technique for the preparation of charged surfaces, similar to that described in [6,7], was used for deposition of monomolecular layers of lyotropic chromonic liquid crystals. The technique was based on electrostatic adsorption of the material by the charged surfaces.

Negatively charged, NE-cells. 5g of KOH dissolved in 30g H_2O and mixed with 400ml of isopropanol charged the glass surface negatively. After etching for 2 h the substrates were removed from the solution, rinsed in deionised water and dried.

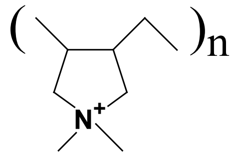
Negatively charged, NP-cells. The surface could be also charged negatively by the electrostatic deposition of negative polyion layer (poly(sodium 4-styrenesulfonate) or PSS). The negative layer could not be deposited directly onto the glass, because the clean glass in itself was particularly charged negatively. However, one can first deposit a polymer layer, exhibiting a positive charge, on the clean glass substrates. Then the monolayer of negatively charged polymer can be deposited on the top of positively charged layer.

To obtain positively charged surface, the NE-substrates were placed into the PDDA solution (see paragraph below for the composition) for 20 min, then removed, rinsed, dried, placed in the PSS solution (0.3g of PSS dissolved in 100ml of water – see Table 1) for 20 min and then again removed, rinsed with water and dried.

Table 1. Structural formulae of the polyions.



poly(sodium 4-styrenesulfonate) or PSS



poly(diallyldimethylammonium chloride) or PDDA

<u>Positively charged, P-cells</u>. The NE-substrates were placed for 20 min in the aqueous solution of the positive polyion PDDA (poly(diallyldimethylammonium chloride – see Table 1) from *Aldrich* (1g of PDDA in 100ml of water), and then removed, rinsed in deionised water and dried.

Four cells of each type were assembled and the LZ solution (the composition see in the subsection below) was filled into the cell and the filling gaps were closed with plasticine. The latter allowed for slow evaporation of the solvent.

<u>Crystal Growth.</u> The crystals were grown from the initial LZ solution (0.12g of LZ dissolved in 2ml of the 50mM Na:Ac buffer). After the full dissolving of LZ, 2ml of 10% NaCl solution was added. The LZ crystals were grown at the temperature 18°C.

Results and discussion

The first nuclei of the LZ crystals are found in the cells after about two weeks. Further growth shows that the six types of the cells described above might be combined into three groups: (1) electrically neutral substrates, (2) positively charged substrates, and (3) negatively charged ones.

<u>Neutral substrates</u>. This group includes *HPl-* and *HPb-cells*. One finds myriads of teeny crystals of the size not larger than a few micrometers, covering the substrates as crystalline sand. Although nuclei grow in time, soon they are covered by new nuclei and, as a result, there are no single crystals, which could be used for further characterization. The conclusion is that non-charged (either hydrophilic or hydrophobic) surfaces favour the nucleation of the LZ crystals too much, producing too many crystalline seeds and are thus not applicable for growing single crystals. Such the result can be explained by good affinity of the protein molecule to the neutral surfaces, independent of their hydrophilic or hydrophobic nature. Indeed, the LZ molecule is composed of different structural (hydrophobic, as well as hydrophilic) units and attaches to the surface, which does not repulse it.

<u>Charged substrates.</u> In water solution, the LZ molecules behave as a zwiterionic: small ions come off the big molecular residue, which obtains several charged centres. Because the zwiterionic LZ residue is electrically charged it can be either attracted to a charged surface or repulsed, depending on their charges. If the surface is charged with the same sign as that of the molecular protein residue, then one can expect that the crystal nuclei would not appear on the surface. As we find, this is the case of positively charged *P-cells*.

<u>Positively charged cells</u>. We first have tested the substrates without assembling them as a cell. The *P-substrates* have been faced up and placed into a Petri dish filled with the LZ solution. We find that the LZ crystal nuclei appear everywhere in the dish, except for the P-substrates. The absence of the nuclei on all four P-substrates confirms that the positively charged

substrate does not favour the deposition of the LZ molecules. After that we have tested the cells assembled from the P-substrates. The observed picture is similar: very few nuclei appear. Moreover, Fig.1 shows that the orientation of the faceted crystalline nuclei is different from that obtained in the other cells described below (compare Fig.1 with Fig.2, 3 and 4).

This result can be used in the following manner. First, while combining the P-layers with those that favour deposition of the LZ molecules, one can control nucleation of the LZ crystals across the cell. Another important application of the result is that P-cells produce some orientation of the crystals, which is different from that usually obtained with untreated glass substrates.

Negatively charged cells. Microscopic observations show that the picture of nucleation in the cells with untreated *Pw*- and negatively charged *NE*- and *NP-cells* is similar: the orientations of the faceted crystalline nuclei in the cells (compare Figs. 2 to 4) are the same. The number of the appearing nuclei is not too large, as in the case of neutral *HPl*- and *HPb*-cells: the nuclei can grow without overlapping. We conclude therefore that the negatively charged surface favours selectively deposition of the LZ molecules, absorbing only the ionic LZ residues, which incidentally contact the surface with their charged centres. This implies that the ionic LZ residues are charged positively. The fact that the number of the nuclei appearing in the cell with the untreated substrates is smaller than for the *NE*- and *NP-cells* can be explained with taking into account that clean untreated glass substrates are charged negatively only locally, because of screening of the glass surface charge by incidentally absorbed ionic impurities. The impurities can even reverse the surface

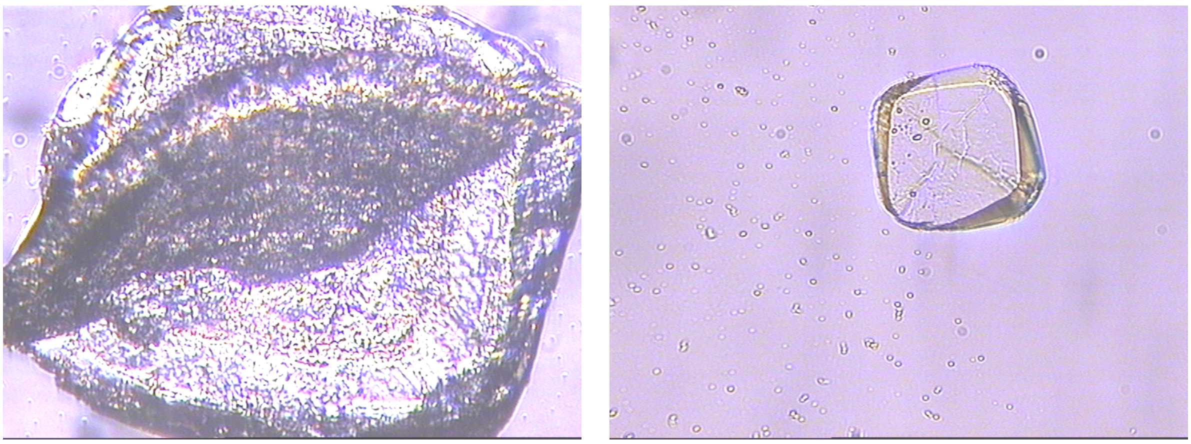


Fig. 1. LZ crystals obtained using the positively charged P-cells.

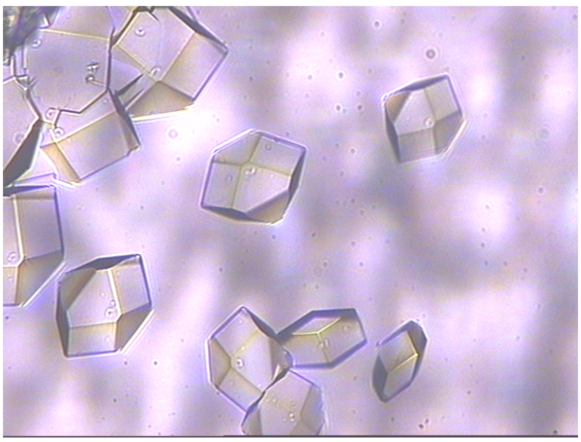


Fig. 2. LZ crystals obtained using the pre-washed cell.

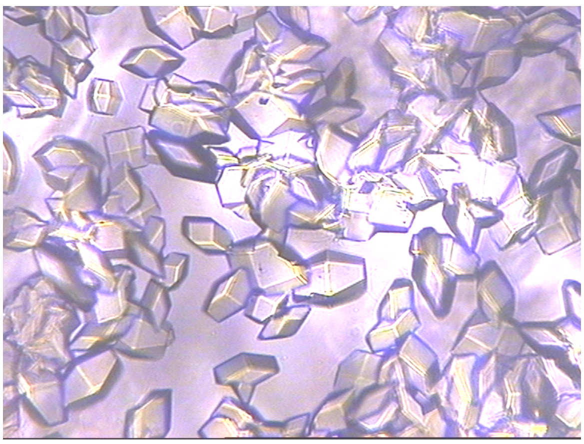


Fig. 3. LZ crystals obtained using the negatively charged NE-cell.

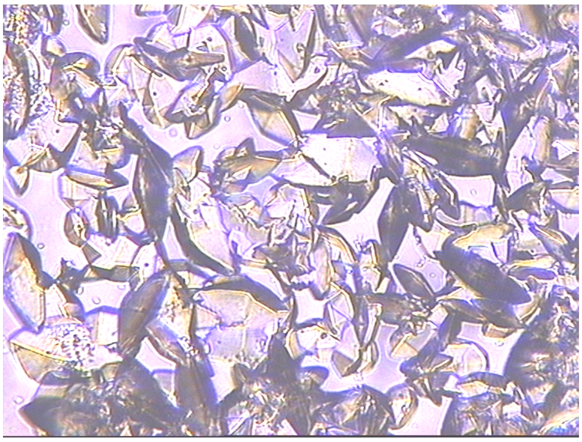


Fig. 4. LZ crystals obtained using the negatively charged NP-cells.

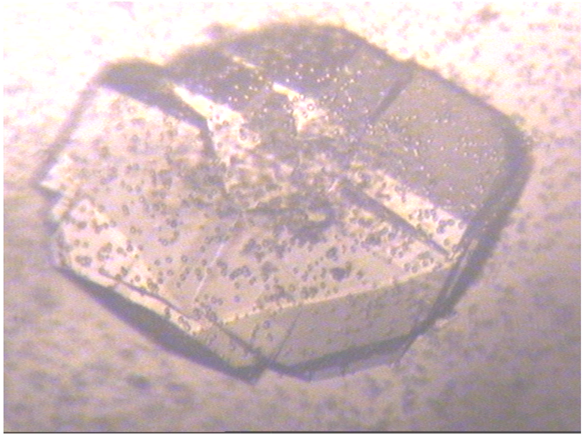


Fig. 5. Large LZ crystals obtained using the pre-washed cell.

charge, thus disfavouring deposition of the LZ molecules onto the glass surface.

Finally, we have found single LZ crystals of the size of about 0.7 mm in *Pw-cells* (see Fig. 5). Such big specimens offer good opportunities for their characterization. The corresponding experiments are in progress in our laboratory.

Conclusion

In conclusion, we state that the negatively charged substrates, including the untreated glass, absorb selectively the LZ ionic residues and so are applicable for growing the BCs in the flat cells. We are aware that the materials studied in this paper are not of the most effective. Nevertheless, we believe that the growth of the BCs inside the cells composed of specially treated substrates would open a possibility for their further characterization with numerous techniques. Many other classes of the materials deposited on the substrate surface can be tested for this purpose. Lyotropic chromonic liquid crystals (LCLC) are among them. The LCLC monolayers deposited electrostatically on the glass from the chromonematic phase, where their rod-like aggregates can be well aligned, preserve the alignment of the aggregates after drying and may be therefore used as a substrate, which is capable of orientating the crystalline axes of the BCs in a desired direction. The experiments testing the LCLCs as substrates for the BC growth are currently in progress in our laboratory.

Acknowledgement

The authors acknowledge financial support from the Ministry of Education and Science of Ukraine (Project No 0103U000698).

References

1. Speziale S., Jiang F., Caylor C.L., Kriminski S., Zha C.-S., Thorne R.E., Duffy T.S. Biophys. J. **85** (2003) 3202.
2. Caylor C.L., Speziale S., Kriminski S., Duffy T.S., Zha C.-S., Thorne R.E. J. Cryst. Growth **232** (2001) 498.
3. Broutin I., Ries-Kautt M., Ducruix A. J. Cryst. Growth **181** (1997) 97.
4. Vlokh R., Marsel L., Teslyuk I., Vlokh O. Ukr. J. Phys. Opt. **2** (2001) 84.
5. Teslyuk I., Nastishin Yu., Vlokh R. Ukr. J. Phys. Opt. **5** (2004) 118.
6. Schneider T., Lavrentovich O. D. Langmuir **16** (2000) 5227.
7. Alignment of lyotropic chromonic liquid crystals at surfaces as monolayers and multilayered stacks, US patent #6673398 (January 6, 2004).