Volume 219, number 2, 472–476 FEB 04913 July 1987

Spectroscopic studies of brunescent cataractous lenses

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Received 8 April 1987

The absorption spectra of brunescent cataractous lenses and their homogenates were analyzed under various conditions by using a double wavelength spectrophotometer. The absorption spectra of the samples were in good agreement with those of synthetic xanthommatin derived from 3-hydroxykynurenine. The results provided evidence that brown pigment in the brunescent cataractous lenses is mainly composed of xanthommatin.

Spectroscopic study; Brunescent cataractous lens

1. INTRODUCTION

Although it has been suggested that metabolites of tryptophan such as *N'*-formylkynurenine, kynurenine and 3-hydroxykynurenine might be involved in the pigmentation of cataractous lenses [1–3], the identity of the brown pigment and the formation mechanism in the brunescent cataractous lenses are still obscure. Van Heyningen [3] showed that yellow compounds such as 3-hydroxykynurenine and *o*-*β*-D-glucoside of 3-hydroxykynurenine are detected in the soluble fractions of the homogenates of cataractous lenses, and suggested that 3-hydroxykynurenine may act as active tanning agent such as occurs in the formation of xanthommatin, a brown pigment, in the insect eye. However, the brown pigment is not present in the soluble fractions of homogenates, but present in the insoluble fractions containing water insoluble proteins [3,4], so that the characterization of this pigment has not been undertaken thoroughly.

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On the other hand, Kurzel et al. [5] showed the fluorescent properties of brunescent cataractous lenses and a build-up in the component giving rise to high wavelength absorption (400–500 nm), but it was difficult to characterize the brown pigment. Since the color of brown pigment in brunescent cataractous lenses is optically analogous to that of xanthommatin, a dimerized chromophore of 3-hydroxykynurenine, we investigated whether brown pigment in brunescent cataractous lenses is composed of xanthommatin, a brown ommochrome.

This manuscript is a preliminary report on spectroscopic properties of intact brunescent cataractous lenses and those of homogenates of the lenses. We were able to provide evidence that xanthommatin is a major component of the brown pigment in brunescent cataractous lenses.

2. MATERIALS AND METHODS

Human brunescent cataractous lenses (highly pigmented) were obtained from patients with senile cataracts after operation. These lenses were stored frozen at −80°C until use. These specimens were thawed and rinsed with distilled water to remove

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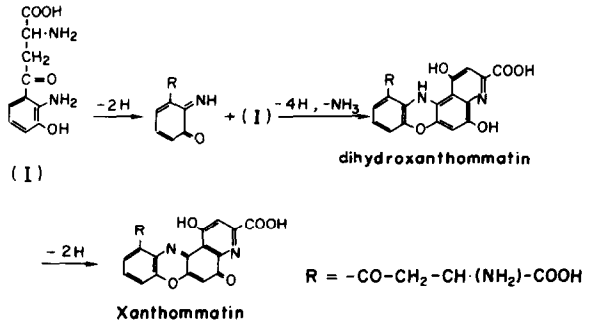
00145793/87/$3.50

fluffy particles. Then, the samples were subjected to measurement of optical analysis. All samples were mounted in a quartz cell cuvette. The absorption spectra of the intact brown cataractous lenses were taken between 350 nm and 600 nm by use of a Hitachi 557 double wavelength spectrophotometer with a data processor.

For the spectroscopic studies of homogenates of brown cataractous lenses, a piece of brunescent cataractous lens was minced and added to 2 ml of 20 mM Tris-HCl buffer (pH 8.0), and then was homogenized by a glass homogenizer. The homogenates were sonicated at 40 W for 5 s in an ice bath. A 0.5 ml of the samples was added separately in glass tubes with 1.5 ml of 20 mM Tris-HCl buffer (pH 8.0), a 5 N HCl solution, a 1 N NaOH solution and a 1:1 chloroform methanol solution, respectively. The absorption spectra of these samples were measured between 350 and 600 nm by using a Hitachi 557 double wavelength spectrophotometer with a data processor. To eliminate turbidity of the lens homogenates, a filter paper was mounted in the reference cuvette.

Synthetic xanthommatin and cinnabarinic acid were obtained by the reactions of 3-hydroxykynurenine (Wako, Tokyo) and 3-hydroxyanthranilic acid (Wako, Tokyo) with ferricyanide [6], and tentatively identified as such on the basis of UV and IR spectra, and retention time on HPLC [7]. The process of xanthommatin formation during the oxidation of 3-hydroxykynurenine (I) with ferricyanide is shown in scheme 1 as proposed by Butenandt [6]. The UV spectra of synthetic xanthommatin, cinnabarinic acid and 3-hydoxykynurenine were spectrophotometrically measured between 350 and 600 nm in different solutions as mentioned above.



3. RESULTS

We studied spectral properties of human brunescent cataractous lenses with high pigmentation (fig.1). Fig.1A shows a typical example of absorption spectra between 350 and 550 nm of a highly pigmented lens and a moderately pigmented lens. Characteristic absorption spectra with a peak near 400 nm appeared in both lenses, but the optical intensity near 400 nm was found to be closely dependent on the extent of the coloration of the brunescent lenses. This result suggests that some chromophore with maximal absorbance near 400 nm is present in the brunescent lenses.

In order to clarify the identity of the chromophore with the absorbance near 400 nm in the brunescent lenses, we studied the absorption spectra of highly pigmented lens at different pHs. As shown in fig.1B, the absorption spectra of the brunescent lens with a peak near 400 nm shifted to the right side and the optical intensity near 400 nm decreased, when the intact lens was immersed in the 5 N HCl solution. The characteristic absorption spectra of the brunescent cataractous lens between 350 and 600 nm disappeared when the cell was immersed in the 1 N NaOH solution. These results strongly suggest that the brown chromophore in the brunescent lens is sensitive to pH. Though a notch was observed at 515 nm in every brunescent lens, this is probably due to unknown causes, because the notch was always observed at different pHs.

Since the color of ommochrome compounds is generally brown at neutral pH and is very sensitive to the changes in pHs, we suspected that the brown coloration of cataractous lenses might be due to the pigmentation of ommochrome compounds in the cells. Fig.2 shows the changes in absorption spectra of homogenates of brunescent lens with high pigmentation at different pHs. The homogenates showed characteristic absorption spectra at 410 nm in the Tris-HCl solution (pH 8.0). In this case, the notch at 515 nm which was observed in the intact lens disappeared. The absorption spectra of the homogenates shifted to the right, i.e., the peak at 410 nm shifted to 475 nm and the optical intensity decreased to some extent, when the homogenates were acidified with the 5 N HCl solution. On the other hand, when the homogenates were alkalified with the 1 N NaOH

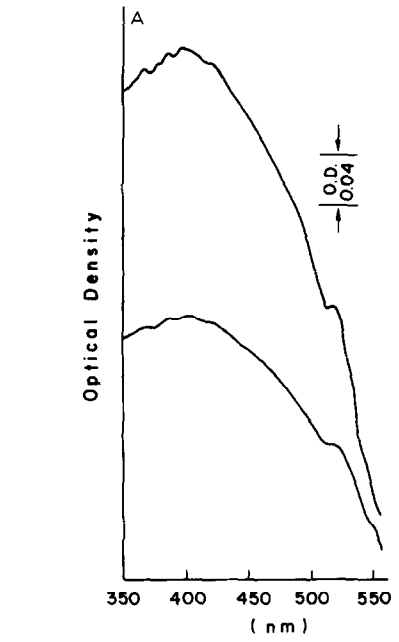


Fig.1. Absorption spectra of intact brunescent cataractous lenses. (A) The absorption spectra of intact brunescent cataractous lenses with different coloration. The upper and lower curves indicate the absorption spectra of highly and moderately pigmented lenses, respectively. (B) Absorption spectra of intact brunescent cataractous lenses with high pigmentation at different pHs.

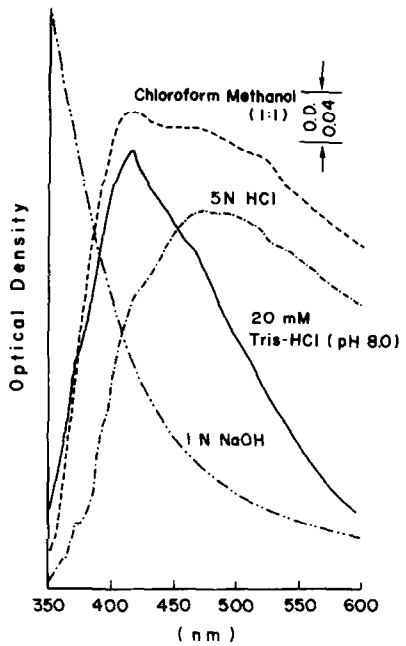
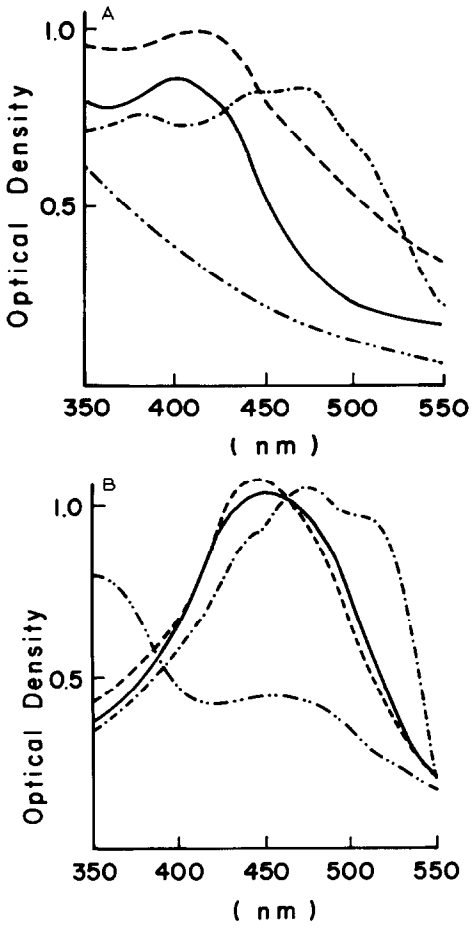


Fig.2. Absorption spectra of the homogenates of brunescent cataractous lenses with high pigmentation under different conditions. The absorption spectra were measured at different pHs and in 1:1 chloroform/methanol solutions.

solution, the peak at 410 nm disappeared. When the homogenates were suspended in 1:1 chloroform/methanol solutions, the shift of absorption maxima was small (about 10 nm to the right), but the optical intensity increased in the visible regions.

The absorption spectra of synthetic xanthommatin from 3-hydroxykynurenine are shown in fig.3A. The absorption spectra of synthetic xanthommatin have a peak at 410 nm in the Tris-HCl solution (pH 8.0), at 420 nm in 1:1 chloroform/methanol solutions and at 475 nm in the HCl solution. The peak at 410 nm disappeared in the 1 N NaOH solution. These results support the view that the chromophore in the homogenates is very analogous to xanthommatin.

Fig.3B and C shows the absorption spectra of synthetic cinnabarinic acid and 3-hydroxykynurenine in the visible regions, respectively. Cinnabarinic acid derived from 3-hydroxyanthranilic acid showed characteristic absorption spectra with maximal absorbance at 455 nm for the Tris-HCl solution (pH 8.0), at 450 nm in 1:1



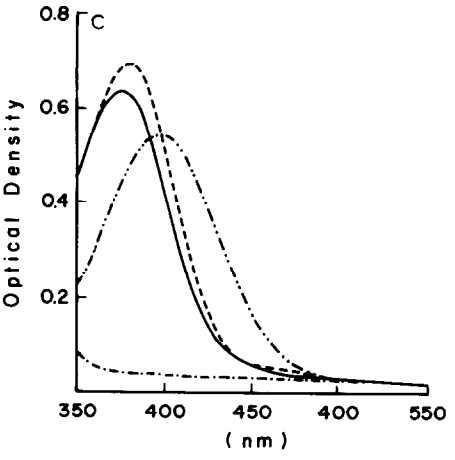


Fig.3. Absorption spectra of synthetic xanthommatin (A), cinnabarinic acid (B) and 3-hydroxykynurenine (C) under different conditions. The absorption spectra were measured in different solutions (20 mM Tris-HCl solution, pH 8.0; 5 N HCl solutions, 1 N NaOH solutions and 1:1 chloroform/methanol solutions). (——) In the solution of 20 mM Tris-HCl (pH 8.0); (– – –) chloroform/methanol (1:1); (-----) in the solution of 5 N HCl; (------) in the solution of 1 N NaOH.

chloroform/methanol solutions, at 475 and 530 nm in the 5 N HCl solution and at 460 nm (but with extensively decreased absorbance) in the 1 N NaOH solution. On the other hand, the absorption spectra of 3-hydroxykynurenine showed maximal absorbance at 370 nm in the Tris-HCl solution (pH 8.0), at 375 nm in 1:1 chloroform/methanol solutions and at 400 nm in 1 N NaOH solutions. The absorbance disappeared in the visible regions when 3-hydroxykynurenine was dissolved in the 5 N HCl solution.

4. DISCUSSION

In spite of many reports on brown pigment of brunescent cataractous lenses, the identification of this pigment in the lenses has not been undertaken thoroughly, probably because the pigment is tightly bound to insoluble proteins which are found abundantly in the brunescent cataractous lenses. Kurzel et al. [5] studied the fluorescent properties of intact brunescent cataractous lenses, but the identification of the brown pigment in the cells was insufficient. Using fluorescent analysis, Van Heyningen [3] observed 3-hydroxykynurenine and its glucoside compound in soluble fractions of the homogenates of cataractous lenses, and suggested that 3-hydroxykynurenine may be involved in the formation of brown pigment. However, a spectral analysis of the brown pigment in the brunescent cataractous lenses was difficult due to much turbidity of insoluble proteins containing the brown pigment. We succeeded in measuring the absorption spectra of the intact brunescent lenses (fig.1) and those of homogenates (fig.2), by using a double wavelength spectrophotometer to cancel spectral turbidity. By this method, we demonstrated that the brown pigment in the brunescent catarac-

tous lenses is mainly composed of xanthommatin (figs 1–3), because the spectral properties of intact brunescent cataractous lenses and their homogenates are in good agreement with those of synthetic xanthommatin. We tried to isolate the pigment from the insoluble proteins, by adding dithiothreitol or mercaptoethanol to cut sulfhydryl bonds, β-glucosidase to cut β-glucoside bonds, and various proteinases. But it was difficult to isolate the brown pigment from insoluble proteins of the homogenates of brunescent cataractous lenses.

As for the mechanism of brown pigment formation in the cataractous lenses, there seems to be the consensus that tryptophan metabolites are involved in the formation mechanism. Pirie [1] showed that the contents of tryptophan in the proteins in cataractous lenses are significantly decreased and that tryptophan is changed to N'-formylkynurenine and to kynurenine. Such changes in protein are shown when protein was irradiated with UV light [8]. Zigman et al. [9] also showed that when the tryptophan solution was irradiated with UV light for long periods, it was changed to some compound like ommochrome. Bando et al. [10] reported that rat lens crystalline, a soluble protein, became brown when the protein was incubated with 3-hydroxykynurenine and was irradiated with UV light. Van Heyningen [3] suggested that 3-hydroxykynurenine, which is present in the soluble fractions of cataractous lenses, may act as a tanning agent such as occurs in xanthommatin formation in insect eye. Therefore, it may be possible that tryptophan residues are changed to 3-hydroxykynurenine residues via N'-formylkynurenines and kynurenines in the insoluble proteins of cataractous lenses, and that subsequently 3-hydroxykynurenines in the soluble fractions are dimerized to the 3-hydroxykynurenine residues to form xanthommatin in the proteins by means of photooxidation with UV light. This view may be coincident with the report of Dillon [11] suggesting the direct photolysis of lens protein and photosensitized reactions involving 3-hydroxykynurenine-glucoside, but remain to be proved further. We also observed that insoluble proteins became brown within several hours, when the insoluble proteins obtained from cataractous lenses were incubated with 3-hydroxykynurenine and irradiated with UV light (unpublished data). The process of xanthommatin formation in the insoluble proteins by photooxidation may be visualized as proposed by Butenandt [6] in the reactions of 3-hydroxykynurenine with ferricyanide (scheme 1).

ACKNOWLEDGEMENT

This work was supported by a grant from the Japanese Ministry (no.60570127).

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