Exp. Eye Res. (1994) 59, 393–400

Combined Elastic and Raman Light Scattering of Human Eye Lenses

I. V. YAROSLAVSKY[a], A. N. YAROSLAVSKY[a], C. OTTO[b], G. J. PUPPELS[b], G. F. J. M. VRENSEN[c*], H. DUINDAM[b,c] AND J. GREVE[b]

[a] *Saratov State University, Department of Optics, Astrakhanskaya 83, 410071 Saratov, Russia;* [b] *University of Twente, Department of Applied Physics, Biophysical Technology, P.O. Box 217, 7500 AE Enschede, The Netherlands and* [c] *The Netherlands Ophthalmic Research Institute, Department of Morphology, P.O. Box 12141, 1100 AC Amsterdam, The Netherlands.*

(Received Norwich 3 December 1993 and accepted 2 June 1994)

The distribution of the scattering coefficient (as defined in the appendix) at a wavelength of 647·1 nm along the visual axis of human eye lenses was investigated using a specially designed set-up for spatially resolved measurements of the intensity of the scattered light. For the same lenses, the distribution of the protein content was measured using confocal Raman microspectroscopy. Data collected by both methods were processed in terms of a recently developed theory of short-range, liquid-like order of crystallin proteins that accounts for eye lens transparency. Seven fresh intact human lenses of varying age have been investigated. In addition, elastic and Raman scattering measurements have been performed on fixed lenses.

The main results and conclusions are: (1) Fixation significantly affects the light scattering properties of the eye lens. The average level of scattering increases and a change in the distribution of scattered light intensity along the visual axis occurs. Protein content and average distribution were not altered by fixation. (2) There are significant differences between the distribution of the scattering coefficient for lenses of different ages. For young lenses (18 and 20 years) regions with a low protein content (anterior and posterior cortex) show a higher level of elastic light scattering, while for older lenses (42–78 years old) there is no obvious correlation between the scattering level and protein content. (3) Changes in the level of light scattering along the visual axis of the lens cannot be explained by protein concentration effects. Therefore, these changes must be caused by changes in the supramolecular organization of lens proteins in the fibre cytoplasm. (4) The observed changes in light scattering may be related to the zones of discontinuity as observed in slitlamp and Scheimpflug photography of human lenses.

Key words: elastic light scattering; Raman light scattering; protein content; human eye lens; zones of discontinuity.

1. Introduction

Transparency is an intrinsic property of the eye lens, which is of vital importance for its function *viz.* focusing of light on the retina. Scattering or absorption of light obviously leads to loss of transparency and reduces the optical quality of the lens. Light scattering is considered to be the main reason for the attenuation of the passing light since normal lenses contain no or only small amounts of chromophores that absorb in the visual region of the spectrum (Tardieu and Delaye, 1988; Duncan and Jacob, 1984).

Many studies have been devoted to the investigation of light scattering by the eye lens and to a clarification of the main principles that govern light scattering or changes in light scattering during ageing. Various experimental techniques were applied in these investigations, including angle-resolved measurements (Tuchin, Shubachkin and Maksimova, 1987; Bettelheim and Paunovic, 1979; Bettelheim and Sali, 1985), X-ray scattering of crystallin extracts (Tardieu and Delaye, 1988; Delaye and Tardieu, 1983), picosecond impulse scattering (Bruckner, 1978), photon correlation spectroscopy (Latina *et al.*, 1987; Van Laetham *et al.* 1991) and others. It was pointed out that short-range order of lens proteins plays an important role in the maintenance of lens transparency (Vérétout, Delaye and Tardieu, 1989; Delaye and Tardieu, 1983; Benedek, 1971).

The development of clinical diagnostic techniques for the lens has led to the appearance of devices, based on the Scheimpflug principle (Hockwin, Sasaki and Lerman, 1990). The principle of the Scheimpflug camera is that an undistorted image of an object is formed in the image plane, if the beam plane, the objective plane and the image plane intersect at one point O, and the angles α and β are equal. The Scheimpflug camera enables the registration of the distribution of light scattering throughout the lens in the plane of the incident beam in vivo. A remarkable feature of the measured light scattering distribution is the presence of so called lines of discontinuity, i.e. peaks in the distribution of the intensity of scattered light (Müller-Breitenkamp and Hockwin, 1992). However, the zones of discontinuity are most pronounced in lenses over 30 years of age.

Raman microspectroscopy is an effective tool in the study of the macromolecular composition of the eye

0014-4835/94/010393 + 07 $08.00/0 © 1994 Academic Press Limited

lens (Mizuno and Ozaki, 1991; Siebinga *et al.* 1992). In particular, it enables the determination of the local protein and water content in a small volume at defined locations inside the lens (Huizinga *et al.*, 1991).

The aim of the present study was to determine relations between the distribution of the light scattering coefficient and the local protein content of human eye lenses.

2. Materials and Methods

For the elastic light scattering measurements a set-up was built that operates on the same principles as the Scheimpflug camera. The lay-out of the set-up is shown in Fig. 1. The laser beam, emitted from a Kr^+-laser (10) (wavelength 647·1 nm, Coherent Innova 90 Series) is modulated by a chopper (9). A narrow collimated beam is produced in the telescope system formed by the lenses (1) and (2). The cuvette containing the eye lens is positioned on a translation stage (8), and the scattered light is collected by the lens (3) and focused on a pinhole (11) by a lens (4). The detector (5) (photodiode integrated with a current-to-voltage converter) is placed behind the pinhole. The motion of the translation stage along the z-axis allows the registration of the image of the light path inside the eye lens. Detector (6) measures the direct transmittance of the eye lens. Detector (7) monitors the fluctuations in the laser power and enables a correction for instabilities in the light source. In practice this was not necessary because of a stable laser output power.

The frequency of modulation of the incident light was 1 kHz. The pinhole diameter was 100 μm. The scattered light registration system was able to detect light levels as low as 50 nW. The estimated measuring volume for a medium with refractive index $n = 1$ was approximately 5×10^{-4} nm^3. The actual measuring volume in the eye lens was larger due to a higher refractive index of lens. The scattered light was collected in a solid angle of about 0·005 sr around a direction of 45° with respect to the direction of the incident beam. The eye lens was held in a specially made cuvet with a hemispherical hole. The cuvette was placed on the translation stage in such a way that the laser beam travelled along the optical axis of the lens. Since the refractive index of the cuvet glass was 1·53, the actual scattering angle was about 60°.

Figure 2 illustrates the spatial resolution that could be achieved in this set up in the direction of the incident beam. The laser light scattering at the glass–water interface was used as a reference point for these measurements. During measurements on eye lenses, the laser power never exceeded 50 mW.

The Raman measurements were performed with a confocal Raman microscope (CRM) described elsewhere (Siebinga et al., 1992; Puppels et al. 1991). A 10× objective (numerical aperture, NA = 0·25) was used. The measuring volume was approximately 90 μm^3, and is much smaller than the measuring volume obtained in the light scattering experiments (5×10^{-4} mm^3). The Raman spectra were recorded in

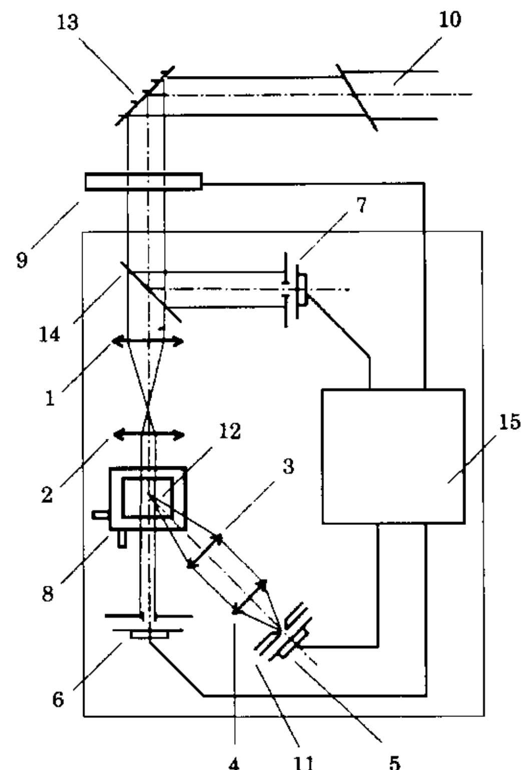

Fig. 1. Diagram illustrating the set up used for measuring the elastic light scattering of lenses in this study.
1–4: lenses; 5–7: photodetectors; 8: translation stage; 9: chopper; 10: laser; 11: pinhole; 12: sample; 13: mirror; 14: beam splitter; 15: control unit. (For further description see Materials and Methods).

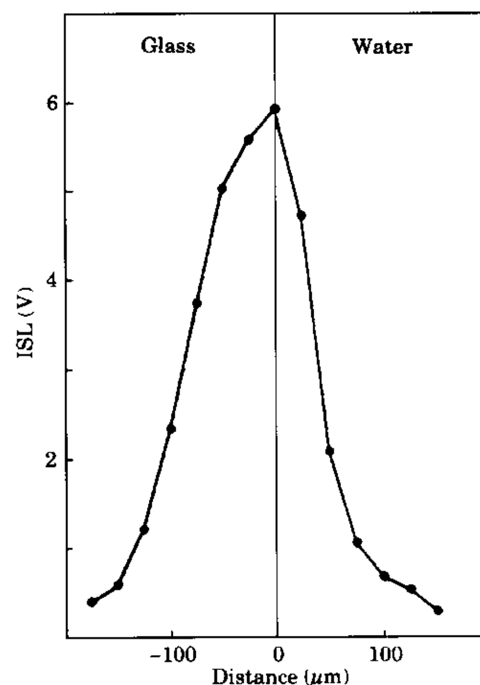

Fig. 2. The distribution of the intensity of scattered light (ISL; in volts) at a glass–water interface. Distance is indicated in micrometres. The diameter of the laser spot used was ~ 500 μm.

the spectral region 2200–3600 cm^{-1}, containing the aliphatic CH stretching modes of proteins (around 2935 cm^{-1}) and the OH stretching mode of water (3390 cm^{-1}). Part of the spectrum in the region 2200–2700 cm^{-1} was used for the precise determination of the background baseline (Fig. 4). Exposure times were between 10 and 15 sec.

Human lenses were obtained from donor eyes spent for cornea transplantation. The lenses were extracted within 24 hr post mortem. After extraction the lenses were kept at 35°C in artificial aqueous humour (AAH) according to Tomlinson and Duncan (1991). Pure AAH was used as a blank sample for direct transmittance measurements.

For both elastic and Raman scattering experiments, lenses were positioned in a cuvette filled with AAH. AAH itself did not show Raman bands in the spectral region of interest (excluding the water band) and had a neglectable level of elastic scattering.

The elastic scattering measurements were carried out first. The cuvette was positioned on the translation stage in such a way that the incident beam entered the anterior pole of the lens. Every lens was scanned along the visual axis twice. Immediately after these measurements, the Raman spectra were recorded. Only for the 18-year-old lens were light-scattering measurements carried out after fixation (see below) that had been shown to have only minor influence on the protein distribution (Huizinga et al., 1989). After the measurements lenses were fixed in a solution consisting of 0·08 M cacodylate buffered 1% paraformaldehyde (pH 7·3). Experiments on fixed lenses were performed with the cuvette containing a phosphate-buffered salt solution (PBS). The Raman data have been used to calculate the protein content at defined locations inside the lens, as described earlier (Huizinga et al., 1989; Siebinga et al., 1991). The following formula was used:

$$P = \frac{1}{1+AR} \times 100$$

where P is the protein content (percent), R is the ratio of the Raman intensities $I(3390)/2935$), A is an empirical constant, established to be equal to $2{\cdot}8 \pm 0{\cdot}1$ (Huizinga et al., 1989).

The exact location inside the eye lens from which the Raman spectra were obtained was determined from the geometrical displacement along the optical axis, taking into account the refractive index of the investigated medium. The small numerical aperture of the 10× objective allow the use of a simplified formula:

$$\Delta = n\Delta'$$

where Δ is the real distance between two points on the optical axis, Δ' is the distance read from the microscope knob, n is the refractive index of the sample. We accepted 1·42 as an average value of the refractive index for the eye lens (Lerman, 1980).

3. Results

An example of a Raman spectrum obtained from a 78-year-old lens from a position 2650 μm below the anterior pole is presented in Fig. 3. This spectrum shows that the CRM allows high quality spectra even from the nuclear region of an elderly lens.

In Fig. 4 the distribution of scattered light is presented for a lens before and after fixation. Although the fixation procedure has been shown not to affect the gross protein distribution it has a pronounced effect on the light scattering from an eye lens.

Fig. 5(A) to (C) show the distribution of scattered light intensity and protein distribution obtained from three human lenses varying in age: 20 years (A), 61 years (B) and 68 years (C). Note that the horizontal axis represents a normalized position in the eye lens. In this way it is possible to compare the results from lenses of different size.

From the elastic scattering data the distribution of

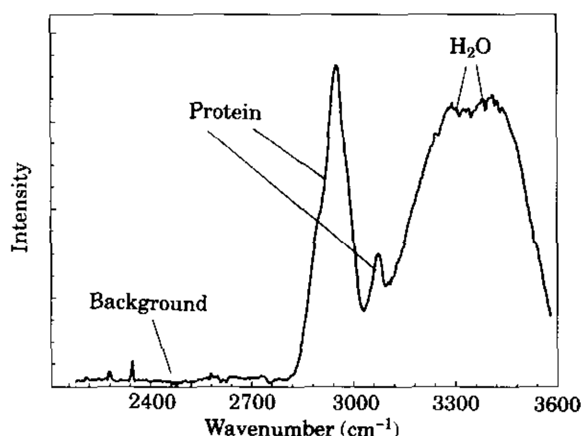

FIG. 3. Raman spectrum in the spectral range 2100–3600 cm^{-1} obtained from a 78-year-old human donor lens at a point 2650 μm below the anterior surface. Note the clear-cut protein peaks (2935 cm^{-1} aliphatic C–H vibration, 3100 cm^{-1}, aromatic C–H vibration) and OH vibrations at 2980 and 3390 cm^{-1}. For calculation of the protein content the ratio of the Raman intensities at 3390 and 2935 cm^{-1} were used (see Huizinga *et al.*, 1989).

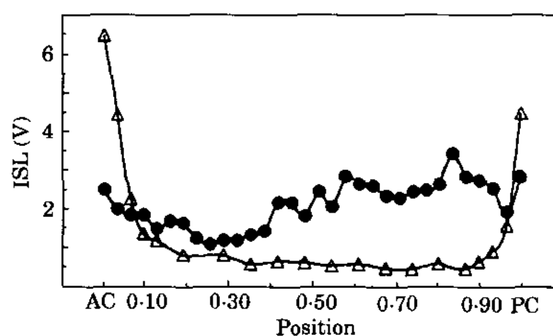

FIG. 4. Intensity of scattered light (ISL) along the visual axis of a human lens. Position normalized with respect to the distance between the anterior (AC) and posterior cortex (PC). ISL was measured in a fresh 18-year-old donor lens in AAH (triangles) and in PBS after prior fixation in cacodylate buffered paraformaldehyde (circles). Note the pronounced effect of fixation on ISL.

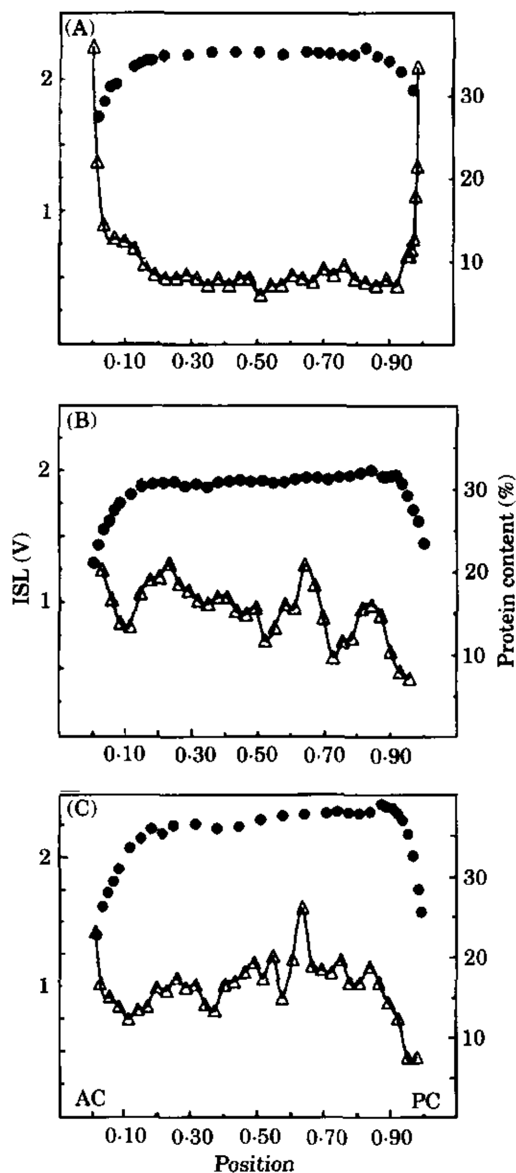

FIG. 5. Variations in the intensity of scattered light (ISL) (triangles) and protein content in per cent (circles) measured along the visual axis of human lenses. [(A) 20 years; (B) 51 years; (C) 68 years]. The visual axis position is normalized, (see legend to Fig. 4). Note the rapid increase in protein at the anterior and posterior pole and the constant level in the core and the significant variations in ISL throughout.

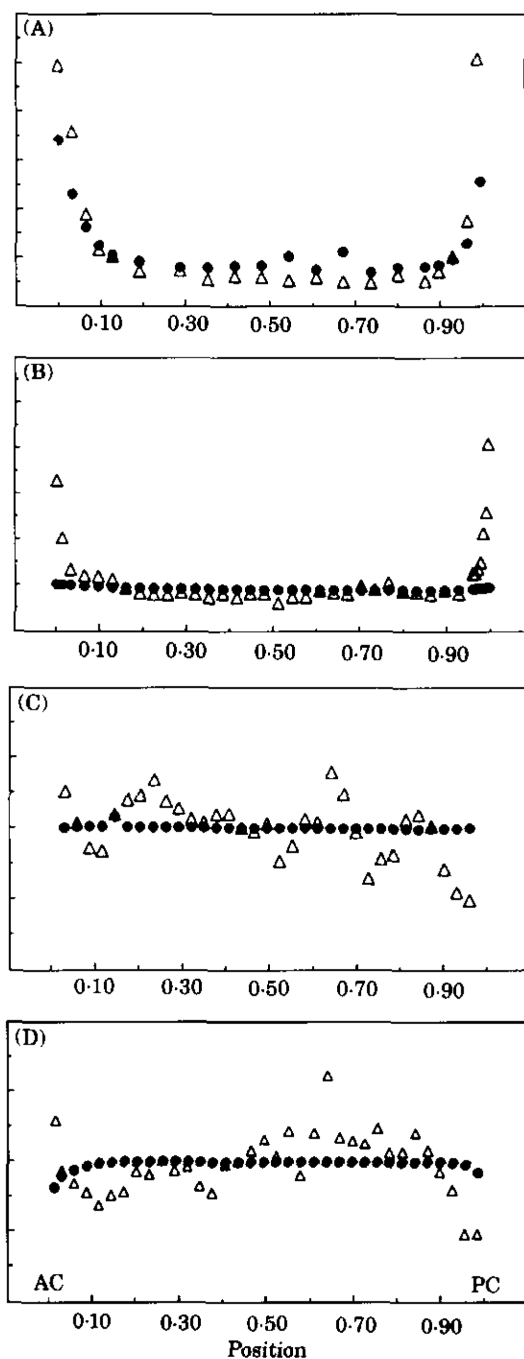

FIG. 6. Comparison of the measured (triangles) and predicted (circles) scattering coefficients (SC) as a function of the position along the visual axis (see legend to Fig. 4) of human lenses of varying age, [(A) 18 years; (B) 20 years; (C) 51 years; (D) 68 years]. The predicted values were calculated taking into account the estimated local protein concentration and eqns (5), (8) and (13) as theoretically outlined in the Appendix to this paper.

the scattering coefficient was calculated using the eqns (5), (8) and (13) with the experimentally obtained protein distribution. For a theoretical outline see the Appendix to this paper. The outermost points (with a distance to the lens surface $< 100\ \mu$m) have been excluded in order to avoid influence of surface scattering. In Fig. 6(A)–(D) the calculated results are compared with the experimental results for four fresh (i.e. not fixed) eye lens differing in age: 18 years, (A), 20 years (B), 51 years (C) and 68 years (D). This analysis has been done only for fresh lenses, since for fixed lenses eqn (5) is not valid due to the high optical density of these lenses. The values of the parameters C and D that are obtained by this method, are presented in Table I, as well as the values of M_w and σ estimated with the help of eqns 14 and 15 using for $c_0 = 1$ g ml^{-1}. This value corresponds to the average density of the human eye lens material (Maltsev, 1988).

4. Discussion

A main purpose of the present in-vitro study was to explore the relation between the local protein content and the local intensity of elastically scattered light in

TABLE I

Best-fit parameters of the lenses investigated in this study. C and D are dimensionless parameters necessary to estimate the average molecular weight M_w and average scatterer's diameter σ (in nm). M_w and σ are calculated for $C_0 = 1$ g ml^{-1}.

Age (years)	C	D	M_w, (g mol^{-1} $\times 10^6$)	σ (nm)
18	62·96	1·66	84·6	76·4
20	3·75	0·56	5·0	20·8
42	1·60	0·29	2·3	12·9
51	1·07	0·46	1·3	12·4
63	5·37	0·89	6·7	26·4
68	1·04	0·29	1·3	10·5
78	1·44	0·34	1·7	12·3

Note: Visual inspection of the 18-years-old lens revealed it to be more turbid than other young lenses for unknown reasons. The increased light scattering may indicate a larger size and weight of the scattering elements.

the human lens. In the two young (18 and 20 years of age) lenses [Figs 5(A) and 6(A) and (B)] a smooth inverse relation between protein content and elastically scattered light was observed. A smooth relation between local protein content and dynamically scattered light was also found for the rabbit (Latina et al., 1987). These observations are in qualitative agreement with predictions based on the theory of the short-range order of crystallins in the eye lens (Delaye and Tardieu, 1983; Tardieu and Delaye, 1988; Vérétout, Delaye and Tardieu, 1989; Van Laetham et al., 1991). For these young lenses a good agreement between the measured and depicted [eqn (13)] scattering coefficients was found, except for the extreme anterior and posterior surfaces which values are disturbed by surface scattering. The observations also agree with the observations in rabbit lenses and thus support the view of a gel-like structure of the eye lens proteins (Latina et al., 1987). However, for older human lenses [Fig. 5(B) and (C)] such a smooth relation between local protein content and light scatter is not observed. Although the overall averaged calculated molecular weight and diameter of scattering elements are close to published data, except for the 18-year-old lens, (Delaye and Tardieu, 1983; Tardieu and Delaye 1988; Vérétout, Delaye and Tardieu, 1989; Mizuno and Ozaki, 1991; Maltsev, 1988) we were not able to obtain a satisfactory fit between the experimental scattering coefficient and the scattering coefficients calculated on the basis of eqn (13) and the known protein content. The present observations reveal marginal variations in protein content between young and old lenses and within individual lenses along the optical axis. Although protein concentration is known to affect the light scattering the small variations observed in the lens core; maximally between 32 and 35%, cannot account for the large variations in light scattering (Bettelheim and Paunovic, 1979). So a more likely explanation for this discrepancy is that in the theory of the short-range order of crystallins as the basis for lens transparency, on which eqn (13) is based, polydispersity, aggregation and non-sphericity of the crystallins are not taken into account. So from the present observations it can be postulated that changes in the protein conformation such as polydispersity, aggregation and non-sphericity of the crystallins must vary among and within human lenses and change with age leading to significant age-related and local variations in light scattering. This is partly in line with the well known age-related increase in high molecular weight and insoluble proteins but additionally shows that crystallin changes are not simply reflecting the age of the particular part of the lens studied but are unevenly distributed within the eye lens. Recently it has been outlined by Tardieu et al. (1992) that the short-range or 'liquid-like' order of lens proteins is due to repulsive interactions between α and β crystallins. They also showed that, in contrast, γ crystallins exhibit attractive interactions depending on protein concentration and osmotic pressure leading to the formation of clusters or domains and consequently to increased light scatter. In view of this the local variations in elastic light scatter may also reflect local variations in γ-crystallin content and osmotic pressure.

The curves representing the variations in elastically scattered light [Figs 5(B) and (C)] show some resemblance with the densitograms obtained from human lens slitlamp and Scheimpflug pictures (Brown, 1973; Hockwin, Sasaki and Lerman, 1990; Müller-Breitenkamp and Hockwin, 1992) and the valleys and peaks in the curves could reflect the zones of discontinuity described for these pictures. This means that the zones of discontinuity possibly reflect local variations in light scatter due to changes in the order and tertiary and quaternary conformation of crystallins. For further proof elastic light scatter, Raman scatter and Scheimpflug photography has to be carried out in vitro on the same human lenses.

The present results on the protein distribution along the visual axis of the human eye lens are in excellent agreement with previous observations using Raman microspectroscopy (Huizinga et al., 1989; Siebinga et al., 1991) and various other techniques (for ref. see Siebinga et al., 1991). Results regarding protein distribution in fresh and fixed lenses (the latter not shown for brevity) indicated that fixation with a paraformaldehyde solution has no or only very minor effects on the average local distribution and content of proteins confirming a previous report on this point (Huzigina et al., 1989). In contrast to this the transmission of fresh eye lenses, averaging about 80%, dramatically dropped to about 15%. This strongly indicates that although the content and average distribution of proteins is not affected by fixation this procedure may lead to a dramatic change in the scattering properties of proteins, possibly due to

the fact that they are no longer in monometric form and that fixation affects the local distribution of proteins. This agrees well with the established view that aldehydes crosslink proteins thus stabilizing the structure of tissues.

Conclusion

The present comparison of the local distribution of protein concentration and the local intensity of elastically scattered light in the human lens reveals that although protein concentration is an important parameter for the overall light scatter the variations in configuration of the lens proteins leads to the significant local variations in light scatter as suggested by Scheimpflug and slitlamp images of human lenses.

Appendix

If the absorption of light in a medium can be neglected, then the attenuation of the incident beam is determined by scattering only. Let us denote the extinction coefficient α_z by

$$\alpha_z = \int_0^z \tau(z')\,dz', \quad (1)$$

where τ is the scattering coefficient, z is the distance along the beam axis, counted from the anterior pole $(0 \leqslant z \leqslant 1)$. α_1 is the optical length when l is the thickness of the lens.

The intensity I_s of light, scattered in a given direction θ from a determined point on the optical axis, is proportional to the scattering coefficient and to the intensity of light I illuminating this point:

$$I_s(z, \theta) = A_1(\theta) I(z) \tau(z), \quad (2)$$

Here A_1 is a constant, determined by the geometry of the experiment and by the scattering phase function $p(\theta)$.

In the case of Rayleigh scattering, eqn (2) takes the following form

$$I_{sR}(z) = I(z) \frac{3}{16\pi r^2} \tau(z) p_R(\theta), \quad (3)$$

where r is the distance from the irradiated volume to the observation point and $p_r(\theta)$ is the phase function for Rayleigh scattering.

If $\alpha_1 \ll 1$, Beer's law is satisfied

$$I(z) = I_0 \exp(-\alpha_z), \quad (4)$$

where I_0 is the intensity of incident light.

Assuming that $p(\theta)$ is independent on the position, it follows that

$$E_j = A_2 \tau_j \exp(-\alpha_j) \quad j = 1, \ldots, N_E \quad (5)$$

where E_j is the experimentally measured intensity of scattered light (volts), A_2 is a new experimental constant, α_j is α_z for the jth experimental point, N_E is total number of experimental points.

α_j may be approximated by

$$\alpha_j = \sum_{k=1}^{j} \tau_k X_k \quad (6)$$

where the set of coefficients X_k is determined by an evaluation of the integral in eqn (1) using the spline integration method.

On the other hand, the measurement of the direct transmittance T gives us value of α_1

$$\alpha_1 = \ln(1/T) \quad (7)$$

At the same time

$$\alpha_1 = \sum_{k=1}^{N_E} \tau_k X_k \quad (8)$$

Hence, we have a system of $N_E + 1$ equations [eqns (5) and (8)] that determine the set of τ_j and A_2. For the solution of the set of equations quasi-Newton's method is used. (Dennis and Schnabel, 1983).

Using the theory developed by Delaye and Tardieu (1983), Tardieu and Delaye (1988) and Véretout, Delaye and Tardieu (1989), a relation can be established between the measured values of τ with structure parameters of the lens protein system. Only a very simplified treatment has been done, assuming that Rayleigh scattering takes place and disregarding the possible polydispersity of the system.

It follows that:

$$\tau = (32\pi^3/3\lambda^4) n_0^2 (\partial n/\partial c)^2 (M_w c_p/N_A) S_0(c_p) \quad (9)$$

where M_w is the average molecular weight, C_p is the protein concentration in g cm^{-3}, n_0 is the refractive index of the solvent, $\delta n/\delta c$ is the refractive index increment due to the presence of the solute, N_A is Avogrado's number, $S_0(c_p)$ is the structure factor for the solution. Both τ and C_p depend on the position of Z.

It has been shown that protein solutions under physiological concentrations can be described by the model of hard spheres. Using the hard sphere model $S_0(c_p)$ can be expressed as a function of excluded volume fraction of solution ϕ

$$S_0(c_p) = (1-\phi)^4/(1+4\phi+4\phi^2-4\phi^3+\phi^4) \quad (10)$$

ϕ is linked to the diameter, σ, of the particle by the relation

$$\phi = (c_p N_A / 6M_w)\pi\sigma^3 \quad (11)$$

The experimentally measured quantities are the protein concentration (in per cent) P_i (1., N_R). N_R is the number of experimental points in the Raman measurements. Then

$$c_{pi} = P_i c_0 \quad (12)$$

where is c_0 is the total density (g cm^{-3}) of the lens material. It is assumed that c_0 is independent of the position. Using a spline interpolation technique, the protein concentration P_j $(j = 1, \ldots, N_E)$ can be estimated in points where the scattering coefficient has been measured. Now τ_j can be written as

$$\tau_j = P_j C S_0(\phi_j) \quad (13)$$

where $\phi_j = P_j D$, C and D are dimensionless parameters, defined by the expressions:

$$C = 1(32\pi^3/3\lambda^4)n_0^2(\partial n/\partial c)/^2(M_w c_0/N_A) \quad (14)$$

$$D = \frac{\pi N_A \sigma^3 c_0}{6M_w} \quad (15)$$

A set of scattering coefficients τ_j has been obtained from eqns (5) and (8). The least squares method is used to calculate parameters C and D. The molecular weight and radius of the scattering particles can thus be determined. The following target function was minimized

$$f(C, D) = \sum_{j=1}^{N_g} (\tau'_j - \tau_j)^2 W_j \quad (16)$$

where τ_j are calculated using eqn (13). W_j is weight, assigned to jth point. If parameters C and D are calculated and c_0 is known, we can estimate M_w and σ using eqns (14) and (15). The value of $(\delta n/\delta c) = 0{\cdot}185$ as taken from Vérétout *et al.*, 1989.

Acknowledgements

The authors are grateful to Dr E. Pels and her coworkers from the Cornea Bank Amsterdam for collecting the human lenses and I. Segers-Nolten and B. Leerkotte from the University of Twente for excellent technical assistance. We are grateful to Dr J. Clauwaert (University of Antwerp) for valuable discussions. We are indebted to the 'Stitching het Haagsch Oogheelkundig Fonds' (HOF) for their financial support regarding the dye laser used in this study.

References

Benedek, G. B. (1971). Theory of Transparency of the Eye. *Appl. Opt.* **10**, 459–73.

Bettelheim, F. A. and Paunovic, M. (1979). Light scattering of normal human lens. 1. Application of random density and orientation fluctuation theory. *Biophys. J.* **26**, 85–99.

Bettleheim, F. A. and Ali, S. (1985). Light scattering of normal human lens. 3. Relationship between forward and back scatter of whole excised lenses. *Exp. Eye Res.* **41**, 1–9.

Brown, N. (1973). Quantitative slit-image photography of the lens. *Trans. Ophthalmol. Soc. U.K.* **42**, 303–17.

Bruckner, A. P. (1978). Picosecond light scattering measurements of cataract microstructure. *Appl. Opt.* **17**, 3177–83.

Delaye, M. and Tardieu, A. (1983). Short-range order of crystallin proteins accounts for eye lens transparency. *Nature*, **302**, 415–7.

Dennis, J. E. and Schnabel, R. B. (1983). *Numerical Methods for Unconstrained Optimization and Nonlinear Equations.* Prentice-Hall: Englewood Cliffs.

Duncan, G. and Jacob, T. J. C. (1984). The lens as a Physiocochemical System. In: *The Eye*, Vol. 1b (Ed. Dawson, H.) Pp. 159–207. Academic Press: Orlando.

Huizinga, A., Bot, A. C. C., De Mul, F. F. M., Vrensen, G. F. J. M. and Greve, J. (1989). Local variation in absolute water content in human and rabbit eye lenses measured by Raman microspectroscopy. *Exp. Eye Res.* **48**, 487–96.

Hockwin, O., Sasaki, K. and Lerman, S. (1990). Evaluating cataract development with the Scheimpflug camera. In *Noninvasive Diagnostic Techniques in Ophthalmology*, (Ed. Masters, B. R.). Pp. 281–318. Springer Verlag: New York.

Latina, M., Chylack, Jr., L. T., Fagerholm, P., Nishio, I., Tanaka, T. and Palmquist, B. M. (1987). Dynamic light scattering in the intact rabbit lens. Its relation to protein concentration. *Invest. Ophthalmol. Vis. Sci.* **28**, 175–83.

Lerman, S. (1980). *Radiant Energy and the Eye.* Macmillan: New York.

Maltsev, E. V., Oud, J. L. and Brakenhoff, G. J. (1988). *The Eye Lens Medicina*: Moscow, (in Russian).

Mizuno, A. and Ozaki, Y. (1991). Aging and cataractous process of the lens detected by laser Raman spectroscopy. *Lens Eye Toxicity* **8**, 177–87.

Müller-Breitenkamp, U. and Hockwin, O. (1992). Scheimpflug photography in clinical ophthalmology. *Ophthalmic Res.* **24**, 47–54.

Puppels, G. J., Colier, W., Olminkof, H. J. M., Otto, C., De Mul, F. F. M. and Greve, J. (1991). Description and performance of a highly sensitive confocal Raman microspectrometer. *J. Raman Spectrosc.* **22**, 217–25.

Siebinga, I., Vrensen, G. F. J. M., Otto, K., Puppels, G. J., De Mul, F. F. M. and Greve, J. (1992). Aging and changes in protein conformation in the human lens: a Raman microspectroscopic study. *Exp. Eye Res.* **54**, 759–67.

Siebinga, I., Vrensen, G. F. J. M., De Mul, F. F. M. and Greve, J. (1991). Age-related changes in local water and protein content of human eye lenses measured by Raman microspectroscopy. *Exp. Eye Res.* **53**, 233–9.

Tardieu, A., Delaye, M. (1988). Eye lens proteins and transparency, from light transmission theory to solution X-ray structural analysis. *Ann. Rev. Biophys. Chem.* **17**, 47–70.

Tardieu, A., Vérétout, F., Krop, B. and Slingsby, C. (1992). Protein interactions in the calf eye lens: interactions between β-crystallins are repulsive whereas in γ-crystallins they are attractive. *Eur. Biophys. J.* **21**, 1–12.

Tomlinson, J. and Duncan, G. (1991). Membrane models for cortical cataract: pCMPS-induced changes in lens Na^+ and transparency can be modified by channel antagonists. In *Eye Lens Membranes and Aging*. (Eds. Vrensen, G. F. J. M. and Clauwaert, J.) Pp. 73–86. Topics Aging Res. Europe. Vol. 15.

Tuchin, V. V., Shubochkin, L. P. and Maksimova, I. L. (1987). Laser light scattering by anisotropic binary biological objects. (eye medium treatment). In *Laser Scattering Spectroscopy of Biological Objects.* (Eds. Stepanek, J., Anzenbacher, P. and Sedlacek, B.) Pp. 611–620. Elsevier: Amsterdam.

Van Laethem, M., Babusiaux, A., Neetens, and Clauwaert, J. (1991). Photon correlation spectroscopy of light scattered by eye lenses in *in vivo* conditions. *Biophys. J.* **59**, 433–44.

Vérétout, F., Delaye, M. and Tardieu, A. (1989). Molecular basis of eye lens transparency: osmotic pressure and X-ray analysis of α-crystalline solutions. *J. Mol. Biol.* **205**, 713–28.

Visser, T. D., Oud, J. L. and Brakenhoff, G. J. (1980). Refractive index and axial distance measurements in 3-D microscopy. *Optik*, **90**, 17–19.