Metabolic Production of a Blue-Green Fluorophor in Lenses of Dark-adapted Mice and Its Increase with Age

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A blue-green fluorophor (496 nm emission/406.7 nm excitation) occurs in the mouse lens; its increase with age is more pronounced in the nucleus than in the cortex. The level of fluorophor and its rate of production are the same for animals reared in the dark as for animals reared in the light. Thus, the fluorophor is not generated by a photochemical reaction but is a purely metabolic product. Invest Ophthalmol Vis Sci 24:1157-1161, 1983.

There is some experimental evidence1-6 that the predominant blue fluorophor in the lenses of human and animal species is a photoreaction product. This is true even for those rodents that normally have no pigment and a low level of fluorescence. The striking visibility of this fluorophor has unduly emphasized its importance over others which may have more significance from the viewpoint of concentration or metabolic involvement. It is usually not acknowledged explicitly that the prominence of a particular fluorophor depends upon another factor in addition to its concentration, namely, the quantum efficiency of the molecule. A molecule-like fluorescein (quantum efficiency ~ 0.90)7 is usually detectable in extremely low concentrations whereas tryptophan (quantum efficiency ~ 0.13)8 at the same concentration fluoresces only very weakly. Furthermore, substances with fluorescence excited at shorter wavelengths1-6 use more energetic photons than those excited at longer wavelengths;9 the greater phototube sensitivity at shorter wavelengths favors the detection of blue fluorophor. Thus, the importance of the blue fluorophor has probably been overestimated; likewise the idea that it is apparently a photoproduct has been extended unconsciously to imply that other fluorophors may be photoproducts. This extension has had the unfortunate result that metabolic involvement in fluorophor production has been deemphasized.

In this paper we show that a blue-green fluorophor (emission maximum at 496 nm with excitation at 406.7 nm) of the mouse lens occurs at the same concentration in mice reared in the dark from birth as in mice reared under the cool-white fluorescent lighting (12 hrs/day) in our animal quarters. Thus, this fluorophor is clearly a metabolic product with no relationship to photochemical effects. It exhibits a steady increase in intensity (hence concentration) with age.

Materials and Methods

Mice of the CFW strain were reared in the dark by putting a pregnant female in a dark room and maintaining the progeny for the desired lengths of time. The control mice were maintained under cool-white fluorescent illumination (12 hrs/day). Both groups were given Purina Rodent Chow and water ad lib.

Lenses were extracted from the enucleated eyes by a posterior approach, and picked clean of clinging materials that may interfere with our measurements. Care was taken to minimize the light exposure of the lenses extracted from dark-adapted mice. The exposure of a lens to the laser beam at 406.7 nm (2.5–4.0
Results

With excitation wavelength at 406.7 nm the mouse lens exhibits a fluorescence maximum at 496 nm, whose intensity increases sharply with age. This is shown in Figure 1, where we compare four spectra taken from the nucleus centers of lenses with the following ages: 2.57, 31.0, 42.0, and 91.0 weeks. Since the instrumental conditions for these measurements were identical and the interference due to clinging materials has been minimized, the intensities displayed in Figure 1 may be compared directly. The fluorescence intensity at 496 nm was found to increase approximately 10 times in going from 2.56-week- to 91-week-old lens. However, for quantitative intensity comparisons under similar, but not necessarily identical conditions, one needs an internal standard. The Rayleigh line is far too intense to be suitable. Fortunately, there appear two prominent Raman signals in the 21,000-22,000 cm\(^{-1}\) region (Fig. 1), due to water \(\nu(O-H)\) and aliphatic \(\nu(C-H)\) stretching vibrations. These two lines (Raman frequency shifts at 3,350 and 2,940 cm\(^{-1}\)) have been employed...
Fig. 2. A plot of relative Raman intensities between proteins ($I_p$) and water ($I_w$) vs. age for dark- and light-adapted mouse lenses. The data were taken from nucleus center.

as internal standards in the present study. The concentrations of water and protein in a lens vary with age, the intensity ratio, $I_p/I_w$, increases slightly from 1 to ~3.5 but becomes constant after 40 weeks of age (Fig. 2).

Fig. 3. Comparison of fluorescence intensities (relative to water signal) at the nucleus center vs. age between dark- and light-adapted mouse lenses.

The blue-green fluorophor of the mouse lens observed here has not been reported previously. There are practically no other detectable fluorophors in the mouse lens with longer wavelength excitation. Plots of fluorescence intensities at 496 nm relative to Raman H2O signal, ie, $I_f/I_w$, are displayed in Figure 3, where data from dark-adapted mice and light-adapted mice are compared. It is clear that there is no significant difference in the rate of fluorophor production between dark and light. In Figure 4 is shown a similar comparison between dark and light, using Raman C-H signal as an internal standard. The fluorescence increases slowly up to about 40 weeks when it suddenly begins to increase at a greater rate but still linearly. Again there is no difference between dark-adapted and light-adapted lenses. Figure 5 shows visual axis scan for fluorescence intensity in dark- and light-adapted mice for several ages up to 72 weeks. It is clear that there is no difference between the two groups of mice with respect to the age-dependent increase in fluorescence intensity, either in the cortex or the nucleus. Thus, the blue-green fluorophor is not a photochemically induced product.

Discussion

The distribution pattern of the fluorophor in the mouse lens (Fig. 5) is not compatible with any sort
Fig. 5. Visual axis profiles of fluorescence intensities (relative to water signal) for dark- and light-adapted mouse lenses. Data points from anterior epithelium have been omitted because their fluorescence intensities are unusually high and the measurement of water signal was difficult.

The changes in the lens during aging and cataract formation have been linked to many different mechanisms including oxidation of SH to SS, conversion of tryptophan and other sensitive components of protein, and condensation of proteins with glucose or malondialdehyde. Among the oxidants implicated are hydrogen peroxide, superoxide anion, and the hydroxyl free radical, all of which are normal oxygen metabolites. In addition to the above reactions, the effects of photochemical reactions in the lens have been investigated widely because the lens is one of the few tissues subjected directly to light action and because of epidemiologic evidence for light-induced cataractogenesis. This interest in photochemical effects has resulted in much evidence, mostly indirect, which has been taken to prove that excessive light exposure causes senile cataract, a conclusion justifiably deplored by Harding and Dilley.

The idea that fluorescence and pigmentation of the lens may both be due to reactions photocatalyzed by light bears the important implication that they may accompany cataract formation and even perhaps be causative. Thus, it is important to determine if such an easily measured substance as a fluorophor is actually dependent upon light for its formation. A fluorophor that is purely metabolic in origin is of practical interest because of the possibility of monitoring a metabolic process by a simple noninvasive procedure. Such a process may or may not be involved in cataractogenesis. We have no evidence concerning the identity or source of the blue-green fluorophor although the possibility exists that it may be the ribonuclease A-malondialdehyde adduct (395/470 nm) involved in the production of lipofuscin. The human lens also contains a blue-green fluorophor that we have no reason to believe is different from the mouse lens fluorophor (unpublished results). If it turns out that the mouse and human blue-green fluorophor are the same we will have an experimental model that can be subject to manipulation and that will be pertinent to the human lens.

An aging study is easily carried out in mice whereas a similar study in human lenses is difficult because of the great variation among lenses and the impossibility of obtaining human lenses which have never been exposed to light. Basic details of fluorophor generation are needed because of the prevalent belief that...
fluorophors are photoproducts and that they are involved in cataractogenesis. The pattern of deposition of fluorophors and pigments is an important clue to their mode of formation. This subject needs to be explored because much basic information is lacking and because of the pertinence of such information to human nuclear cataracts which are characterized by abnormally increased accumulation of both fluorescent and colored materials in the nucleus. Although there is wide-spread acceptance of the hypothesis that such enhanced accumulation in some human cataracts is light induced, the evidence is mostly indirect and far from conclusive as Harding and Dilley13 have indicated. The symmetrical distribution in the mouse lens correlates with its spherical shape and the spherical shape of the nucleus. It is quite unlike the distribution of fluorescence and pigment in the human lens. The deposit, viewed parallel to the equatorial plane, is not symmetrical but conforms to the shape of the nucleus (when the nucleus is defined in terms of hardness alone) which in turn conforms fairly closely to the overall shape of the human lens. That is, the anterior face is flatter than the posterior face, which often has a distinct bulge. If the pigment were light generated in situ, the bulge should occur in the anterior segment since the pigment in the older nucleus center would prevent light from reaching the posterior center. Any theory of fluorophor and pigment formation in the human lens must explain the peculiar shape of this deposit. Our results on the mouse lens are equivalent to that in the human lens in that the deposit is spherical in a spherical lens with a spherical nucleus. Thus, the deposit in the human lens could arise in the same way and the elucidation of the mechanism remains a subject for our future research.

Key words: lens, mouse, aging, Raman, fluorescence, fluorophor

References