Causes of Decreased Phase Transition Temperature in Selenite Cataract Model

K. P. Mitton, J. L. Hess, and G. E. Bunce

Purpose. Lenses from selenite-treated animals develop reversible "cold cataract" at a lower temperature than is required for lenses from age-matched control animals. This unexplained, stabilized phase transition is readily observed in intact lenses 36 to 48 hours after treatment and occurs in lenses before the appearance of the irreversible nuclear opacity observed 72 to 96 hours after treatment. The objective of this study was to investigate factors that may be responsible for this difference.

Methods. Preweanling rats were injected with sodium selenite. Lens extracellular water volume was measured using 14C-inulin. Free amino acids were analyzed using precolumn derivatization and high performance liquid chromatography. Soluble protein was isolated from lenses of control, and treated animals and temperature-dependent changes in light scattering were measured at 490 nm.

Results. Lens extracellular water was increased by the selenite treatment, with a concurrent 10% decrease in intracellular volume. Solutions of soluble protein from lenses of selenite-treated animals after postinjection hours 24 and 48 had higher critical phase transition temperatures (Tc) compared to similar proteins from control lenses. From 24 to 72 hours after injection, the free amino acid content of the lens increased 42%. Taurine levels were unchanged over the same period. The addition of 7 mM glycine and 7 mM proline to solutions of soluble protein (96 mg ml⁻¹) decreased the phase transition temperature. Taurine (14 mM) had a similar effect. Combining taurine and the glycine plus proline solutions had an additive effect in lowering the Tc.

Conclusions. Increases in free amino acid concentration occur in lenses in response to the stress imposed by a systemic dose of selenite. The altered polyion content in lenses from selenite-treated animals, before nuclear cataract formation, contributes to the greater thermal stability of transparency in these lenses, thus lowering the temperature at which "cold cataract" forms. Invest Ophthalmol Vis Sci. 1995;36:914–924.

Subcutaneous injection of sodium selenite (Na₂SeO₃) in a single dose induces the formation of nuclear cataract in neonatal rats within 3 to 4 days. The following changes 0 to 48 hours after injection precede cataract formation: increased selenium content, decreased ATP content, glutathione loss, increased NADP⁺—NADPH ratio, elevated glycerol-phosphate content, and DNA double-strand breaks. From 24 to 48 hours after injection, alterations occur in lens Ca²⁺ homeostasis: decreased Na⁺—Ca²⁺ exchange, decreased Ca²⁺—ATPase activity, and increased Ca²⁺ permeability.

The appearance of nuclear cataract 72 to 96 hours after injection is accompanied by some recovery from glutathione and ATP loss, increases in lens Ca²⁺ and inorganic phosphate content, increased insoluble protein content, and elevated proteolysis in the nuclear region of the lens. The current hypothesis regarding the nuclear cataract is that elevated Ca²⁺ activates calpain II and proteolysis of β-crystallins, causing their insolubility.

The lenses from selenite-treated rats contain β-crystallins missing portions of their N-termini, similar to those
obtained by treatment with purified calpain in vitro. Mature, but clear, rat lenses contain similar amounts of insoluble γ-crystallin and insoluble, fragmented β-crystallin as are present in cataractous lenses from younger selenite-treated rats. This recent finding shows that this cataract does not depend solely on insoluble protein content. The developmental stage at which proteolysis occurs may also be a factor in the selenite model.

Rat lenses exhibit a phenomenon called "cold cataract" in which phase separation occurs as a function of temperature. At 10 to 15 days of age, cold cataract appears below $32^\circ$C and is reversible upon elevation of temperature. The temperature of phase transition drops to $26^\circ$C in selenite-treated rats at 36 to 48 hours after injection but increases sharply above physiological temperature just before the emergence of nuclear cataract.

Direct effects of temperature on light scattering properties of protein solutions provide information about protein–solute interactions. Solutions of bovine lens extracts or pure bovine γ-crystallin display a temperature-dependent phase transition in vitro. This property of crystallin interactions may explain the formation of cold cataract in the intact lens. Two phases, a protein-rich phase and a protein-poor phase, coexist at and below the critical temperature ($T_c$) and cause light scattering. All γ-crystallin amino acid sequences are extremely homologous. The high-$T_c$ crystallins (γIIIa- and γIVa-) and low-$T_c$ crystallins (γIIa- and γIIIb-), however, have very different maximum $T_c$ values of approximately $35^\circ$C and $5^\circ$C, respectively.

Lens age is an important factor for cataractogenesis in the selenite model. The rat lens is less susceptible to selenite after 16 days of age, which corresponds to the end of the critical maturation period of the lens. During this period, the lens changes from a state of uniform hydration to a state in which the nucleus is less hydrated relative to the cortex.

The decreased phase transition temperature in the selenite model before nuclear cataract formation suggests a transient environmental change of the lens cytosol. These studies were undertaken to examine the regional intracellular and extracellular hydration states of lenses from selenite-treated animals compared to age-matched controls and also to examine how alterations in free amino acids correlated with the temperature-dependent phase transition characteristics of the intact lens. Temperature-dependent phase transition properties revealed early alterations of the soluble protein population before nuclear cataract formation.

**METHODS**

**Cataract Induction**

Sprague–Dawley rats were housed in polypropylene shoe box cages in rooms maintained at $24^\circ$C and 80% relative humidity with a 12-hour light–12-hour dark cycle. Animals were fed Agway 3000 laboratory rat chow (Agway, Syracuse, NY) and distilled water. Ten-day-old animals (13-day-old animals for the phase transition temperature experiments) were injected subcutaneously with 20 mM sodium selenite ($Na_2SeO_3$), 0.9% NaCl, for a total dose of 30 nmol g$^{-1}$ body weight (approximately 30 μl injection). Uninjected control animals from the same litter were used for all experiments. These animals served as appropriate controls because injections with 0.9% NaCl or 20 mM sodium sulfite do not cause lens pathology. At designated times after injection, animals were decapitated and lenses were removed immediately for extraction of free amino acids or proteins. Lenses were observed and photographed with an Olympus SZH zoom-stereo microscope equipped with a model SZH-ILLD illumination base (Olympus Optical, Tokyo, Japan). All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Amino Acid Extraction and Analysis**

Free amino acids were measured by precolumn derivatization and high-performance liquid chromatography. Pairs of lenses were removed by a posterior approach into 0.9% NaCl, blotted, and homogenized on ice in a 0.5 ml volume of 10% trichloroacetic acid using a glass Potter–Elvehjem homogenizer. Supernatant was collected after centrifugation (10,000g X 5 minutes). Aliquots of supernatant were dried under vacuum (70 mTorr), combined 1:1 with norleucine internal standard (0.40 mM 0.1 N HCl), and again dried under vacuum (70 mTorr). The material was washed with 40% MeOH (vol/vol), 40% 1M sodium acetate, 20% triethylamine, and again dried under vacuum. Amino acids were converted to phenylthiocarbamyl amino acids by reaction with 10% phenylisothiocyanate, 70% MeOH, 10% triethylamine, and filtered using microcentrifuge filters (Ultra-Free-MC; Millipore, Milford, MA) before chromatography. All detectable amino acids were quantified by calibration with physiological amino acid standards (Sigma, St. Louis, MO) run with each set of analysis. The high-performance liquid chromatography system was a Waters WISP/Pico-Tag system using the Waters free amino acid analysis column (#10950, 3.9 × 300 mm). (Waters, Millipore)

**Osmolarity Measurement**

Osmolality of buffers was measured using an Osmette A automatic osmometer (Precision Systems, Natick, MA) calibrated with 100 and 500 mOsm kg$^{-1}$ standards.
Protein Determinations

Protein content was measured by using the Bradford dye-binding assay using bovine serum albumin as a standard and by monitoring the A_{280}:A_{260} ratio.

Determination of Lens Extracellular Space

The extracellular volume in the rat lens was quantified by equilibrating lenses individually in Hank's medium containing inulin (14C-carboxylated, 0.5 μCi ml⁻¹; DuPont NEN, Boston, MA), which is permeable to the lens capsule but not to the plasma membrane. Uptake by lenses from age-matched control and selenite-treated rats was determined after 24 hours at 30°C. Lenses, suspended on a glass loop, were rinsed twice on Parafilm M (American National Can, Greenwich, CT), transferring the appropriate sections of the lens capsule but not to the plasma membrane. Uptake by lenses from age-matched control and selenite-treated rats was determined after 24 hours at 30°C. Uptake by lenses from age-matched control and selenite-treated rats was determined after 24 hours at 30°C.

Preparation of Lens Total Soluble Protein Extract

Lenses were obtained as above and frozen using liquid nitrogen for storage at -80°C to obtain sufficient tissue for the protein extract (22 lenses/extract). Pooled lenses were homogenized on ice in a glass Potter-Elvehjem homogenizer after the addition of 0.5 ml of buffer: 100 mM sodium phosphate, 5.0 mM ethylenediaminetetraacetic acid, 0.01% NaN₃, pH 7.2 with HCl, (295 mOsm kg⁻¹). The homogenate was warmed to 15°C during the homogenization. Samples from selenite-treated rats were processed simultaneously with their controls.

Insoluble cell debris and capsular material were removed after centrifuging (12,000g × 5 minutes) at 15°C. The supernatant fraction was transferred directly into Spectra/por-1 dialysis tubing (6 to 8 kd cutoff; Spectrum, Houston, TX.) and dialyzed against 3-1 changes of buffer, equilibrated with argon gas. Dialysis and concentration steps were performed at 21°C. After 14 hours, samples were concentrated in dialysis tubing against Sephadex G-200 (Pharmacia Biotech, Piscataway, NJ) followed by 30 minutes of dialysis against buffer.

Phase Transition Temperature Curves

Phase transition was monitored at 490 nm as percent transmittance (%T) of protein samples at various temperatures. Samples were diluted with dialysis buffer to equivalent protein concentration (96 mg/ml⁻¹) with or without the inclusion of various amino acids as specified and transferred into 0.300 ml volume quartz cuvettes with teflon-plug lids to prevent evaporation. The microcuvettes were placed in the cuvette holder assembly of a Gilford Thermoset Temperature Controller installed in a Gilford 2600-UV/VIS-Spectrophotometer. The assembly included a bolt-on lid to minimize temperature gradients; integrated bidirectional, solid-state heat pumps; and an internal thermistor. The internal thermistor was calibrated for linearity from 0°C to 99°C with a National Bureau of Standards probe. Each cuvette measured 12.2 × 5.5 × 7.1 mm external dimensions. The exposed optical surface was 24 mm², only 5% of the total surface area. The maximum surface contact with the temperature control block and the small sample volume ensured rapid thermal equilibration.

The cuvette holder temperature, measured with a thermometer, was within 0.15°C of the thermistor readout. Argon was introduced (450 cm³ minute⁻¹) into the spectrophotometer cell thermal cavity to provide a dry atmosphere for operation between 1°C to 15°C. Light transmission was monitored from high to low temperature after equilibrating samples for 1.5 minutes at a new set temperature. All solutions were transparent at 36°C and were referenced to 100% T at this temperature.

The phase transition data could be fitted to the sigmoidal equation,

\[ \%T = \left( \%T_{\text{max}} \times k(T° - a)^b \right) / \left( 1 + k(T° - a)^b \right) \]

with an \( r^2 > 0.997 \). Differences between phase transition temperature curves were evaluated using a balanced two-way analysis of variance and Duncan’s new multiple range test. Seven to 10 temperatures that determined the solution transmittance properties between 76%T and 90%T blocks were used in the analysis of variance and served as the basis for comparing the average Tc between group treatments.

Isolation of Lens Nuclear–Cortex Tissue

For obtaining primarily nuclear and primarily cortical tissue, the greatest concern was metabolic alterations to amino acid concentrations during dissection. A technique was developed that allowed for complete freezing of the very fragile neonatal rat lens and separation of tissue. After washing the lenses in saline and blotting gently, they were frozen quickly at the surface.
of a liquid nitrogen bath. The lens was held with pre-cooled forceps and carefully pierced along the posterior–anterior axis with a 27-gauge needle and cooled in the liquid nitrogen bath. A small scalpel blade was used to shave off the equatorial cortex first, followed by the anterior cortex–epithelium and then the posterior cortex so that the lens nucleus, a sphere of 1.5 mm, remained. Tissue shavings were transferred to a smooth forceps cooled in liquid nitrogen. Isolated nuclear and cortical samples, each from 12 lenses, were stored at −80°C until preparation for amino acid analysis. Both samples contained perinuclear material.

RESULTS

Appearance of the Lens
In Figure 1, we present both a posterior and a side view of the lens from control and selenite-treated rats. Forty-eight hours after treatment with selenite, before nuclear cataract formation, the posterior subcapsular cataract is apparent, as is the greater clarity of the nuclear region at 28°C compared to the age-matched lens from control animals. The temperature must be decreased to 24°C for the lens from selenite-treated animals to have a similar cold cataract, as seen in these lenses from the control animals.

Lens Hydration: Intracellular and Extracellular
Table 1 shows the weight data for lenses from control and selenite-treated animals. Although lens growth was attenuated in the selenite-treated animals, no difference in the ratio of dry–fresh tissue weight was observed. Significantly greater extracellular water was detected in the lenses from selenite-treated animals (Table 2). The increased extracellular volume occurred in both the capsule–epithelium–cortex and nuclear regions of the lens (Table 2) before nuclear cataract formation, 48 hours after injection (age 12 days), and when nuclear cataract had fully formed, 96 hours after injection (age 14 days).

Amino Acid Analysis
The results of amino acid analysis of whole lenses from selenite-treated animals and age-matched controls are reported for 6, 12, 24, 48, 72, 96, and 192 hours after injection (Fig. 2). Elevation of total amino acids occurred as early as 24 hours, well before the nuclear cataract stage. After postinjection hour 72, the total free amino acid content was 142% of controls in lenses from selenite-treated rats (38.1 ± 0.7 μmol g⁻¹ versus 25.1 ± 0.9 μmol g⁻¹).

The ratio of amino acid content of nuclear to cortical tissue (Fig. 3) was less than unity for control lenses at all three time periods examined. Although total amino acids were elevated at 24 hours after injection (Fig. 2), the nuclear–cortical ratio remained less than unity in lenses from selenite-treated animals (Fig. 3a). However, after entry into the nuclear cataract phase (96 hours after injection), the ratio became greater than unity in the selenite-treated group. This ratio became similar to control values at 10 days after
Selenite treatment was administered on the tenth day after birth. Lenses were weighed immediately after enucleation and after drying at 100°C to constant weight. Values are the mean ± SE for a minimum of eight lenses in each determination using lenses from at least two different litters. * Fresh tissue weight for the number of lenses designated by (n).

**Phase Transition Studies In Vitro**

Plots of light transmission as a function of temperature, for soluble protein solutions from lenses of selenite-treated and age-matched control animals, are presented in Figure 4. Solution turbidity was stable and apparent when light transmission (490 nm) decreased to 80%T. Therefore, the T_c (°C) at 80%T was compared. Statistical comparisons were based on the average T_c calculated from this most stable region of transmittance change between 76%T and 90%T (Table 3). Once light transmission fell below 75%T, solutions displayed a continuously decreasing transmittance at constant temperature. Protein solutions (without amino acid addition) from lenses of selenite-treated animals had a T_c approximately 2°C greater than protein solutions from lenses of age-matched controls after both 24 and 48 hours after injection (Table 3, Fig. 4). This selenite-dependent difference is inconsistent with the formation of cold cataract at lower temperatures compared to control lenses (Fig. 1).

Elevated amino acid concentration stabilized the transparency of lens soluble protein solutions, seen as a shift of the T_c to lower temperatures (Fig. 5). Solutions contained glycine, proline, and taurine concentrations similar to those in lenses from selenite-treated rats. Specifically, an equimolar mixture of glycine and proline (14 mM total) or taurine (14 mM) reduced the T_c of lens protein solutions from control and selenite-treated animals (Table 4A). Taurine was more effective than the equimolar glycine–proline mixture. Further effects on T_c were examined in a solution of taurine, glycine, and proline (28 mM total) (Fig. 6). There was an additive effect on the reduction of T_c of soluble lens protein in this solution (Table 4B).

**TABLE 1. Selenite-Dependent Decrease in Lens Growth**

<table>
<thead>
<tr>
<th>Animal Age (days)</th>
<th>Control</th>
<th>Selenite Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (mg) (n)</td>
<td>Dry/Wet Ratio</td>
</tr>
<tr>
<td>11</td>
<td>9.2 ± 0.2 (8)</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>10.8 ± 0.2 (7)</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>13</td>
<td>11.5 ± 0.4 (9)</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>14</td>
<td>14.3 ± 0.3 (7)</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>21</td>
<td>16.2 ± 0.5 (9)</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>

Selenite treatment was administered on the tenth day after birth. Lenses were weighed immediately after enucleation and after drying at 100°C to constant weight. Values are the mean ± SE for a minimum of eight lenses in each determination using lenses from at least two different litters.

**TABLE 2. Selenite-Dependent Increase in Extracellular Lens Water**

<table>
<thead>
<tr>
<th>Animal Age (days)</th>
<th>Lens Water (μl/lens)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraocular</td>
</tr>
<tr>
<td></td>
<td>Whole Lens</td>
</tr>
<tr>
<td>Control (12)</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>Selenite-treated (12)</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>Control (14)</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>Selenite-treated (14)</td>
<td>7.6 ± 0.7</td>
</tr>
</tbody>
</table>

Selenite treatment was administered on the tenth day after birth.

* Volume, estimated by equilibrating lenses in 14C-inulin and from tissue weights (1 mg = 1 μl), is reported as mean value ± SE; n = 6 for all measurements. Similar overall errors occurred with the dissected tissue, although variation was greater with the estimation of the nuclear volume in the cataractous (14-day) tissue.

† Extracellular volume in lenses from selenite-treated animals was significantly less compared to this volume in lenses from control animals (P < 0.05, t-test).
DISCUSSION

Hydration

Decreased growth (Table 1) characterizes lenses from selenite-treated animals. The dry mass concentration at various points along the lens axis is not altered in the selenite model until 6 days after injection, after the formation of nuclear cataract. These lenses showed no change in the wet–dry weight ratio (Table 1); therefore, hydration effects, typical of an osmotic cataract, do not occur in this model. The extracellular compartment water volume increases before nuclear cataract formation in the capsule–epithelium–cortex and nuclear regions of the lens (Table 2). The posterior liquid-filled vacuoles identified by 2 days after selenite treatment are consistent with the increase in extracellular water in these lenses. Posterior delivery of metabolites and selenite would be provided by the tunica vasculosa lentis at this stage of lens development. The posterior subcapsular cataract and the open appearance of the posterior suture (Fig. 1) indicate that this region of the lens is susceptible to local increases in the extracellular space. These changes may reflect interrupted cell maturation so that normal cell-to-cell interactions do not occur.

The posterior subcapsular cataract decreases before nuclear opacification, yet an abnormal distribution of extracellular space persists (Table 2). The nature of the extracellular space during early lens development and perturbations of this space in lenses from selenite-treated rats may provide exposure of the lens nucleus to abnormal levels of calcium that are required for nuclear opacification. Modifications of membrane lipids after exposure of the lens to selenite in vivo could also influence the cell-to-cell associations, permeability to ions and metabolites, and the distribution of lenticular water.

The critical maturation period lasts from approximately 12 to 16 days of age and is characterized by a transient sharp increase in the rate of lens dehydration, a temporary plateau in growth in the equatorial diameter and the posterior–anterior axis, a primarily intracellular water loss with constant extracellular water, a decreased penetration of extracellular space by procion yellow, and a constant increase in lens dry weight (1 to 21 days). During maturation, the lens epithelium displays junctions of focal adhesions on basolateral borders of adjacent cells that decrease in number with age as desmosomes appear to replace...
them. The decreasing susceptibility of lenses to systemic selenite exposure beyond 18 days of age may be a result of decreasing access of selenite into the extracellular space related to the regression of the tunica vasculosa lentis.

Temperature-dependent Phase Transition

Proteins. The temperature sensitivity of the phase transition of the intact rat lens decreases with age. This age-dependent change correlates with the lower Tc for "older" (by 24 hours) lenses of control and selenite-treated animals (Table 3, Fig. 4). Age-dependent changes in Tc have been observed in the bovine lens, probably in response to an altered distribution of proteins among the chaperone a-crystallins and the high-Tc and low-Tc γ-crystallins. Modifications to existing proteins may also cause these effects, although the relationship between γ-crystallin primary structure and Tc is not known.

The phase transition properties of the soluble proteins from lenses of selenite-treated rats cannot explain the requirement for a lower temperature to cause onset of cold cataract in these lenses (Fig. 1). These protein solutions became cloudy 2°C higher than solutions of proteins isolated from control lenses (Table 4). Protein modification has not been well characterized in lenses before nuclear cataract formation. No additional disulfide bond formation occurs in soluble proteins from the lenses of selenite-treated animals. Although elevated selenium content is associated with the crystallins in lenses from treated animals, there exists no more than one Se atom per 10,000 protein molecules. It is unlikely that this modification could account for the observed change in phase transition properties of these protein solutions. Other uncharacterized modifications to proteins must cause the increased Tc of solutions of protein isolated from lenses of selenite-treated rats.

Amino Acids. Tc was decreased in vitro using elevations of amino acid concentrations similar to those found in vivo (Table 4A, Figure 5). Glycine and proline were selected because they displayed the largest individual increases in the selenite model in vivo. The effect of taurine was also examined because it is the most abundant small molecular weight organic substance in the rat lens. All three amino acids possess uncharged side chains and an α-amino and an acidic group; therefore, all are zwitterions at physiological pH. Altered water distribution across fiber cell membranes in the lenses from selenite-treated animals (Fig. 2) would additionally increase the effective intracellular concentration of free amino acids and, thus, favor a lower Tc.

TABLE 3. Increased Apparent Tc of Solutions of Proteins Isolated From Lenses of Selenite-Treated Rats

<table>
<thead>
<tr>
<th>Tc, °C</th>
<th>Group (hours PI)</th>
<th>Average (80% T to 90% T) (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 80% T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Control†</td>
<td>11.9</td>
<td>11.3</td>
</tr>
<tr>
<td>Selenite-treated†</td>
<td>13.9</td>
<td>12.9</td>
</tr>
</tbody>
</table>

* Values of Tc at 80% T are estimated from the phase transition curves in Figure 4. In addition, the average Tc is calculated for seven temperatures determining 80% T to 90% T. The average Tc for solutions of protein from lenses of selenite-treated rats is significantly higher than for solutions of protein from lenses of control rats (P < 0.05). The average Tc for the samples from lenses of 15-day-old animals (48 hours after injection) is significantly lower than the comparable sample from 14-day-old rats (24 hours after injection) (P < 0.01, Duncan's test).† Soluble lens protein concentration of 96 ± 1 mg ml⁻¹. PL = after injection.
FIGURE 5. Amino acids stabilize phase transition properties of solutions of soluble lens proteins. Amino acid additions—7 mM glycine and 7 mM proline or 14 mM taurine—decrease the \( T_c \) of solutions containing 96.0 ± 1 mg protein ml\(^{-1}\). Soluble protein is extracted from (A) age-matched control rats and (B) selenite-treated rats 48 hours after injection.

Taurine was more effective than an equimolar mixture of glycine and proline using extracts of the preweanling rat lens (Table 4A, Fig. 5). Taurine is also superior to glycine and glutamate in lowering \( T_c \) of soluble protein extracts from calf lens nuclear homogenates.\(^\text{30}\) The proportion of taurine to other amino acids increases substantially in the developing rat lens, from 15 mol% at day 14 to 32 mol% by day 20 (data not shown), and to about 75 mol% of the entire free amino acid pool in the adult rat lens.\(^\text{35,36}\) Taurine in the lens may function in osmoregulation.\(^\text{37}\) In the diabetic rat model of cataract, sorbitol elevation is balanced by decreased taurine concentration before initial vacuole formation\(^\text{38}\) and after vacuole formation.\(^\text{37}\) Taurine is a conditionally essential nutrient implicated in osmoregulation in the brain,\(^\text{39}\) membrane stabilization—antioxidation,\(^\text{40}\) and stimulation of cellular proliferation in retinal pigment epithelium.\(^\text{40}\) Our data support taurine’s possible contribution to increased temperature stability (decreased \( T_c \)) of the lens fiber cytosol.

The ratio of amino acids contained in the nucleus to those in the capsule–epithelium–cortex fraction remained constant through the age of 23 days (240 hours after injection). Only in the lenses with nuclear cataract did the population of free amino acids become greater in the nucleus relative to the cortex (Fig. 3a). This effect was not seen with taurine and ornithine. These data suggest that extensive proteolysis in the lens nucleus leads not only to the accumulation of water insoluble products\(^\text{40}\) but also to the degradation of peptides to amino acids. Furthermore, these amino acids are not freely equilibrated throughout the lens at this stage of development.

The addition of glutathione and amino acids lowers the \( T_c \) of calf lens nucleus extracts.\(^\text{30}\) Because glutathione content is decreased in lenses from selenite-treated rats,\(^\text{1}\) it cannot account for lower \( T_c \). Glutathione degradation may, in part, contribute to the elevated glycine content in the lenses from selenite-treated rats.
TABLE 4. Amino Acids Decrease Estimated $T_c$ of Lens-Soluble Protein Solutions

A. Solutions containing 96 ± 1 mg protein ml$^{-1}$

<table>
<thead>
<tr>
<th>Composition</th>
<th>Control</th>
<th>Se-treated</th>
<th>Average (80% $T$ to 90% $T$) (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein†</td>
<td>11.3</td>
<td>12.9</td>
<td>11.9$^b$ 15.8$^b$</td>
</tr>
<tr>
<td>Protein + 7 mM gly, 7 mM Pro</td>
<td>11.0</td>
<td>12.8</td>
<td>11.6$^b$ 15.7$^b$</td>
</tr>
<tr>
<td>Protein + 14 mM Tau</td>
<td>10.9</td>
<td>12.5</td>
<td>11.3$^b$ 13.3$^b$</td>
</tr>
</tbody>
</table>

B. Solutions containing 93 ± 1 mg protein$^{-1}$

<table>
<thead>
<tr>
<th>Composition</th>
<th>At 80% $T$</th>
<th>Average (76% $T$ to 90% $T$) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein†</td>
<td>11.3</td>
<td>11.4$^c$</td>
</tr>
<tr>
<td>Protein + 14 mM Tau</td>
<td>10.9</td>
<td>11.0$^c$</td>
</tr>
<tr>
<td>Protein + 14 mM Tau + 7 mM Gly, 7 mM Pro</td>
<td>10.7</td>
<td>10.8$^c$</td>
</tr>
</tbody>
</table>

Values of $T_c$ (at 80% $T$) are estimated from the phase transition curves in Figures 5 and 6. In addition, average values are calculated from (n) values of temperature that determine 76% $T$ through 90% $T$. Values within a column with different superscript letters are significantly different at $P < 0.05$. (Duncan’s test).

† Soluble proteins extracted from lenses of selenite-treated rats 48 hours after injection and age-matched (15-day-old) control rats.

SUMMARY

We consider three changes in lenses from selenite-treated rats that relate to the requirement for a lower temperature to cause “cold cataract” in these lenses: intracellular volume, amino acid concentration, and changes to soluble proteins. Of these, the selenite-dependent decrease in intracellular volume and the increase in amino acid content affect greater stability of protein associations, detected as lower $T_c$ in temperature-dependent changes in light scattering. Changes to the lens proteins, however, result in solutions that have a higher $T_c$. This unexpected result reveals that lens transparency may not be driven by the nature of protein properties alone but, rather, by their interaction with the environment as influenced by metabolite concentrations and the properties of cellular membranes.

FIGURE 6. Additive stabilization, caused by amino acids, of the temperature-dependent phase transition of solutions of lens soluble proteins. Amino acid additions—7 mM glycine and 7 mM proline plus 14 mM taurine or 14 mM taurine—decrease the $T_c$ of solutions containing 93.0 ± 1 mg protein ml$^{-1}$. Protein was extracted from lenses of 15-day-old control rats, comparable to 48 hours after injection.
Phase Transition in Selenite Cataract

Key Words
amino acids, cataract models, lens proteins, phase transition, selenite

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