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DEPOLARIZED LIGHT SCATTERING INTENSITY TEMPERATURE ANOMALIES IN THIN ORIENTED PURPLE MEMBRANE FILM

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Abstract The depolarized light scattering intensity (I^S) temperature dependence was investigated in suspension of purple membranes from Halobacterium hallobium, sandwiched as a thin oriented film by the glass plate walls. Three anomalies of $I^S=f(T)$ were registered at (33-37)°C, (66-71)°C and (92-95)°C. The third anomaly was connected with irreversible changes in bacteriorhodopsin state, probably due to its denaturation. The other anomalies were connected with changes in orientation, mobility and structure of membrane lipids and the bacteriorhodopsin, i.e. they appear like smeared second order phase transitions with partial bacteriorhodopsin denaturation.

INTRODUCTION

Purple membrane fragments (PM) from Halobacterium hallobium are composed of single protein (Bacteriorhodopsin) and membrane lipids[2]. Bacteriorhodopsin molecules form a two-dimensional hexa-

gonal lattice with the space group P_3 in the purple membrane[10,13]. It was demonstrated[9] that the PM have a large permanent electric dipole moment ($\vec{P}$), perpendicular to the membrane plane[1]. X-ray and thermal analysis investigations indicated phase transitions at temperature variations in PM suspension. However, the exact phase transition temperatures are controversial[3,12,7].

A sensitive method for the phase transition study is the depolarized light scattering thermal analysis. We have examined the PM phase transitions in the temperature interval 25-95°C using this method. The light scattering intensity temperature dependence indicates fluctuations in the orientation and anisotropy of the medium.

Since the oriented PM fragments are useful for optical investigations of the membrane structure, a variety of methods has been employed to produce oriented samples. One popular method is drying of PM suspension onto surface. This surface induced orientation method characterizes with penetration of the orienting influence of the surface into the volume. The volume in the PM system is usually composed of physically separated membrane sheets. The steric interaction of the anisotropically shaped membrane fragments with themselves and with the surfaces is also an important factor in the process of orientation. Up to now, however, the physical mechanism of the PM orientation is not yet clear.

The physics of the system as liquid crystals oriented by the surface[6] can be a basis for the understanding of the thermodynamical and optical properties of the oriented PM system, assuming that separate membrane fragments (thickness a and diameter D as $a < 0{,}1\ D$) appear like a determine liquid crystal unit with anisotropic form[4].

MATERIAL AND METHODS

Sample and thin oriented film preparation

The PM used in the present investigation were kindly supplied by Keszthelyi et al. and the detailed method of cell growth, membrane preparation etc., by modification of Oesterhelt procedure were described[9]. Measurements were performed on PM suspension with triple distilled water.

In order to obtain a thin oriented PM film, the suspension was inserted by capillary forces between two glass plates, treated with SiO, obliquely evaporated, forming microchannels on the surfaces[11]. The Mylar spacers fixed a thickness $d = 50\ \mu m$. The section of the thin oriented cell, connected with a laboratory coordinate system, is presented on Fig.1. The Y-axis coincides with the direction of the microchannels and the hydrodynamic flow orienting the PM, the Z-axis - with the normal to the glass plates. At heating the solvent (distilled water) evaporates more intensively which squeezes the membrane fragments. This process reduces the volume and builds loose arrays. The solvent meniscus with continually de-

creasing radius of curvature further compacts the arrays. For destroying the empty space between the membrane sheets we use capillary suction as was mentioned above accompanied by drying. Since the concentration of fragments is raised at these conditions, the orienting process depends on the reorientation of the fragments caused by steric interaction, thus increasing the local parallel orientational correlation. So, orientationally correlated groups of PM fragments interact with the surfaces.

Since the PM fragments are concentrated by drying and capillary forces, we accept that they are orientationally frozen for a given temperature into a liquid crystal-like structure - a state with minimum elastic energy (when the larger area of the membrane disk is parallel to the surface (XOY) Fig.1) and higher degree of orientation[4].

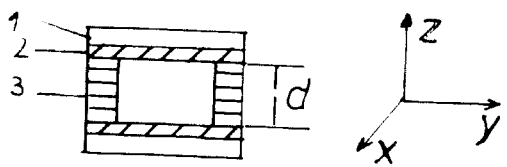

FIGURE 1. PM thin oriented cell.
1 - glass plates; 2 - thin (50 nm) layer of SiO;
3 - Mylar spacers; d - the PM cell thickness.

Since the PM fragment structural orientation can not be observed directly by the corresponding optical microscopy or electromicrograph textures

we use the known polarization method, convenient for 50 μm thick sample. A system of vertical fractures extended along the film of width of approximately 0,5 - 0,7 μm is seen under microscope. The so prepared dried PM film is quite rigid at higher temperatures and resembles a thin crystal. A polarization study indicated that the 50 μm PM oriented film is optically uniaxial, with an optic axis parallel to Z-axis and to the dipole moment $\vec{P}$ respectively.

To maintain the PM suspension temperature, the sample is kept in hot stage temperature regulation equipment.

Experimental set-up and results

The experimental equipment for depolarized light scattering study is presented in Fig. 2. The He-Ne laser (λ =632,8 nm) was used, the laser light with intensity I_0 directed on the sample (PMS),which passed through the diaphragm D_1 and the polarizer P_1. The laser power is less than 1 mw, in order to avoid sample heating, which eventually can contribute in producing additionally thermal fluctuations of PM.

The temperature dependence of the scattered light intensity $I^s = f(T)$ is shown in Fig. 3: in the course of heating three anomalies are observed - at temperature interval $(33^o-37^o)C$, $(66^o-71^o)C$ and $(92^o-95^o)C$ respectively. The I^s value sharply decreases just above 95^oC. At further heating of the sample the typical for the strong anisotropic scattering medium noise disappears.

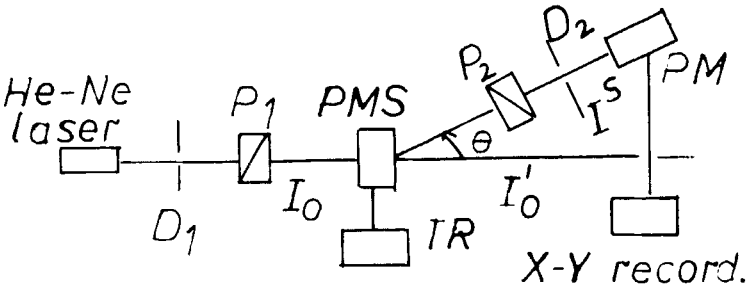

FIGURE 2. The experimental apparatus. I^s is the scattered laser light intensity; θ is the scattering angle (with a value of about 10^o). The scattered light passed through the diaphragm D_2 and the polarizer P_2, perpendicular to P_1 and was detected by the photomultiplier FEU 28. The scattering light intensity was recorded by means of XY-recorder.

The scattering intensity (I_o^s) is similar to that of an isotropic liquid. It was established experimentally that the I_o^s value is approximately equal to that of distilled water but a bit higher. At the same time $I_o^{'}$ is considered as zero level of the transmitted light intensity.

Cooling from temperatures less than 95^oC always produces scattering intensity higher than I_o^s and $I_o^{'}$. Only a slight decrease was recorded compared to the first heating intensity level. After cooling from temperatures higher than 95^oC a constant intensity level was preserved, i.e.

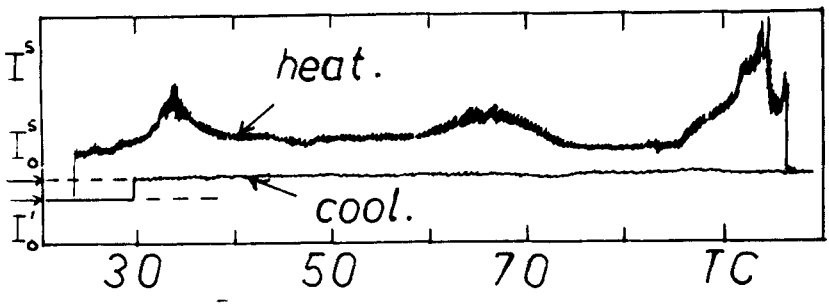

FIGURE 3. The light scattering intensity temperature dependence.
I^s - the scattering intensity of light; I_o^s - the scattering light intensity after 92-95°C phase transition; I_o' - zero level of the intensity measurements.

the I_o^s level.

DISCUSSION

To characterize the local PM fragment orientation in analogy with liquid crystal systems, we accepted an unit vector $\vec{n}$, giving the average orientation of the disklike PM fragments. This vector is a director and in the oriented system coincides with the direction of Z-axis and the permanent dipole moment $\vec{P}$ and its components are $(0,0,n_z)$. Adopting the liquid crystal model[4] $\vec{n}(r)$ is considered as a continuous unit vector field.

The energy connected with eventually drastic

Changes of $\vec{n}$ orientation must be unnormally big, so, nowadays it is accepted that real situation in an oriented system like ours is rather continuous and this can be regularly described with angular correlated thermal or another type of fluctuations of $\vec{n}(r)$ in the time and space. Therefore, at present the scattering of the light can be interpreted as a scattering with small amplitude fluctuation of $\vec{n}$ orientation in the frame of the continual theory. The intensity of such a scattering is bigger than the intensity of the light scattered by the fluctuation of a sample density, and the contribution of the latter can be neglected. Our scattering geometry is as follows (Fig. 4).

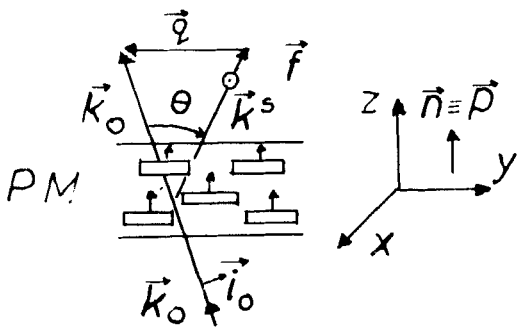

FIGURE 4. The light scattering geometry. Purple membrane sample is rotated around X-axis. The molecular director $\vec{n}$ (the small arrow perpendicular to the plane of the disk-like PM) coincides with the permanent dipole moment $\vec{P}$ ($\vec{n}\equiv\vec{P}$). The optical axis of the PM oriented system is also the Z-axis. $\vec{K}_o$ and $\vec{i}_o$ are the wave and unit vectors of the polarization of incident monochro-

matic light, $\vec{k}^s$ and $\vec{f}$ - the same vectors for scattered light.

The scattering process is connected with the change of the scattering vector $\vec{q}$ as $\vec{q}=\vec{k}_o-\vec{k}^s$. In our scattering geometry the lengths of $\vec{k}_o$ and $\vec{k}^s$, Fig.4, are determined from the combination of the refraction index of the PM medium - extraordinary $n_{\parallel}$ and ordinary $n_{\perp}$, respectively parallel and perpendicular to the optical axis or to the PM fragment director $\vec{n}\equiv\vec{p}$. The plane of the vectors $\vec{k}_o$ and $\vec{k}^s$ is the scattering plane. The light scattering intensity is a result of the director $\vec{n}$ fluctuations leading to fluctuations of $n_{\parallel}$ and $n_{\perp}$. The refraction index fluctuation is equivalent with the fluctuation of the dielectric susceptibility tensor. The director $\vec{n}$ and the dielectric constant ε are connected with equation[5]

$$\varepsilon = \varepsilon_{\alpha}\delta_{\alpha\beta} + \varepsilon_a(n_{\alpha}n_{\beta}) \qquad (1)$$

where $\varepsilon_a=\varepsilon_{\parallel}-\varepsilon_{\perp}$ is the dielectric anisotropy, $\varepsilon_{\parallel}$ and $\varepsilon_{\perp}$ are dielectric constants parallel and perpendicular to $\vec{n}$ (and $\vec{P}$). The angular deviations of the $\vec{n}$ director from the equilibrium are also accompanied by elastic deformations of the medium, connected with the system free energy. The mean square amplitude[5] of $\vec{n}$ thermal fluctuations can be written, i.e. Fourier component of the amplitude, $\langle|\delta n(q)|^2\rangle$

As the intensity of the scattering is proportional to $\langle|\delta n|^2\rangle$ and $(\langle|\delta n|^2\rangle)\sim K_B T/K$ whe-

re K_B is the Boltzman's constant, K - elastic constant, so

$$I^s \sim \varepsilon_a^2 K_B T/K \qquad (2)$$

or

$$I^s/I_o^s \sim \varepsilon_a^2 K_B T/K \qquad (3)$$

where $I_o^s \approx I_o^{'}$ ($I_o^{'} \neq 0$)

Our experimental results ($I^s/I_o^s = f(T)$, Fig. 3) demonstrated that the physical parameters following the temperature variations are ε_a (or the refraction index anisotropy respectively) and layer system elastic constant K.

Let us consider the anomaly at 92-95°C. It is an irreversible phase transition between the anisotropic PM system and the almost isotropic medium. Starting at 86°C, I^s/I_o^s rapidly increases. So, in the temperature interval 86-95°C ε_a^2/K increases with the temperature (see Formula 3). At 95°C, where I^s tends to I_o^s, the rate ε_a^2/K also tends to I_o^s. We can conclude that above this temperature point the anisotropy expressed by ε_a is very small but does not disappear. The PM system above 95°C is not fully isotropic. Presumably the K elastic constant diverges above this point since $I^s \to 0$.

It was reported[7] that in the PM suspension they have not registered a phase transition at about 30°C, at the same time[3,12] reported about such a transition. Our previous results point out to a temperature anomaly at 34°C[11]. Something else, the temperature about 40°C was mentioned as

a peculiar one too[10], at which the X-ray scattering intensity has an anomaly.

The intensity anomaly at 66-71°C is smaller than the one at 33-37°C.

Probably the observed anomalies are generally connected with changes in orientation, mobility and structure of membrane lipids and the bacteriorhodopsin which contribute to the PM fragments director $\vec{n}$ angular correlated temperature fluctuations but at a different degree. For the first anomaly (33-37)°C, it is acceptable that changes in scattered intensity occur mainly due to orientation and mobility changes in the lipid bilayer and only partially in the bacteriorhodopsin (with accordance[7] and our own results[11]).

The laser wavelength used is in the absorption band of K intermediate of bacteriorhodopsin. Hence, a significant contribution to the scattered component could be eventually related to retinal conformation, which is in progress in further investigations.

The anomaly 33-37°C is reversible and it is probably a smeared second order phase transition.

It is possible to conclude for the second anomaly at 66-71°C that in general, the two-dimensional crystal of bacteriorhodopsin transforms into a two-dimensional liquid[8,10]. The anomaly is reversible and we can treat it as a second order phase transition under the condition that a partial denaturation of the bacteriorhodopsin occurs

The transition at 92-95°C is irreversible and

probably connected with a total protein denaturation (in accordance with the results, obtained by means of other methods[7]). As mentioned above, I^s, after heating above 95°C, has the value I_o^s close to the scattering intensity of an isotropic liquid. Probably the intensity I_o^s is due to scattering from fully denaturated bacteriorhodopsin as well as to the scattering from disordered distribution of lipids in the medium.

CONCLUSION

Using depolarized light scattering from thin oriented PM film, we found I^s anomalies with maximum when the incident light polarization $\vec{i}_o$ is perpendicular to the polarization $\vec{f}$ of the scattered light, meaning that the thermally induced correlated angular fluctuations of the PM fragment orientation is the reason for the event. It is clear from the experimental geometry that the fluctuations in the plane perpendicular to the scattering plane is energetically favourable. Naturally, such a mode introduces a periodical orientational deformations of the medium. In an oriented PM film this medium could be considered as a continuous one.

A slight scattering intensity can be registered also in unoriented samples without clear information about correlated fluctuations at temperature close to phase transitions. And since the membrane phase transitions are too weak, that is the reason to use experimental methods in condition

of their topic sensitivity, i.e. under best orientation conditions in our case.

Using a liquid crystal approximation, we found that the PM medium possesses elasticity participating in the light scattering process.

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