Small-angle light scattering studies on xylose cataract formation in bovine lenses

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Using polarized light scattered in the \( I_1 \) and \( I_\perp \) modes, we determined the relative contributions of density and orientation fluctuation to the opacity of bovine lenses. In both the cortex and the nucleus of bovine lenses cultured in xylose medium, we found that the increase in hydration effects the optical anisotropy (birefringence) first, before the lake formation will contribute heavily to the density fluctuation.

Key words: bovine lens, cataract, cortex, light scattering, nucleus, xylose

Lenses cultured in xylose medium demonstrate a dramatic increase in water content accompanied by the opacification of the lens. Obazawa et al.¹ have shown that the increase in water content is due to the accumulation of xylitol and an increase in the total electrolyte content.

The increased water content is collected in vacuoles, and eventually lake formation occurs. Vacuoles filled with water provide structures comparable in size to the wavelength of the light that differ significantly in refractive index from the surrounding medium. Such structures give rise to density fluctuations and can account for the opacity observed.² On the other hand, vacuole and lake formation can alter the form birefringence of lens fibers and give rise to an additional scattering component due to orientation fluctuation.³

We have demonstrated that in the galactose cataract formation in rat lenses there is, in fact, a component of scattered light that is due to orientation fluctuation.⁴

The purpose of this investigation was to determine the relative contribution of the two components of scattered light in a cataract formation that can quickly develop and be strictly controlled. The organ culture technique in xylose medium enables the observation of controlled cataract formation.

Materials and methods

Bovine eyes were obtained from a slaughterhouse less than 12 hr post mortem. In two sets of experiments (runs II and III), lenses were removed with the lens capsule intact, with a small amount of adhering vitreous in the manner of Chylack.⁵ In the first set of experiments, lenses were used without lens capsule. Xylose and fructose media were prepared according to Obazawa et al.¹ and Chylack⁵ as follows: 128 ml of TC 199 media without phenol red (Grand Island Biological Co.); 10 ml of fetal calf serum; 50.2 ml of bicarbonate buffer; 0.216 gm of glucose; 0.023 gm of glutamine; 0.055 gm of CaCl₂; 0.4 ml of PSN antibiotic mixture 100X (Grand Island Biological Co.); plus sufficient water to dilute to 200 ml. Upon addition of the above, the xylose medium contained 0.908 gm of xylose and the fructose medium 1.0809 gm of fructose.

The media were filtered into sterilized vessels through a Millipore 800 μm filter.

The lenses were transferred, after being washed...
Fig. 1. Densitometric scans of small-angle light-scattering patterns of cortical sections in the \( I_e \) and \( I_0 \) modes. Intensity of the scattered light in arbitrary units against scattering angle \( \theta \).

A, Fresh cortex. B, Cortex in xylose media for 23 hr. 

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Results and discussion

In the simplest model for light scattered in both the \( I_e \) and \( I_0 \) mode, one may assume that the scattering is caused by inhomogeneities twice in respective medium into the organ culture media and incubated at 37° C in 5% CO\(_2\) atmosphere.

Four lenses, two from the xylose medium and two from the fructose medium, were removed at set intervals. Thin sections of the cortex and the nucleus of each lens were cut with a double-bladed knife. The sections were placed between a microscope slide and cover glass and exposed to a He-Ne laser beam. The magnet was removed from the laser beam to allow adjustment of direction of polarization by simply rotating a Polaroid filter.

First the sample was placed between crossed polarizers, and both the polarizer and analyzer were rotated in tandem to get a minimum of transmittance. This was done to minimize the effect of any possible birefringence upon the angular distribution of the intensity of the scattered light.6

The light scattering pattern recorded with this setting was designated as \( I_e \) mode. Turning the analyzer 90 degrees, we obtained a pattern designated as \( I_0 \) mode. Four-second exposure time was employed on Kodak Plus X film held at a 40 cm film-to-sample distance in cassettes.

The films were developed in DK50 developer for 2 min, and the negatives were scanned by a Photovolt densitometer (Photoset Corp., New York, N. Y.) to obtain scattered light intensity in arbitrary units as a function of scattering angle. Fig. 1 provides a sample of such primary data obtained on fresh cortex (A) and on a cortex which was organ-cultured for 23 hr in xylose medium(B).
Fig. 2. The relative contribution of optical anisotropy, $I_s/I_0$, to the light scattered by bovine lens. A, cortex. B, Nucleus as a function of hours in xylose media. •, at 1 degree and ○, 3 degrees scattering angle. ●, Experiment I lenses cultured without lens capsule; ○, experiment II and, ○, experiment III lenses cultured with lens capsule and adhering vitreous.
that give rise both to density and optical anisotropy fluctuations.7 In such a model, both fluctuations are random and are not correlated. The intensities of the scattered light in the $I_0$ and $I_+$ modes are given as

$$I_0 = K \left\{ \frac{4}{45} \int f(r) \left[ \frac{\eta^2}{\alpha^2} \gamma(r) + 1 \right] \frac{\sin hr}{hr} r^2 dr \right\}$$

$$I_+ = K \left\{ \frac{15}{2} \int f(r) \left[ \frac{\eta^2}{\alpha^2} \gamma(r) + 1 \right] \frac{\sin hr}{hr} r^2 dr \right\}$$

$$I_+ / I_0 = \left( \frac{15}{4} \right) \int \left[ \frac{\eta^2}{\alpha^2} \gamma(r) + 1 \right] \frac{\sin hr}{hr} r^2 dr + \frac{4}{3}$$

In the above equations, $h$ is a variable that depends upon the scattering angle, $\theta$:

$$h = (4\pi/\lambda) \sin (\theta/2)$$

where $\lambda$ is the wavelength of the light. $r$ is a scalar distance along the path of the beam in the lens section; $\alpha$ is the average polarizability of the lens section. Two parameters describe the density fluctuations: $\eta^2$ is the mean squared average density fluctuation, and $\gamma(r)$ is the correlation function between the density fluctuations of two volume elements. Two similar parameters describe the optical anisotropy (or orientation) fluctuations: $\alpha^2$ is the mean squared average optical anisotropy fluctuation, and $f(r)$ is the correlation function between the optical anisotropy fluctuations of two volume elements separated $r$ distance apart.

The density fluctuation in the lens may be identified as vacuole or lake formations in the case of sugar cataracts or as caused by aggregates of lens proteins that grow to a size comparable to the wavelengths of the light and are embedded in a media of different refractive index.2 The optical anisotropy fluctuation may be identified with birefringent entities in the lens that may be due to particular orientations of supermolecular structures or to form birefringence.3

In the absence of orientation fluctuations, only the density fluctuations contribute to the turbidity. The correlation function $f(r)$ is zero in this case, and therefore, equation 2 equals zero. This means that no scattering in the $I_+$ mode will occur.

If only orientation fluctuations are present, the first term in the right hand side of equation 1 is zero, and the ratio of $I_+/I_0 = 4/3$.

If both density and orientation fluctuations contribute, the relative contribution of the two can be qualitatively assessed by the $I_+/I_0$ ratio (equation 3). In this ratio, the nominator is influenced by the density fluctuation only, while the denominator is influenced by a combination of factors attributable to both density and orientation fluctuations. Therefore, the ratio of $I_+/I_0$ or its inverse, $I_0/I_+$, can be taken as a qualitative indicator for the relative contribution of these two fluctuations.

Using this intensity ratio as a function of cataract development serves a further purpose. Since both $I_0$ and $I_+$ are obtained on the same section, the ratio of the two will not be influenced by the thickness of the sections, and therefore, small variations in the thickness of sections obtained on different days will not influence the results.

In Fig. 2, A the change in the $I_+/I_0$ ratio is given for the sections of cortex as a function of hours in xylose media. Fig. 2, B represents the same diagram for the nucleus also in xylose media. In each diagram the combined data of three experiments that were run 1 year apart are presented. The first experiment was performed on lenses from which the lens capsules had been removed. In the second and third experiments, lens capsules and some adhering vitreous were retained. The opacity developed much faster in lenses organ-cultured without the capsule. This has been previously observed by Chylack and Kinoshita.8 Therefore, the duration of the first experiment was shorter than that of the subsequent experiments.

In presenting the data, we selected the $I_+/I_0$ ratio as qualitative indicator for the two contributions in accordance with our previous paper on galactose cataract.4 Each point on the diagram represents average values obtained from two lenses in xylose medium.

In general, as the opacity begins to devel-
op, the contribution of the $I_\perp$ component toward the total scattering increases. This is especially noticeable, since in normal lenses in the scattering the $I_\perp$ mode is minimal (Fig. 1, A). Not only is the intensity of the $I_\perp$ component increased, but it spreads to higher angles (Fig. 1, B). It must be emphasized, however, that the $I_\perp$ component is strictly a low-angle phenomenon; even in lens sections with high opacity, there is very little scattering in this mode above a 10 degree scattering angle. The $I_\perp/I_\parallel$ ratio shows an initial increase in both the cortex and nucleus of lenses cultured in xylose medium. This increase is faster in the cortex than in the nucleus, but after a certain time, the increase levels off or even decreases.

As emphasized before, the $I_\parallel$ and $I_\perp$ components both have contributions from density as well as optical anisotropy fluctuations, the $I_\parallel$ being more heavily influenced by the density fluctuation. However, in the absence of any optical anisotropy fluctuation, there would be no $I_\perp$ component. Therefore, an increase in $I_\perp/I_\parallel$ can be qualitatively interpreted as indication that at the beginning of the xylose cataract formation, optical anisotropy fluctuation is an important contributor.

In order to eliminate the effect of organ culture, in general, from the observed light scattering, we also used lenses cultured in fructose medium as controls. These lenses maintain their transparency throughout the experimental runs, and according to Oba-
zawa et al., they do not gain water. In order to eliminate possible variations in thickness of sections when comparing lenses from different media, we designated a relative measure for the $I_+ / I_-$ ratios:

$$\Delta Q = Q_x - Q_0$$

and

$$Q = \left[ I_+ I_x(\theta) - I_- / I_x(0) \right] / \left[ I_+ / I_x(0) \right]$$

(6)

The $Q$ is the measure of $I_+ / I_-$ at a set $\theta$ scattering angle relative to the same ratio at a 0 degree scattering angle. The subscripts $x$ and $f$ in equation 5 refer to xylose and fructose media respectively. The data presented in Fig. 3 describe the variation in $\Delta Q$ with the development of xylose cataract. Fig. 3, A indicates that the $\Delta Q$ value in the cortex is increasing rapidly at the beginning of the experiment and later levels off. This behavior was observed at all scattering angles measured. When the lens was organ-cultured without the lens capsule, the $\Delta Q$ indicator observed at wide scattering angles increased more than at low scattering angles. In lenses cultured with the lens capsule and adhering vitreous, the reverse was true, although the differences at different scattering angles were small. The nucleus showed a different behavior. The initial increase in $\Delta Q$ was followed by a decrease and further increase as the cataract developed. Furthermore, the increase in $\Delta Q$ in the nucleus still proceeded, while in the cortex this had already leveled off.

We may assume that the $\Delta Q$ represents a measure of the relative contribution of the optical anisotropy fluctuation to the total scattering in xylose cataract. It has been observed that the cortical region shows the development of the opacity before it spreads to the nuclear region. The sharper rise of $\Delta Q$ in the cortical region than in the nuclear region indicates that the optical anisotropy fluctuation is an important contributor to the opacity, especially at the beginning of cataract formation. Opacity develops as the osmotic pressure created by the accumulation of xylitol enhances the entrance of water and causes swelling. We may visualize the following scenario. At the beginning, the water accumulation is small and does not form large vacuoles that will contribute heavily to the density fluctuations; that is a later process. In the incipient cataract formation, the accumulation of water sufficiently changes the refractive index around the lens fiber membranes to cause a change in the form birefringence. This in turn upsets the balance between form and intrinsic birefringence, and a net total birefringence will be observable. These changes in total birefringence over areas large enough to be comparable to the wavelength of the light will give rise to the optical anisotropy fluctuations.

In the later stages, with more water influx into the lens, larger vacuoles will form, and some swelling within the fiber cells will occur. This will enhance the density fluctuation, although the orientation fluctuation may also increase. The fact that $\Delta Q$ levels off in the cortical region is in agreement with this explanation.

The nuclear region shows a two-stage development in the cataract formation (Fig. 3, B). The hydration of the nuclear region is a slower process, and therefore the $\Delta Q$ increases more slowly. A decrease in the $\Delta Q$ may mean a much larger contribution of the density fluctuation than that of optical anisotropy fluctuation or an actual decrease in the optical anisotropy fluctuation. It is interesting to note that in galactose cataract formation in rat lenses in vivo, there was a steady decrease in the $I_+ / I_-$ ratio with cataractogenesis, implying that, in vivo, part of the water accumulation in the cortex was at the expense of the dehydration of the nucleus. Here, in the organ-cultured lenses in xylose medium, there is sufficient water entering the lens from the medium, and therefore, no dehydration of the nucleus occurs as vacuolization appears in the cortex.

In summary, it was demonstrated that the $I_+$ component of light scattering increases as xylose cataractogenesis develops, and that the optical anisotropy fluctuation is an important contributor to the opacity, especially at the beginning of cataract formation.

These results encourage further studies to
quantitatively evaluate the individual scattering parameters $\bar{\eta}^2$, $\gamma(r)$, and $\delta^2(r)$. Only when this is accomplished will a full description of the physical process in cataractogenesis be possible.

REFERENCES


