Protein Synthesis in X-irradiated Rabbit Lens

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The present study deals with the incorporation of $^{35}$S methionine into lens crystallins as a function of time after x-irradiation. Crystallin synthesis is first affected approximately 4 weeks following x-irradiation. This coincides with the time period at which the ratio of the two cations in the lens is affected, as shown in earlier studies. A greater decrease in $^{35}$S-methionine incorporation into crystallins is observed between 5-7 weeks following x-irradiation in good agreement with a cation imbalance at these time intervals. These studies also revealed for the first time that the change in cation distribution can affect not only crystallin synthesis, but also the synthesis of certain polypeptides of lens membranes. No alteration in protein synthesis could be detected in lens epithelium even after 7 weeks following irradiation. In addition to the effect of Na$^+$ and K$^+$ levels on protein synthesis, an impaired transport of amino acids into the x-rayed lens was also found to be a factor in the observed reduction in synthesis of the crystallin, cytoskeletal and membrane proteins of the fiber cells. It is concluded that Na$^+$/K$^+$ ratio as well as the availability of amino acids in the lens are important factors in protein synthesis of x-ray cataracts. Invest Ophthalmol Vis Sci 25:147-152, 1984

A decrease in lens growth rate is a common feature of many types of cataracts such as those that are x-ray induced in the rabbit, the diabetic and galactosemic type in the rat, and the genetic form in the Philly and Nakano mouse. The reduced rate of growth of the cataracts may be due to a combination of factors including reduction in protein synthesis, increased proteolysis, or the leakage of proteins from the hydrated lens. The mechanism by which protein synthesis is affected in these cataracts is not well understood. Recent studies involving several cataract models have suggested that alterations in the concentrations of cations in the lens may influence the rate of protein synthesis. Results of Piatigorsky and co-workers also suggest that the ratio of the levels of Na$^+$ to K$^+$ may be an important factor besides the absolute levels of these ions.

An unusual feature of the x-ray-induced cataract is that, although it is an osmotic type, no significant increase in hydration occurs in the lens until the development of mature cataract, 8 to 9 weeks after exposure to x-ray. However, prior to the increase in hydration there is evidence of pronounced change in lens permeability and in the transport of cations resulting in significant alterations in the concentrations of Na$^+$ and K$^+$. Despite changes in the levels of individual cations in the lens prior to formation of the mature cataract, the total content of Na$^+$ and K$^+$ remains constant as a result of the ability of the lens to compensate for the gain in Na$^+$ with an equivalent loss of K$^+$. Thus, the premature stage of x-ray cataract offers a means of studying the effect of an altered ratio of Na$^+$ and K$^+$ levels on protein synthesis since changes in the ratio of the cations occur without a significant change in either hydration or total Na$^+$ and K$^+$ content.

In the present study, we have examined the rate of incorporation of $^{35}$S-labeled methionine into crystallin proteins of x-irradiated lenses prior to development of mature cataract. The findings indicate that a decrease in $^{35}$S-methionine incorporation coincides with an alteration in Na$^+$/K$^+$ ratios in the x-irradiated lens. We also report for the first time the effect of cation changes on the synthesis of lens cytoskeletal and membrane proteins as studied in the x-ray-induced cataract. In addition to the effect of Na$^+$ and K$^+$ levels on protein synthesis, an impaired transport of amino acids into the x-rayed lens was also found to be a factor in the observed reduction in synthesis of the crystallin, cytoskeletal, and membrane proteins of the fiber cells.

Materials and Methods

X-ray induced cataractous lenses and the contra-lateral controls from albino rabbits were obtained as described earlier. Lenses were incubated in 4 ml of bicarbonate buffer containing TC 199 medium, 30 mM fructose and $^{35}$S-methionine (25 uCi/ml, specific activity approximately 400 Ci/mmol, New England Nuclear, Boston, MA), in a humidified atmosphere of 5% CO$_2$ and 95% air, at 37°C. Incubation was generally...
Fig. 1. Total cpm of the $^{35}$S-methionine incorporated into crystallin, cytoskeletal and membrane proteins of normal rabbit lens fiber cells, expressed as percent of the total radioactivity in the lens. Bars represent mean ± SD of four separate experiments.

Fig. 2. Specific activity (cpm/µg protein) of the $^{35}$S-methionine incorporated into crystallin proteins as a function of time after x-irradiation, expressed as percent of the control lens. Values represent means of n = 2–4 separate experiments, except at 7 weeks post-x-ray where n = 1.

Results

$^{35}$S-methionine incorporation into normal rabbit lens represented in Figure 1, showed that, of the total radioactivity transported into the fiber cells, 29% was incorporated into the crystallin fraction (LSF), 1.1% into the cytoskeletal (USF) and 0.15% into the membrane fractions (LMF).

In order to observe the effect of x-irradiation on protein synthesis in the lens, we examined the specific activity of the $^{35}$S-methionine incorporated into the soluble proteins of the fiber cells (LSF), shown in Figure 2. A decrease in the specific activity (cpm/µg protein) of the crystallin proteins was first observed approximately 4 weeks after x-irradiation. The incorporation decreased further as a function of time after irradiation, until 1 week prior to formation of the mature cataract. By the end of this period, $^{35}$S-methionine activity was reduced to 20% of that in the control lens.

To the extent that the rate of transport of the radioactive amino acid entering the lens may also be affected as a result of x-irradiation, the total radioactivity present in the lens was examined. As seen in Figure 3, the amount of $^{35}$S-methionine entering the lens decreased as a function of time after exposure to x-rays.

Since the total radioactivity entering the lens decreases as a result of x-irradiation, in comparing the
incorporation of \(^{35}\)S-methionine into proteins in the control and x-rayed lenses, the total amounts of radioactivity in the lenses were taken into consideration. The results (Fig. 4), showing the ratio of \(^{35}\)S-methionine incorporated into crystallin proteins (LSF), to the total \(^{35}\)S-methionine found in the lens, indicate that protein synthesis is indeed affected due to x-irradiation, although to a lesser extent than indicated by data based on specific activity of the proteins alone (Fig. 2). The decrease in the incorporation of \(^{35}\)S-methionine and presumably a decrease in protein synthesis at the end of 7 weeks following irradiation is 50% of the control value.

In addition to the crystallins, incorporation of \(^{35}\)S-methionine in the membrane and cytoskeletal fractions was also affected as a result of x-irradiation. Figure 5 shows the decrease in specific activity of the \(^{35}\)S-methionine incorporated into cytoskeletal proteins (USF), as a function of time after x-irradiation. By the end of 7 weeks post-irradiation, specific activity in the x-rayed lens decreases to 20% of the control value. This effect was also observed in the membrane protein fraction (LMF) (not shown).

An initial increase in \(^{35}\)S-methionine incorporation in the above figures was noted at 1 and 2 weeks post-irradiation, but the significance of this increase is not known.

The incorporation of \(^{35}\)S-methionine into individual polypeptides of the various protein fractions of fiber cells as well as the capsule-epithelium of normal lenses, separated by SDS-PAGE, is seen from the autoradiographs in Figure 6A. The epithelial fractions had the highest specific activity (approximately 80%) as revealed by a comparison of the density of the Coomassie blue stained bands (Fig. 6B) and their corresponding autoradiographs (Fig. 6A). The similarity between the autoradiographs of the fiber cell and epithelial fractions (Fig. 6A, lanes a-c, b-d) indicate that the epithelial cells synthesize polypeptides similar to those found in the lens fibers, such as the crystallin and cytoskeletal proteins. This observation is supported by studies on bovine epithelial cells in culture. However, the fiber cell membrane protein, MP 26, (Fig. 6A, lane e, arrowhead), does not appear to be a major component of the CE-insoluble polypeptides (CEIF).

The effect of x-irradiation on \(^{35}\)S-methionine incorporation into individual polypeptides of lenses at various periods following x-irradiation, is seen from the autoradiographs of SDS-PAGE separation of the proteins (Fig. 7–10). The decrease in incorporation of radioactivity into crystallin polypeptides, which was
first observed around 4 weeks, decreases further at 5 weeks and 6 weeks following x-irradiation (Fig. 7). Similarly, incorporation of $^{35}$S-methionine into the fiber cell membrane polypeptides also decreased, starting around 4 weeks and continuing at 5 and 6 weeks following exposure to x-rays (Fig. 8). These results also revealed that the decreased incorporation was a generalized phenomenon, indicating that incorporation into specific polypeptides was not differentially affected.

The addition of cycloheximide to the incubation medium resulted in complete inhibition of the incorporation of $^{35}$S-methionine into proteins (Figs. 7-9). This observation clearly suggests that the radioactivity incorporated into the polypeptides was due to protein synthesis, and not due to non-specific binding of $^{35}$S-methionine.

In contrast to the fiber cells, protein synthesis in the epithelium remained unaffected by x-irradiation as seen in Figure 9 during the same period of cataract development (ie, 4 weeks, 5 weeks, and 6 weeks post-irradiation). Even at 7 weeks post-irradiation, while amino acid incorporation into fiber cell membrane is appreciably reduced, incorporation into the epithelial protein fractions was unaffected (Fig. 10).

Discussion

The results of this investigation show that incorporation of $^{35}$S-methionine into proteins of the lens fiber cells is reduced as a result of x-irradiation. Along with a decreased incorporation, a decrease in transport of the amino acid into the lens was also observed. This decrease in protein synthesis is consistent with the reduced growth rate of the lenses following exposure to x-ray, as observed in earlier studies.\(^1\) The decrease in protein synthesis was not restricted to the crystallins alone (Fig. 2), but was a generalized phenomenon affecting other proteins of the lens including the cytoskeleton (USF) (Fig. 5) and membrane (LMF) (Fig. 8).

The major objective of this study was to examine the relationship between changes in Na$^+$/K$^+$ ratios and protein synthesis in the lens during development of the x-ray-induced cataract. Previous studies in various
other types of cataract models have emphasized the effect of cations on crystallin synthesis. It is apparent from the present study that changes in cation distribution also affect the synthesis of the cytoskeletal and membrane proteins (Figs. 5, 8).

The decreased incorporation of the $^{35}$S-methionine, which was first observed around 4 weeks post-irradiation (Fig. 2), coincides with the changed Na$^+$/K$^+$ ratios in the x-irradiated lens that become manifest around the same time, and continue until formation of the mature cataract. The absence of hydration during this period of cataract development suggests that there is a correlation between the decreased incorporation of $^{35}$S-methionine into proteins and the altered Na$^+$/K$^+$ ratios. The decrease in dry weight of the lens also observed during this period may be largely due to decreased crystallin synthesis, which represents the bulk of the lens proteins. While the possibility of protein degradation cannot be excluded from the present results, the decreased growth rate of the lens in x-ray induced cataract cannot be due to leakage of protein, since hydration does not occur until the development of mature cataract.

In earlier studies, the decrease in crystallin synthesis in osmotic cataracts has been attributed mainly to the influence of cations. A recent study by Reszelbach and Patterson considers also the possible effect of decreased amino acid transport which results from lens swelling, in altering protein synthesis of galactose-induced cataracts. The present study not only confirms the cation correlation with amino acid incorporation into proteins, but also supports the notion that decreased transport of amino acids (Fig. 3) is also a contributory factor in the decreased synthesis of proteins (Fig. 2), and thereby, in the growth rate of cataractous lenses. The decreased transport of amino acids into the lens observed in this study cannot, however, be related to lens swelling since no hydration occurs in the precataractous lens. The exact mechanism by which amino acid transport decreases in x-rayed lens remains to be determined.

A surprising finding was that, despite the pronounced effects on $^{35}$S-methionine incorporation into proteins of fiber cell, synthesis of proteins in the epithelium, even in the near-mature cataract, is not reduced (Fig. 10). This behavior was also reported by Shinohara and co-workers in the galactosemic cataracts in rat, and in the hereditary cataracts in mice. Earlier work by

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Fig. 8. Representative autoradiograph of lens fiber cell membrane proteins at 4, 5 and 6 weeks after x-irradiation. c = control lens; x = x-irradiated lens. Cyhx- e represents 0.01 M cycloheximide added to the incubation medium.

Fig. 9. Representative autoradiograph of lens epithelial insoluble proteins, 4, 5 and 6 weeks after x-irradiation. c = control lens; x = x-irradiated lens. Cyhx- e represents 0.01 M cycloheximide added to the incubation medium.
von Sallmann has shown that exposure to x-irradiation, after an initial suppression, causes an increase in mitosis of the epithelial cells. This mitotic activity may account for the unchanged protein synthesis observed in the present study. It is also possible that, despite the lowered Na-K ATPase levels evident in the epithelial cells from earlier studies, these cells may maintain a normal Na⁺/K⁺ ratio. Since the cation content of these cells has not been studied successfully, the possibility that the unchanged protein synthesis in the epithelium is due to unchanged Na⁺/K⁺ ratios remains to be examined. The possibility that cell to cell communication between the epithelial layer and the underlying fibers may be interrupted as a result of x-irradiation, thus uncoupling the transport between these two regions of the lens, also requires further exploration.

While the present study clearly supports the ideas that changed Na⁺/K⁺ ratios and availability of amino acids are responsible for decreased protein synthesis, the possibility that other unknown factors may also influence protein synthesis during cataract development cannot be ruled out.

Key words: protein synthesis, x-irradiation, cataracts, crystallins, membranes, transport, cations, rabbit

References