

## Dynamic light-scattering measurement for a salt-induced cataract in the eye lens of a chicken

K. Hamano, N. Kuwahara, B. Chin, and K. Kubota

*Department of Biological and Chemical Engineering, Faculty of Technology, Gunma University, Kiryu, Japan*

(Received 23 January 1990; revised manuscript received 31 July 1990)

We study the dynamical behavior in the eye lens of a chicken inflicted with a cataract induced by a salt solution. The mean diffusion coefficient  $\bar{D}$  in the cataracted lens estimated in this work is approximately expressed by a power law as a function of the salt concentration of  $|C - C^*|$ . Our experimental findings suggest that the salt concentration should be a relevant parameter for describing the dynamical behavior in a cataracted lens. The magnitude of  $\bar{D}$  obtained for the cataracted lens is approximately five times smaller than that for the normal one, which is almost compatible with the earlier work of Tanaka and Benedek [*Invest. Ophthalmol.* **14**, 449 (1975)]. The diffusive decay in the salt-induced cataract, which should be regarded as an osmotic type of cataract, has been discussed in terms of the phase separation associated with the lens opacification following the concept of Tanaka, Ishimoto, and Chylack [*Science* **197**, 1010 (1977)].

### I. INTRODUCTION

Great attention has been directed to understanding the mechanisms of the cataract in the eye lens, for example, of humans and cattle,<sup>1,2</sup> rabbits,<sup>3,4</sup> and rats.<sup>4,5</sup> A cataract is clinically defined as an opacity of the lens that impairs vision. The origins and processes of the opacity in the lens have been extensively investigated from the biochemical and morphological standpoints.<sup>6</sup> For example, biochemical investigators have suggested the accumulation of polyols in the lens plays an important role in the formation of diabetic and galactosemic cataracts. The increase in polyols in the lens may result in osmotic swelling. This type of cataract has been recognized as an osmotic cataract.<sup>3</sup>

The main components of the lens are about 65 wt. % water, 35 wt. % proteins, and a small amount of salts. The proteins in the transparent eye lens consist of about 90% of the soluble structural proteins called crystallins and a small percentage of insoluble proteins called the albuminoid fraction whose physical properties are still unknown.<sup>7,8</sup> The  $\alpha$ -crystallin has a high molecular weight of about  $10^6$  daltons and the  $\gamma$ -crystallin has a small one of about  $2 \times 10^4$  daltons. The  $\beta$ -crystallins have a roughly intermediate one between the  $\alpha$ - and  $\gamma$ -crystallins, some of which elongate into lens fiber cells and play an important role in an interaction of these structural proteins with the cytoskeleton and plasma membranes. These proteins comprise at least 50% of the soluble proteins in the mature lens of most vertebrates. The  $\delta$ -crystallins are highly lens-specific substances in birds and reptiles, which are the principal protein of the embryonic chicken lens and constitute a major soluble protein of the mature chicken lens.<sup>9</sup> These crystallins also consist of several subunits; for example, the  $\delta$ -crystallins consist of four subunits with about  $5 \times 10^4$  daltons.<sup>10</sup> The crystallins are quite various and comprise a complex system with different types of these structural proteins and the

aqueous humor in the lens. The transparency and refractive properties of the lens have been attributed to a gentle concentration gradient associated with the crystallins, along the radius from the periphery to the center of the lens.<sup>6</sup>

An attractive approach has been made for the cataract induced experimentally by lowering the temperature of the excised lens, the so-called cold cataract, in terms of vapor-liquid phase transition and phase separation in a binary mixture, by Tanaka and co-workers.<sup>11</sup> They have found a remarkable resemblance between the development of opacity in the cold cataract and the appearance of turbidity in protein-salt-water mixtures. Their experimental result suggests that the opacification point of the lens may depend on the radial position in the lens, which could be a function of the concentration of proteins in water. This leads us to infer a possible phase boundary of mixing, whose order parameter could be regarded as the concentration difference between two different radial positions with the origin of the opacity at the same temperature. Such a situation may be interpreted as the relaxation behavior at the fixed radial position of the lens depending on the temperature along the isochore in analogy with the phase separation of a fluid mixture. They have suggested that the temperature-induced cataract is essentially due to a phase separation of a quasibinary mixture of proteins and water in the lens. It is quite interesting to investigate the cataract in the lens in terms of critical dynamics, not only to reveal the cooperative properties in the cataracted lens, but also to obtain information associated with the nature of phase separation in a complex system such as that of biological structural substances. In addition, it is of interest to study the cataracted lens in the chicken, from the viewpoint of a comparison with those in the mammalian lenses. In this work we try to investigate the relaxation behavior of the fluctuations in the salt-induced cataracted lens of a chicken by use of the dynamical light-scattering measurement.

It is thought that the eye lens consists of a great num-

ber of individual fiber cells with an approximately homogeneous mixture of lens proteins and water. Therefore, the mixture in a single cell should be regarded as a quasi-binary mixture, which consists mainly of lens proteins and water. The decay rate  $\Gamma$  associated with the diffusive decay of the concentration fluctuations can be determined by measuring the time-dependent correlation function of the scattered light from the lens. In general, the dynamic phenomenon of a critically binary mixture is that the concentration fluctuations decay very slow in time, the so-called critical slowing down.<sup>12</sup> The asymptotic behavior of  $D = \lim_{k \rightarrow 0} (\Gamma/k^2)$  with  $k$  being the magnitude of the momentum-transfer wave vector, which is related to the correlation length  $\xi$  and the viscosity  $\eta$ , can be represented by the Einstein-Stokes-Kawasaki law as<sup>13</sup>

$$D = \frac{Rk_B T}{6\pi\eta\xi}, \quad (1)$$

where the universal amplitude  $R$  has a value of a few percent larger than unity. In the region far from the critical point of mixing, the diffusion coefficient estimated from the  $k^2$  dependence of  $\Gamma$  may be approximately equivalent to that in the hydrodynamic limit.

## II. EXPERIMENT

The lens employed in this work was removed carefully from the eye of a mature chicken. The excised lens was quite transparent and a strong enhancement of turbidity was observed when the concentration of NaCl in water was increased. Dynamic light-scattering measurements were performed to obtain the autocorrelation function of the scattered light from the lens by use of a cylindrical cell with an optical-path length of 0.6 cm. The autocorrelation function was measured for the normal lens immersed in silicon oil and the cataracted one immersed in a NaCl aqueous solution over the concentration range from 0.1 to 0.7 mol/liter in NaCl with water at the fixed angle of  $90^\circ$ , which corresponds to a wave number of  $k \approx 2.5_9 \times 10^5 \text{ cm}^{-1}$  with an argon-ion laser as the light source ( $\lambda_0 = 488 \text{ nm}$ ). We used the value of  $n \approx 1.4_2$  as the refractive index for the lens-protein mixture. The incident light beam was passed through each lens surface at the center of the lens. We carefully adjusted the position of the sample cell to observe the center of the lens. We measured the autocorrelation function for the cataracted lens over a fixed range of total delay time from approximately 3 to 30  $\mu\text{s}$  waiting 3 h after the preparation of the sample. The autocorrelation function was also measured for the contents of the lens, i.e., the lens-protein mixture with the aqueous humor, having removed the crystalline capsule from the intact normal lens at four different angles of  $\theta = 30^\circ, 45^\circ, 60^\circ,$  and  $90^\circ$ . In order to compare the experimental results we performed light-scattering measurements for the intact normal lens and the interior lens-protein mixture without the crystalline capsule at two different angles of  $\theta = 60^\circ$  and  $90^\circ$  using a He-Ne laser ( $\lambda_0 = 638 \text{ nm}$ ). Water and silicon oil baths were used for light-scattering photometers, with Ar-ion and He-Ne lasers, respectively, as a light source. The temperature of

each bath for the sample cell was controlled to  $20^\circ\text{C}$ .

The phototube signal was analyzed by a 48-channel, single-clipped correlator. The observed autocorrelation function for the lens showed systematic deviation from a single exponential decay law. Therefore, we estimate the mean decay rate  $\bar{\Gamma}$  from an analysis of the correlation function  $F(k, \tau)$  with a cumulant expansion truncated after the second term by<sup>14</sup>

$$F(k, \tau) = B[1 + A |g(\tau)|^2], \quad (2)$$

$$\ln g(\tau) = -\bar{\Gamma}\tau + \frac{1}{2}\mu_2\tau^2,$$

where  $B$  is the base line and  $A$  the coherence factor that depends on the instrument geometry and the experimental condition. The normalized second cumulant  $k_2 = \mu_2/\bar{\Gamma}^2$  is used to characterize the magnitude of the deviations from the exponential decay. If  $g(\tau)$  has exponential decay, then  $\mu_2 = 0$ , i.e.,  $k_2 = 0$ . The optical alignment and the test of the correlator were achieved using a centrifuged colloidal silica solution and polystyrene latex with a diameter of  $0.091 \mu\text{m}$  (Dow Chemical Company, Lot No. 1A82). In light-scattering experiments, the light scattered from dust in the sample and from the reflected beam at the glass surface may apparently cause additional effects in the value of  $k_2$ . It is considered that the interior of the eye lens should be essentially dust free. We have tried to estimate the  $k_2$  value for the polystyrene latex following Eq. (2) by measuring over the angular range of  $45^\circ \leq \theta \leq 125^\circ$  in a step of  $10^\circ$ ; for example,  $k_2 = 0.01 \pm 0.01$  at  $\theta = 45^\circ$ ,  $k_2 = 0.012 \pm 0.011$  at  $\theta = 90^\circ$ , and  $k_2 = 0.013 \pm 0.010$  at  $\theta = 125^\circ$  for the light-scattering photometer with the He-Ne laser. The quoted error represents a standard deviation. The  $k_2$  value averaged over the experimental angular range was estimated to be  $k_2 = 0.017 \pm 0.009$ , which was in reasonable agreement with that expected for our sample of the latex. We have detected no contributions due to the reflected light in the magnitude of the effect in the case of the analysis for the polystyrene latex. The coherence factor  $A$  calculated from the magnitude of the correlation function was about 0.4 at  $\theta = 90^\circ$  for the system using the He-Ne laser. On the other hand, the  $k_2$  in the polystyrene latex yielded a slightly larger value; for example,  $k_2 = 0.05 \pm 0.01$  at an angle of  $\theta = 90^\circ$  using the photometer with the Ar-ion laser with the water bath. The diameter of the latex was estimated to be  $0.092 \pm 0.003 \mu\text{m}$  with the relation  $\bar{D} = \bar{\Gamma}/k^2$  and the viscosity of pure water. A slightly larger value in  $k_2$  would be attributed mainly to the reflected light at the glass surface in the water bath. We will treat this magnitude of deviation as an error included in the analysis of our experimental data for the eye lens. In this case, the factor  $A$  was about 0.76, which corresponds to roughly one coherence area. The light-scattering photometer, the digital autocorrelator, the optical alignment, the thermometer, and experimental details have been fully described elsewhere.<sup>15</sup>

## III. RESULTS AND DISCUSSION

In order to investigate the  $k^2$  dependence in the decay rate, the mean decay rate  $\bar{\Gamma}$  for the lens-protein mixture

without the crystalline capsule is plotted against  $k^2$  in Fig. 1, in which the open circle denotes the value of  $\bar{\Gamma}$  estimated following Eq. (2). A typical error in  $\bar{\Gamma}$  was evaluated to be about 2%. The mean decay rate is acceptable when the  $k_2$  value is fairly small, but care must be taken to account for the distribution in the decay rate in the case of a large value of  $k_2$ .<sup>16</sup> In the present analysis the  $k_2$  values measured for the lens-protein mixture without the capsule were significantly large, for example,  $k_2=0.62\pm 0.08$  at  $\theta=90^\circ$  and  $k_2=0.7\pm 0.2$  averaged over the experimental angular range, that it would be advantageous to try an additional analysis in terms of the decay-rate distribution.<sup>17</sup>

The lens of the eye is one of the few structures whose cells are preserved without turnover of their contents. Growth of the lens depends on the proliferation of these cells at the front, some of which are pushed from this region around the rim, toward the back. As cells move to the rear, they stop dividing, synthesize crystallins, and differentiate into lens fibers. Therefore, the types of crystallins filling the earlier generations of the lens fibers are different from those of the later generations. Thus it is natural to infer that the scattered light could be collected from the scattering region, including a number of individual fiber cells with approximately homogeneous constituent lens proteins, suggesting a superposition of exponentially relaxing processes that leads to a distribution of the decay rate, i.e., the relaxation times. If the relaxing quantity is considered as arising from the additive contributions of exponentials, the time-dependent correlation function  $g(\tau)$  would be written in the form

$$g(\tau) = \int G(\Gamma) \exp(-\Gamma\tau) d\Gamma, \quad (3)$$

where  $G(\Gamma)$  is the decay-rate distribution function with the normalization condition  $\int G(\Gamma) d\Gamma = 1$ . We have tried to analyze tentatively our experimental data with the help of a multiexponential expression of the measured autocorrelation function for simplicity, as shown in Fig. 2, in which the effective distribution function  $G(\Gamma)$  con-

structed from the measured correlation function  $g(\tau)$  is represented by the height of a segmented  $\delta$  function varied from the lower to the upper boundaries in the  $\Gamma$  space.<sup>18</sup> Our result shows that the distribution in the decay rate is significantly widespread in the  $\Gamma$  space. To investigate the validity in  $\bar{\Gamma}$  and  $k_2$  following Eq. (2), we calculated the average decay rate  $\bar{\Gamma} = \int \Gamma G(\Gamma) d\Gamma$  and  $\mu_2 = \int (\Gamma - \bar{\Gamma})^2 G(\Gamma) d\Gamma$  in terms of  $G(\Gamma)$ . The values calculated for  $\bar{\Gamma}$  and  $k_2$  were  $\bar{\Gamma} \approx 1.66 \times 10^3 \text{ s}^{-1}$  with  $k_2 \approx 0.7$  at  $\theta=30^\circ$ ,  $\bar{\Gamma} \approx 4.21 \times 10^3 \text{ s}^{-1}$  with  $k_2 \approx 0.8$  at  $\theta=45^\circ$ ,  $\bar{\Gamma} \approx 9.46 \times 10^3 \text{ s}^{-1}$  with  $k_2 \approx 0.6$  at  $\theta=60^\circ$ , and  $\bar{\Gamma} \approx 1.79 \times 10^4 \text{ s}^{-1}$  with  $k_2 \approx 0.6$  at  $\theta=90^\circ$ , in excellent agreement with those obtained by Eq. (2). In the present case the experimental data could be described by a quadratic in  $\tau$  following Eq. (2), as evidenced by the fact that the values of  $\bar{\Gamma}$  and  $k_2$  were virtually consistent with those averaged in terms of the distribution function of  $G(\Gamma)$ . This suggests that the mean decay rate  $\bar{\Gamma}$  approximately obeys the  $k^2$  dependence, as shown by the dotted line in Fig. 1. We estimated the value of  $\bar{D} = (2.65 \pm 0.10) \times 10^{-7} \text{ cm}^2/\text{s}$  for the mean diffusion coefficient in the lens-protein mixture without the capsule by the relation  $\bar{D} = \bar{\Gamma}/k^2$ . On the other hand, the most probable decay rate  $\Gamma_m$  is also plotted against  $k^2$ , designated by the closed circle in Fig. 1. The ratio of  $\Gamma_m$  to  $\bar{\Gamma}$  varied from about 1.7 to 1.4 over the entire experimental range of angles.  $\Gamma_m$  was also able to fit the straight line with the slope corresponding to the most probable diffusion coefficient of  $D_m = \Gamma_m/k^2 \approx 3.7 \times 10^{-7} \text{ cm}^2/\text{s}$ , as shown by the solid line in Fig. 1. In Fig. 3 we have shown the profiles of the distribution in terms of  $G(D)$  with the  $D$  space, in comparison with those analyzed for the intact normal lens. The circles and squares represent the results measured at  $\theta=60^\circ$  and  $90^\circ$  for the intact normal lens using a He-Ne laser as a light source. The hexagons and triangles represent the results measured at  $\theta=60^\circ$  and  $90^\circ$ , respectively, for the lens-protein mixture without the crystalline capsule using the Ar-ion laser. For comparison of the present results with those of a

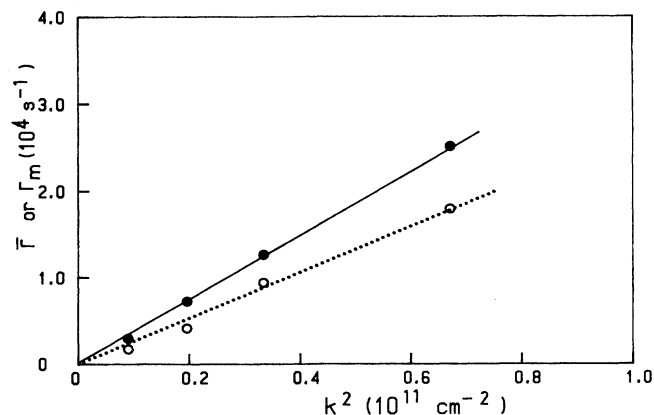


FIG. 1. The plots of the mean decay rate ( $\circ$ ) and the most probable decay rate ( $\bullet$ ) against  $k^2$  for the lens-protein mixture without the crystalline capsule at  $20^\circ\text{C}$ .

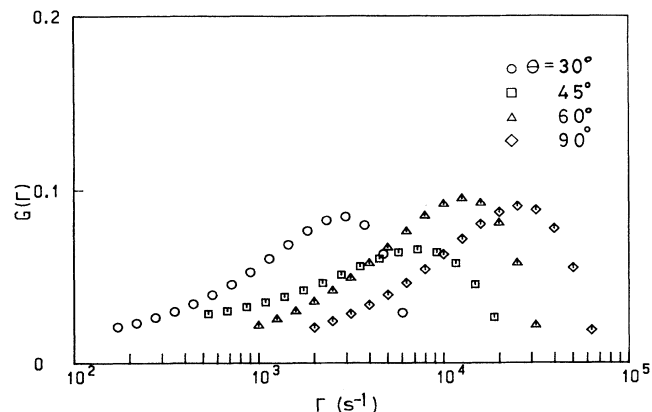


FIG. 2. The effective distribution function  $G(\Gamma)$  in  $\Gamma$  space for the lens-protein mixture without the capsule at the scattering angles of  $\theta=30^\circ$  ( $\circ$ ),  $45^\circ$  ( $\square$ ),  $60^\circ$  ( $\triangle$ ), and  $90^\circ$  ( $\diamond$ ).

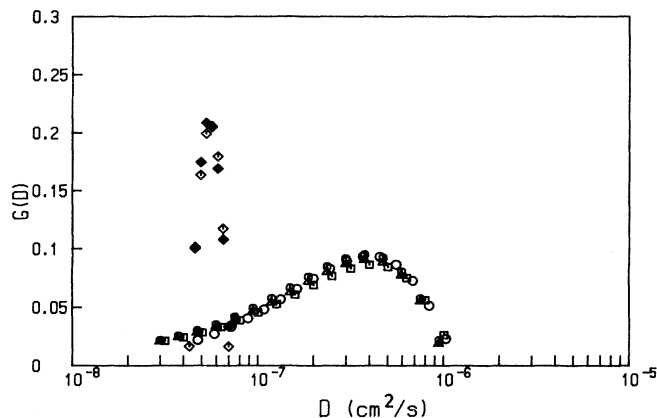


FIG. 3. The effective distribution function  $G(D)$  in  $D$  space for the intact normal lens at the angles of  $\theta=60^\circ$  ( $\circ$ ) and  $90^\circ$  ( $\square$ ) using the He-Ne laser and for the lens-protein mixture without the capsule at the angles of  $\theta=60^\circ$  ( $\diamond$ ) and  $90^\circ$  ( $\triangle$ ) using the argon-ion laser, respectively. The function  $G(D)$  is normalized as  $\int G(D)dD=1$ . The open and closed diamonds denote the narrow distribution function for the polystyrene latex as a reference substance.

reference substance we have shown the results for the polystyrene latex analyzed in a similar way, represented in the figure by open and closed diamonds. The ratios  $D_m/\bar{D}$ , i.e.,  $\Gamma_m/\bar{\Gamma}$ , appearing in Fig. 3 have almost the same value of about 1.4, irrespective of the condition measured here. In this estimation the mean diffusion coefficient  $\bar{D}$  evaluated for the intact lens was about 10% larger than that for the lens-protein mixture without the capsule. For the intact lens there may exist an ambiguity in the observed angle of scattering due to a specific form such as a convex lens in silicon oil. We roughly estimated this uncertainly in the wave number  $k$  to be about 3% with a refractive index of  $n \approx 1.39$  for silicon oil and radii of curvature of 10 and 6 mm in each lens surface, which yielded an error of about 6% in the  $\bar{D}$ . The displacement from the center of the lens along the incident beam in the observed region was also estimated to be about 0.1 mm, which was about 5% of the thickness of the lens and which is ignored in the present analysis. Thus we are able to estimate  $\bar{D}=(3.34 \pm 0.54) \times 10^{-7}$  cm<sup>2</sup>/s with  $k_2=0.6 \pm 0.2$  for the intact normal lens, including a 6% error in the  $\bar{D}$  value, and  $\bar{D}=(2.64 \pm 0.70) \times 10^{-7}$  cm<sup>2</sup>/s with  $k_2=0.7 \pm 0.4$  for the lens-protein mixture without the crystalline capsule by averaging over the values in each of the three samples of the chicken lenses. Both numerical values in the diffusion coefficient for the normal lens-protein mixture are in rough agreement within estimate errors.

In the present analysis, the effect of possible nonergodicity,<sup>19,20</sup> which could result from the restricted concentration fluctuations within the fiber cells, is not considered. Regarding this point, the works of Pusey and van Megen<sup>19</sup> are worth noting, and the very large value of the obtained  $k_2$  may be due partly to this nonergodic behavior. In such a treatment, the correction to  $\bar{D}$  for this effect following Pusey and van Megen was evaluated

to be about 20% and 4% for the results with the He-Ne and the Ar-ion lasers, respectively. These magnitudes of the correction were within the estimated errors of the present analysis, and this nonergodic effect could be included.

When the excised lens was immersed into the NaCl aqueous solutions the appearance of turbidity in the lens was observed. We call this opacification in the lens a salt-induced cataract, which should be regarded as an osmotic type of cataract. In fact, we observed a swollen and cataracted opacity in the lens when the normal one was immersed into the NaCl solution. A noticeable shrunken and fully developed cataract was also observed at a higher concentration of about 0.7 mol/liter. The mean diffusion coefficients evaluated from  $\bar{\Gamma}$  following Eq. (2) in such a cataracted lens are examined as a function of the concentration of NaCl with water in Fig. 4, in which the data points plotted are averaged over the values measured for two or three cataracted lenses. The mean diffusion coefficient in the cataracted lens decreases monotonously from  $\bar{D}=(3.10 \pm 0.09) \times 10^{-7}$  cm<sup>2</sup>/s at  $C=0.1$  mol/liter to  $\bar{D}=(6.9 \pm 0.7) \times 10^{-8}$  cm<sup>2</sup>/s at  $C=0.43$  mol/liter and, then, increases from  $\bar{D}=(8.5 \pm 0.7) \times 10^{-8}$  cm<sup>2</sup>/s at  $C=0.45$  mol/liter to  $\bar{D}=(2.3 \pm 0.2) \times 10^{-7}$  cm<sup>2</sup>/s at  $C=0.6$  mol/liter. These behaviors are shown as open and closed circles in Fig. 4, respectively. The value of  $\bar{D} \approx 7 \times 10^{-8}$  cm<sup>2</sup>/s at  $C \approx 0.43$  mol/liter, which was also observed for the fully developed cataracted lens at  $C=0.7$  mol/liter, is approximately five times smaller than that for the intact normal lens. We have tried the plot of the  $\bar{D}$  as a function of the concentration difference  $|C-C^*|$  with  $C^*=0.44$  mol/liter in double logarithms in Fig. 5, including the value for the intact normal lens in silicon oil. The corresponding values of  $k_2$  are plotted in the figure as triangles. This figure shows that the diffusion coefficient  $\bar{D}$  in the salt-induced cataract may be approximately

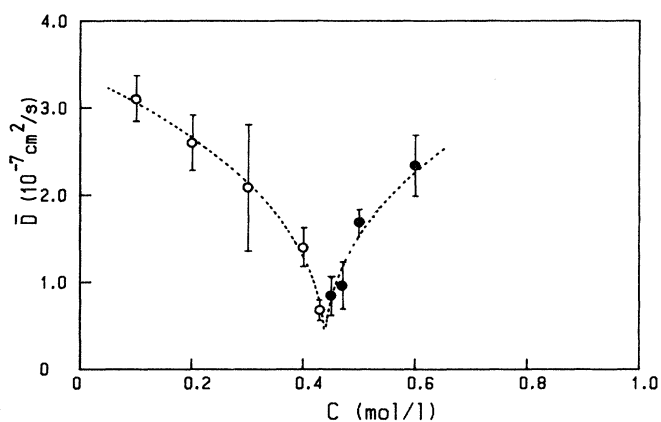


FIG. 4. The mean diffusion coefficient  $\bar{D}$  as a function of the concentration of NaCl in water for the cataracted lens induced by the NaCl aqueous solution. The open and closed circles represent the values at concentrations lower and higher than the salt concentration of  $C=0.44$  mol/liter, respectively.

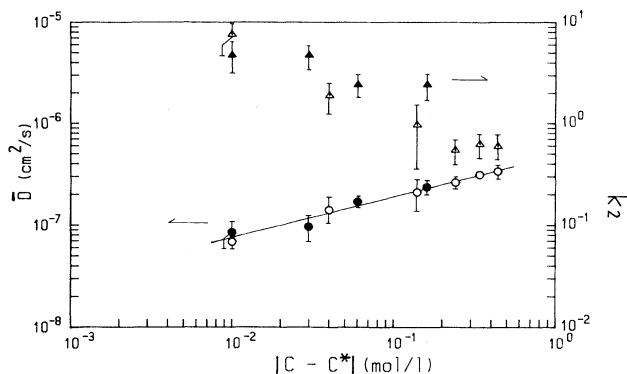


FIG. 5. The mean diffusion coefficient  $\bar{D}$  and the normalized second cumulant  $k_2$  as a function of the salt-concentration difference  $|C - C^*|$  with  $C^* = 0.44$  mol/liter. The circle and the triangle represent the values for  $\bar{D}$  and  $k_2$  deduced from Eq. (2), respectively. The solid line denotes the simple power-law behavior of  $\bar{D} = D_0 |C - C^*|^{\nu^*}$  with  $D_0 = (4.71 \pm 0.44) \times 10^{-7}$  cm<sup>2</sup>/s and  $\nu^* = 0.40 \pm 0.05$ .

represented by a simple power law as a function of  $|C - C^*|$ . The solid line in Fig. 5 denotes the power-law behavior of  $\bar{D} = D_0 |C - C^*|^{\nu^*}$  with  $D_0 = (4.71 \pm 0.44) \times 10^{-7}$  cm<sup>2</sup>/s and  $\nu^* = 0.40 \pm 0.05$ , which is also represented in Fig. 4 by the dotted curve. Such results indicate that the concentration of NaCl in water should be a relevant parameter to describe the diffusive decay in our cataracted lens.

It is worthwhile comparing our results with the work of Tanaka and Benedek.<sup>11</sup> They have found that the mean diffusion coefficient in the human lens inflicted with a cold cataract is about 5.5 times smaller than that in the intact normal one, which is in close agreement with our factor of 5. Their results may be interpreted as indicating that the relaxation behavior along the radius of the lens at the fixed temperature would correspond to that along the isotherm by varying the composition in an ordinary fluid mixture with a critical point of mixing. If we observe a fixed radial position of the lens, the composition of the lens protein in water may depend on an influx of water. Therefore, it could be considered that our situation is partially analogous to that of approaching a possible phase boundary of mixing along the isotherms in terms of critical phenomena.<sup>21</sup>

Many investigators have suggested important roles of the inorganic ions such as Na, K, and Ca in a stage of the cataract in the lens, which could be deeply related to the enzyme Na, K- and Ca-ATPase (adenosine triphosphatase) activities.<sup>32</sup> Kinoshita and Merola have reported that the increase in Na and the decrease in K in the cataracted lens is not due to deterioration of the Na, K-ATPase activity, but to the permeability of the lens.<sup>23</sup> It has been suggested that the membrane permeability of human cataracted lenses depends on the internal Na content of the lens; that is, cataracted lenses with high Na concentration have very low membrane potentials, contrary to relatively high membrane potentials in lenses with low Na contents.<sup>24</sup> In such a cataract a large influx

of water may be caused by an increase in lenticular NaCl. This allows us to infer that an influx of water is closely related to the concentration of Na, contrary to the decrease in K. This should cause the changes of not only the composition in the lens proteins with water but also the ionic strengths in the cataracted lens. The existence of a critical point of mixing has been established even for such structural proteins (i.e., lysozyme) with electrical charge in the NaCl solution.<sup>25</sup> Nicoli and Benedek have suggested that the denaturation temperature in the structural proteins is nearly independent of ionic strength from the examination of the extent of physical swelling and the conformational changes as a function of temperature and ionic strength for the proteins such as lysozyme, ribonuclease, and chymotrypsinogen.<sup>26</sup> Though it is questionable to apply this suggestion straightforwardly in our case with the lack of information on the lenticular pH, it is noted that the opacification indicating the thermal denaturation of the lens proteins was not observed for the intact normal lens immersed in silicon oil at our experimental temperature of 20°C. It seems likely that the change in the ionic strengths, which may affect molecular interactions in the lens, mainly cause the shift of phase-separation temperature.

The effect of salt species and concentration on phase separation has been examined recently for macromolecular solutions<sup>27</sup> and aqueous C<sub>8</sub>-lecithin solutions, which create aggregates of surfactant molecules called micelles.<sup>28</sup> A dramatic change of the phase-separation temperature as the salt concentration is increased has been observed. An experimental investigation, which is partly related to this effect, has been reported also for the dependence of aspirin and vitamin E on the lens opacification in terms of phase-separation temperature.<sup>29</sup> It is important to evaluate such effects on the lens proteins in connection with the phase separation in the lens; however, here we are concerned with a more general investigation of the cataract behavior.

On the other hand, Barber has observed the reversible pressure-induced opacification in the lens, which was attributed to the migrations of water between the lens membranes.<sup>30</sup> Another possible explanation for this effect was presented suggesting that this opacification could be attributed to the distortions of protein ordering with the long wavelength by reason of the instant responsiveness.<sup>31</sup> A quick response on the applied pressure has been also observed in a critical mixture, which actually has been applied to investigate the dynamics of phase separation.<sup>32</sup> The pressure dependence of the phase-separation temperature evaluated, for example, in a macromolecular solution is at most of a magnitude on the order of a hundredth kelvin/atom near a critical point of mixing,<sup>33</sup> which could be relatively insensitive to our cataracted lens. Thus the present work should be regarded as an experimental investigation of dynamical behavior in the cataracted lens along a certain path associated with a possible phase boundary of mixing, involving the composition changes in the lens proteins with water.

In general, the asymptotic power-law behavior becomes valid in a fluid mixture near the critical point of mixing. Some mixtures forming the aggregate structure

as shown, e.g., by micelles or microemulsion have a relatively wide range, satisfying the power-law behavior associated with the phase boundary of mixing. It is noted that there exists an apparent parallelism between the present result and the critical behaviors, as shown by a micellar solution in the range far from a critical point of mixing, except for the difference in corresponding variables used.<sup>34</sup> If we assume that the diffusion coefficient in a single cell or an effective domain with crystallins and water obeys Eq. (1), then it would be possible to evaluate an effective size of the fluctuations in the cataracted lens with a knowledge of the viscosity associated with the eye lens. As far as we observed, the lens-protein mixture without the crystalline capsule was a highly viscous fluid, which allows us to infer a possible gel-like structure with a "network" due to lens fibers. It would be desirable to study the behavior of the viscosity in the lens-protein mixture from the experiment, in consideration of a possible large dependence on shear gradients.<sup>34,35</sup> Since the lens-protein mixture must be fairly packed with fiber membranes, the viscosity expected in the present work could be higher than that for pure water. Therefore, the effective size of the fluctuations associated with the cataracted lens studied here is presumably much smaller than 60 nm, evaluated tentatively with the viscosity of pure water. This could be interpreted as indicating that the effective size of the fluctuations is a certain average over the effective domain with different relaxing processes, the maximum relaxation time of which diverges at a possible critical point of mixing (if it exists on the lens in a chicken). We feel that a new theoretical approach would be necessary to describe the relaxation behavior in such biological structural substances, in consideration of a superposition of the exponential relaxing processes with a critical slowing down. A similar approach has been made in a glassy transition in terms of the breakdown of ergodicity,<sup>36,37</sup> which could be applicable to the relaxing

processes in a large  $\tau$ . Detailed work would be desirable to investigate diffusive properties in the lens-inflicted cataract in terms of a nonergodic correlation function.

In this work we have examined the behavior of the diffusive decay in a salt-induced cataract of a mature chicken lens following the concept of Tanaka and co-workers, in terms of phase separation associated with a possible phase boundary of mixing. Our analysis suggests that the decay rate has a distribution that is quite widespread, even for a normal lens. The mean diffusion coefficient for the cataracted lens estimated in this work is approximately represented by the power law as a function of the concentration difference in the NaCl aqueous solution.

Our experimental findings suggest that a salt concentration should be a relevant parameter for describing the dynamical behavior in a cataract induced by a NaCl solution. Our value in the diffusion coefficient estimated within the cumulant expansion for the cataracted lens is approximately five times smaller than that for the normal one, which is almost compatible with the work of Tanaka and Benedek. It may be concluded that the diffusive behavior in the salt-induced cataract, which should be regarded as an osmotic type of cataract, could be principally interpreted within the same scheme of phase separation, in conjunction with the cold cataract.

#### ACKNOWLEDGMENTS

We have benefited from many illuminating discussions with Professor T. Shinozawa, Professor T. Endo, Professor R. Masho, and Professor K. Kobayashi. The authors also wish to thank the Ministry of Education in Japan for supporting this work with a grant-in-aid for scientific research.

- <sup>1</sup>L. Takemoto, B. Straatsma, and J. Horwitz, *Exp. Eye Res.* **48**, 261 (1989); A. Kamei, *Atarashii Ganka* **5**, 1749 (1988); P. Vidal and J. Cabezas-Cerrato, *Acta Ophthalmol.* **66**, 589 (1988).  
<sup>2</sup>J. E. Amoore, W. Bartley, and R. von Heyningen, *Biochem. J.* **72**, 126 (1959); H. L. Kern, *Invest. Ophthalmol.* **1**, 368 (1962).  
<sup>3</sup>H. N. Fukui, L. O. Melora, and J. H. Kinoshita, *Exp. Eye Res.* **26**, 477 (1978); J. H. Kinoshita, L. O. Merola, and S. Hayman, *J. Biol. Chem.* **240**, 310 (1965).  
<sup>4</sup>A. Mizuno, Y. Okazaki, Y. Kamada, H. Miyazaki, K. Itoh, and K. Iriyama, *Eye Res.* **1**, 609 (1982).  
<sup>5</sup>R. E. Perry, M. S. Swamy, and E. C. Abraham, *Exp. Eye Res.* **44**, 269 (1987); Y. Ozaki, A. Mizuno, K. Itoh, S. Matsushima, and K. Iriyama, *Appl. Spectrosc.* **41**, 597 (1987); T. Lee, T. Iwamoto, E. Hitomi, T. Kanematsu, M. Yoshiura, S. Onimura, and K. Iriyama, *Jikeikai Med. J.* **35**, 225 (1988); K. Iriyama, A. Mizuno, Y. Ozaki, K. Itoh, and H. Matsuzaki, *Curr. Eye Res.* **2**, 489 (1983).  
<sup>6</sup>R. von Heyningen, *Sci. Am. (Japan)* **6**, 62 (1976); M. Delaye and A. Tardieu, *Nature* **302**, 415 (1983).  
<sup>7</sup>*Molecular and Cellular Biology of the Eye Lens*, edited by H. Bloemendal (Wiley, New York, 1981), p. 469.  
<sup>8</sup>H. Bloemendal, *CRC Crit. Rev. Biochem.* **12**, 1 (1982).

- <sup>9</sup>J. Piatigorsky, *Differentiation* **19**, 134 (1981).  
<sup>10</sup>J. Piatigorsky, P. Zelenka, and R. T. Simpson, *Exp. Eye Res.* **18**, 435 (1974).  
<sup>11</sup>T. Tanaka and B. Benedek, *Invest. Ophthalmol.* **14**, 449 (1975); T. Tanaka, C. Ishimoto, and L. T. Chylack, Jr., *Science* **197**, 1010 (1977).  
<sup>12</sup>For a review, see P. C. Hohenberg and B. I. Halperin, *Rev. Mod. Phys.* **49**, 435 (1977).  
<sup>13</sup>For a review, see J. V. Sengers, *Int. J. Thermophys.* **5**, 803 (1985).  
<sup>14</sup>D. E. Koppel, *J. Chem. Phys.* **57**, 4814 (1972).  
<sup>15</sup>K. Hamano, T. Nomura, T. Kawazura, and N. Kuwahara, *Phys. Rev. A* **26**, 1153 (1985); K. Hamano, S. Teshigawara, T. Koyama, and N. Kuwahara, *ibid.* **33**, 485 (1986).  
<sup>16</sup>K. Kubota, H. Urabe, Y. Tominaga, and S. Fujime, *Macromolecules* **17**, 2096 (1982).  
<sup>17</sup>S. W. Provencher, J. Hendrix, and L. de Maeyer, *J. Chem. Phys.* **69**, 4273 (1978); S. W. Provencher and V. G. Dovi, *J. Biochem. Biophys. Methods* **1**, 313 (1979).  
<sup>18</sup>Erdogan Gulari, Esin Gulari, Y. Tsunashima, and B. Chu, *Polymer* **120**, 347 (1979).  
<sup>19</sup>P. N. Pusey and W. van Meegen, *Physica A* **157**, 705 (1989).

- <sup>20</sup>P. N. Pusey and W. van Meegen, *Phys. Rev. Lett.* **59**, 2083 (1987); W. van Meegen and S. M. Underwood, *J. Chem. Phys.* **88**, 7841 (1988); I. Nishio, J. C. Reina, and R. Bansil, *Phys. Rev. Lett.* **59**, 684 (1987).
- <sup>21</sup>J. V. Sengers, in *Critical Phenomena, Proceedings of the International School of Physics Enrico Fermi, Course LI*, edited by M. S. Green (Academic, New York, 1972); *Ber. Bunsenges. Phys. Chem.* **76**, 234 (1972); K. Hamano, N. Kuwahara, and M. Kaneko, *Phys. Rev. A* **20**, 1135 (1979); K. Hamano, T. Kawazura, T. Koyama, and N. Kuwahara, *J. Chem. Phys.* **82**, 2718 (1985).
- <sup>22</sup>H. L. Kern, *Invest. Ophthalmol.* **1**, 368 (1962); S. L. Bonting, L. L. Caravaggio, and N. M. Hawkins, *Arch. Biochem. Biophys.* **101**, 47 (1963); A. Iimura, M. Takehashi, and S. Iwata, *Ophthalmic Res.* **19**, 95 (1987); S. Zheng, S. Li, Y. Li, J. Yang, and Z. Zhang, *Fenxi Shiyanshi* **7**, 61 (1988).
- <sup>23</sup>J. H. Kinoshita and L. O. Merola, *Invest. Ophthalmol.* **3**, 577 (1964).
- <sup>24</sup>G. Duncan and K. R. Hightower, *Inst. Nat. Sante Rech. Med. Colloq.* **147**, 341 (1986).
- <sup>25</sup>C. Ishimoto and T. Tanaka, *Phys. Rev. Lett.* **39**, 474 (1977).
- <sup>26</sup>F. Nicoli and G. B. Benedek, *Biopolymers* **15**, 2421 (1976).
- <sup>27</sup>S. Saeki, N. Kuwahara, M. Nakata, and M. Kaneko, *Polymer* **18**, 1027 (1977).
- <sup>28</sup>Y.-X. Huang, G. M. Thurston, D. Blankshtein, and G. Benedek (unpublished).
- <sup>29</sup>S. Eccarius and J. I. Clark, *Ophthalmic Res.* **19**, 65 (1987).
- <sup>30</sup>G. W. Barber, *Arch. Ophthalmol.* **91**, 141 (1974).
- <sup>31</sup>R. A. Schacher and S. A. Solin, *Invest. Ophthalmol.* **14**, 380 (1975).
- <sup>32</sup>N.-C. Wong and C. M. Knobler, *J. Chem. Phys.* **69**, 725 (1978).
- <sup>33</sup>S. Saeki, N. Kuwahara, K. Hamano, Y. Kenmochi, and T. Yamaguchi, *Macromolecules* **19**, 2353 (1986).
- <sup>34</sup>J. S. Huang and M. W. Kim, *Phys. Rev. Lett.* **47**, 1462 (1981); K. Hamano, T. Sato, T. Koyama, and N. Kuwahara, *ibid.* **55**, 1472 (1985); K. Hamano, N. Kuwahara, T. Koyama, and S. Harada, *Phys. Rev. A* **32**, 3168 (1985).
- <sup>35</sup>A. Onuki and K. Kawasaki, *Phys. Lett.* **75A**, 485 (1980), and references therein.
- <sup>36</sup>R. G. Palmer, D. L. Stein, E. Abrahams, and P. W. Anderson, *Phys. Rev. Lett.* **53**, 958 (1984); G. Palmer, *Adv. Phys.* **31**, 669 (1982).
- <sup>37</sup>C. P. Lindsey and G. D. Patterson, *J. Chem. Phys.* **73**, 3348 (1980).