Alterations of lens protein synthesis in galactosemic rats

Peter F. Kador, J. Samuel Zigler, and Jin H. Kinoshita

A differential effect on protein synthesis has been demonstrated in the lenses of galactosemic rats. During galactose cataract development the synthesis of lens crystallins is depressed, whereas that of noncrystallin proteins is unaffected. This effect correlates with the influx of Na⁺ and loss of K⁺ from the lens. Removal of the galactose diet results in a gradual recovery of crystallin synthesis to normal levels. In vitro the nuclear cataractous lenses leak crystallins into the media; however, upon 5 day's recovery no leak-out of crystallins could be detected. Both decreased synthesis and leak-out probably account for the marked loss of dry weight of cataractous lenses. These results support the hypothesis that crystallin synthesis may be affected by cation imbalance or changes concomitant with such an imbalance.

Key words: sugar cataracts, galactosemic rats, protein synthesis, lens crystallins, cation imbalance

Cataracts, or lens opacities, cause visual impairment by interfering with the passage of light to the retina. One well-understood class of cataracts is associated with increased levels of blood sugar.¹ Such cataracts include those associated with diabetes and galactosemia and are collectively called sugar cataracts.²

Sugar cataract formation is initiated by the aldose reductase-mediated accumulation of polyols in the lens.³ These polyols are not capable of readily diffusing out of the lens or of being rapidly metabolized. Their accumulation therefore causes a hypertonicity in the lens leading to hydration, lens swelling, and opacity formation. At the same time, there is an increase in Na⁺ and a decrease of K⁺, amino acids, and glutathione in the lens. In the galactose cataracts the cataractogenic process can be reversed during the early stages of sugar cataract formation by decreasing the dietary amounts of aldoses.⁴

Recently, alterations in protein synthesis in cultured embryonic chick lenses have been associated with changes in the concentrations of intracellular Na⁺ and K⁺.⁵ Furthermore, in vitro incubation of mouse lenses with ouabain, a Na⁺, K⁺-ATPase inhibitor which causes an increase of Na⁺ and a decrease of K⁺ in the lens, differentially decreased crystallin synthesis and stimulated degradation and leakout of crystallins from the lens.⁶ Here we report the in vivo observation of selective cut-off and recovery of crystallin synthesis in the lenses of galactosemic rats.

Material and methods

Sprague-Dawley rats initially weighing 50 gm were fed a diet consisting of a pulverized mixture of 50% galactose and 50% Purina Lab Chow while control rats were fed only Purina Lab Chow. Both experimental and control animals had free access...
to both diet and water. In the reversal experiments the experimental rats were placed on the control diet after 12 days of the galactose diet.

At specified intervals groups of control and experimental rats were killed, and their lenses were removed via a posterior approach. The paired lenses from individual animals were then divided, with one being analyzed for the cations Na⁺ and K⁺ and the other used for the measurement of protein synthesis.

**Cation determination.** Rat lenses placed in quartz crucibles were dried overnight at 100° and then ashed at 600° with a drop of concentrated sulfuric acid. The residues were dissolved in 1N HCl, and the cation concentrations were measured with a Coleman Model 51 flame photometer.

**Protein synthesis.** Rat lenses were incubated for 6 hr in 1.5 ml of bicarbonate buffer containing TC-199 media, 30 mM fructose, and 200 μCi of 35S-methionine (specific activity approx. 400 Ci/mmole, New England Nuclear). The incubations were carried out in plastic dishes containing 16 mm diameter wells (Castor tissue culture cluster dishes) at 37° in a humidified environment of 5% carbon dioxide and 95% air. After incubation the lenses were homogenized in a 0.5 ml solution containing 2% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol, 8M urea, 10% glycerol, and 1.2% Tris-HCl, pH 6.8, and then centrifuged for 15 min at 12,000 × g for 4°. After centrifugation only a slight precipitate, which did not increase with cataract development, remained. The supernatant was decanted, and aliquots were prepared for SDS electrophoresis by mixing 0.1 ml of supernatant with 0.15 ml of a mixture containing 2% SDS and 2% 2-mercaptoethanol in 0.02M Na⁺ phosphate buffer, pH 7.0. The incubation medium used for each lens was stored frozen for subsequent testing for lens proteins.

SDS-polyacrylamide gel electrophoresis was performed essentially according to the method of Weber and Osborn. All samples were heated in a boiling water bath for 2 min prior to being run on analytical disc gels (5 by 100 mm) of 10% polyacrylamide. An electrophoretic run was complete in 3.5 to 4 hr.

Gels to be autoradiographed were stained with Coomassie blue, destained, and then sliced lengthwise with a Gel Slicer II (Miles Laboratories). The gel slices were mounted on filter paper and dried under heat and vacuum on a Gel Drying Plate (Miles Laboratories). Corresponding slices were aligned and autoradiographed on Kodak XR-1 x-ray film. The completed autoradiograms were scanned densitometrically with a Quick Scan Jr (Helena Laboratories).

Immunodiffusion was performed according to the method of Ouchterlony on commercially prepared plates (Hyland Laboratories). Specific antisera prepared previously against calf lens α-, β-, and γ-crystallin were used.

**Results**

When placed on a 50% galactose diet, all rats developed bilateral nuclear opacities within 9 to 12 days. When the rats were returned to a normal diet after 12 days of the galactose diet, no change in the nuclear opacity could be detected. Some clearing in the cortical regions, however, was observed after 27 to 34 days of recovery.

The concentration of K⁺ in the lens rapidly dropped, reaching a minimum of 10 mEq/kg of total lens water compared to a concentration of 111 mEq/kg for the controls (Fig. 1). Correspondingly, the concentration of Na⁺ in the lens increased to a maximum of 160 mEq/kg of total lens water compared to a control value of 22 mEq/kg. Upon recovery, the K⁺ concentration increased, reaching a six-fold increase by day 27 (day 39, Fig. 1), while the concentration of Na⁺ gradually decreased, being halved by day 27. These findings closely agree with previously published results.

The incorporation of 35S-methionine through short-term in vitro organ culture incubations was used as a measure of protein synthesis. Lens homogenates were electrophoretically separated on analytical SDS-polyacrylamide gels and autoradiographed. The results are presented as densitometric scans of the developed x-ray films (Figs. 2 and 3). The more rapidly migrating lens crystallin polypeptides were located at the right half of the scans, and the left half corresponded to noncrystallin proteins. As an indication of selective synthesis the integrated areas of crystallin vs. noncrystallin proteins were compared. The initial peak, corresponding to the top of the gel, was ignored.

Scans of the autoradiograms representing protein synthesis during galactose cataract development are shown in Fig. 2. Striking-
Fig. 1. Concentration of cations in control (A) and experimental (B) lenses expressed as milliequivalents per liter of total lens water (n = 3 to 5 except for day 46, where n = 1).

ly evident is a marked decrease in crystallin synthesis as the cataractogenic process evolved. The respective ratios of crystallin vs. noncrystallin protein synthesis decreased from a value of 4.9 for the control to 4.2 after 3 days of galactose feeding, 2.3 after 6 days, 0.1 after 9 days, and 0.2 after 12 days. However, crystallin synthesis rapidly recovered upon removal of the galactose diet. As seen in Fig. 3, the ratios increased from a value of 0.3 at day 0 to 1.7 after 5 days, 3.4 after 10 days, 3.8 after 27 days, and 5.2 after 34 days, compared to a control value of 4.9.

Fig. 4 shows the staining pattern for representative SDS gels. Clearly there was a decrease in total protein during cataract devel-
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Fig. 2. Densitometric scans of autoradiogrammed gels representing lens protein synthesis as measured by 6 hr in vitro incorporation of $^{35}$S-methionine in rats fed a 50% galactose diet. Region A represents noncrystallin polypeptides; region B represents crystallin subunits. Arrows give the location of the following marker proteins: bovine $\gamma$-crystallin (20,000), carbonic anhydrase (29,000) and ovalbumin (43,000).

opment, with some polypeptides being affected more than others. Most notable was the virtually complete disappearance of the slowest migrating crystallin polypeptide, which is a constituent of the $\beta$H-crystallin. Less evidence of protein degradation was found in these patterns than that reported for the Nakano mouse and ouabain-treated mouse lenses. It should be noted, however, that two of the $\beta$-crystallin bands (arrows) did not become labeled during the 6 hr incubation and thus may represent postsynthetically modified components. The increase in the concentration of the faster of these two bands in the cataracts may indicate that it was the result of partial degradation of a larger polypeptide, as occurs in the Nakano cataract.

To investigate the possibility that the decrease in crystallins was due to increased leak-out from the lens, the media of cultured lenses were analyzed by immunodiffusion for the presence of $\alpha$, $\beta$, and $\gamma$-crystallins. No protein was detected in the media of lenses taken from animals which were on a galactose diet for less than 9 days. After 9 to 12 days of galactose feeding, $\gamma$, $\beta$, and a trace of $\alpha$-crystallins were detected. Analysis by SDS-gel electrophoresis indicated over 50% of the crystallins present in the media to be $\gamma$. Furthermore, the crystallin bands present had normal mobilities, indicating that no degradation had occurred. After 5 days removal from the galactose diet, no crystallins could be detected in the media.

Discussion

Our investigation demonstrates that in the formation of galactose cataracts, changes in protein synthesis occur. The synthesis of crystallins, the major proteins of the lens,
Fig. 4. Staining patterns from SDS gels of various rat lens homogenates after 6 hr of incubation. Each gel was loaded with 12 μl of lens homogenate as follows: (1) control lens 6 days, (2) galactose-fed 6 days, (3) galactose-fed 9 days, (4) galactose-fed 12 days, (5) 5 days' reversal (6) 17 days' reversal, (7) 34 days' reversal, (8) control for 34 days' reversal. Arrows represent β-crystallin bands which are not labeled in a 6 hr in vitro incubation with 35S-methionine.

rapidly decreases, whereas that of the non-crystallin proteins appears to be unaffected. Moreover, upon reversal, crystallin synthesis returns to control levels.

Other biochemical changes associated with galactose cataract formation and recovery have been previously described. These include the decrease and essentially complete recovery of amino acids and glutathione along with changes in the cation distribution which remain after recovery and possibly lead to continued hydration of the lens.

Our results are consistent with those of Shinohara and Piatigorsky, who reported that under in vitro conditions changes in the intracellular concentration of Na⁺ and especially K⁺ lead to altered crystallin synthesis in the chick lens, and with those of Piatigorsky et al., who correlated a selective decrease in crystallin synthesis with altered Na⁺ and K⁺ concentrations in Nakano and ouabain-treated mouse lenses. Furthermore, the results of the present experiments on galactose cataracts closely agree with those on the Nakano lens with respect to the leak-out of crystallins in cultured, cataractous lenses. It is not known whether the changes in protein synthesis in the galactose cataracts are a primary or a secondary result of the altered cationic concentrations or the result of other concomitant changes.

Care, however, must be exercised in our qualitative correlation, since the intracellular concentration of cations is not known and the intercellular spaces are known to significantly increase during the galactose cataract development. This is especially true in the recovery phases, in which crystallin synthesis returns to control levels while the concentration of cations does not. It should be noted that protein synthesis in the lens occurs predominantly in the peripheral cortex, where it has been shown that lens fibers with normal morphology and clarity are present during the reversal of galactose cataracts. This zone of normal cortical fibers broadens throughout the reversal phase as a result of the repair of existing fibers and/or the formation of new ones. Cation levels for individual regions of galactose cataracts have not been determined; therefore the possibility exists that the Na⁺/K⁺ ratio is normal in cells with active crystallin synthesis.

To ascertain any potential significance of the observed decrease and recovery of crystallin synthesis, the dry weights of the lenses were compared (Fig. 5). These may be interpreted as an indicator of lenticular protein levels, since proteins account for 96% of the dry weight and crystallins account for virtually all of the lens proteins. After 6 days the dry weights of the galactosemic lenses compared to the normal lenses were depressed, and after 9 days a significant difference (p < 0.1) in the dry weights was observed. Between 9 and 12 days a sharp drop in the dry weights was noted, and no increase in the dry weights was observed until after day 29.
The protein concentration in the lens reflects the existing balance between synthesis and degradation; therefore changes in the synthesis, breakdown, and leak-out of proteins could all account for a reduction of lens dry weights. Differences in the protein content before day 9, however, are probably due to decreased crystallin synthesis, since little evidence for increased protein degradation was observed and crystallin leak-out, as measured in vitro, was not detected until days 9 to 12. This leak-out probably accounted for the sharp drop in the dry weights during this period. No leak-out of crystallins could be detected at day 17 (day 5 of recovery), probably due to the synthesis of a new layer of fibers in the cortex. Although crystallin synthesis had partially recovered by day 17, the dry weights did not increase until after day 29. This may be due to the depressed levels of amino acids or other unknown factors.

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REFERENCES


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