Fluorescence emission and excitation spectra of fluorophores of lipofuscin granules isolated from retinal pigment epithelium of human cadaver eyes

T. B. Feldman, M. A. Yakovleva, A. E. Dontsov, and M. A. Ostrovsky*

N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 4 ul. Kosygina, 119334 Moscow, Russian Federation. Fax: (499) 137 4101. E-mail: feldman@sky.chph.ras.ru

Visible light-induced changes in fluorescence characteristics of lipofuscin granules (LG) isolated from retinal pigment epithelium of human cadaver eyes are compared with the analogous age-related changes and correlated with the content and photooxidation of LG main fluorophore, A2E. We used HPLC to examine changes of LG fluorophore composition with donor age, as well as before and after visible-light irradiation (the latter HPLC tests were also done with synthetic A2E). Visible light induces oxidation of LG fluorophores. As a result, their fluorescence characteristics change: the emission spectrum is blue-shifted by 25—40 nm. The observed age-dependent changes in the relative content of LG fluorophores and their oxidized derivatives were qualitatively similar with those caused by irradiation. To improve the accuracy of a new noninvasive diagnostic method, fundus autofluorescence imaging, it is important to know the ratio of nonoxidized and oxidized fluorophore derivatives depending on age and eye pathology.

Key words: retinal pigment epithelium, lipofuscin granules, fluorophores, all-*trans*-retinal derivatives, fundus autofluorescence, age-related macular degeneration, visible light.

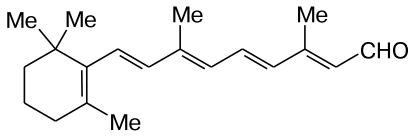
Lipofuscin granules (LG) or age pigment are complex lipid-protein aggregates that accumulate in retinal pigment epithelial cells of human eyes with aging.[1] Recent studies have revealed the correlation between LG accumulation in retinal pigment epithelium (RPE) and degenerative retinal diseases, including such a severe and widespread retinopathy as age-related macular degeneration.[2]

LG have been long believed to be just a cell metabolism by-product. It turned, however, that they are photochemically active. As we have shown in the early 1990-s,[3,4] LG can generate oxygen reactive species upon photoexcitation with visible light. Now there is abundant evidence of their phototoxic and pathogenic effect.[5,6]

LG exhibit distinct fluorescence in the visible region. Based on its measurement, fundus autofluorescence imaging is a new noninvasive diagnostic method for revealing age-related base changes and degenerative retinal and RPE pathology. Autofluorescence allows assessment of the condition, integrity and viability of the photoreceptor-RPE complex.[7]

The major LG constituents responsible for its fluorescence are different all-*trans*-retinal (ATR) conjugates.

As yet, the only lipofuscin fluorophore that has been identified and characterized is the di-retinal conjugate A2E (*N*-retinyl-*N*-retinylidene-ethanolamine) which is produced by two molecules of ATR and amino group of a lipid



ATR

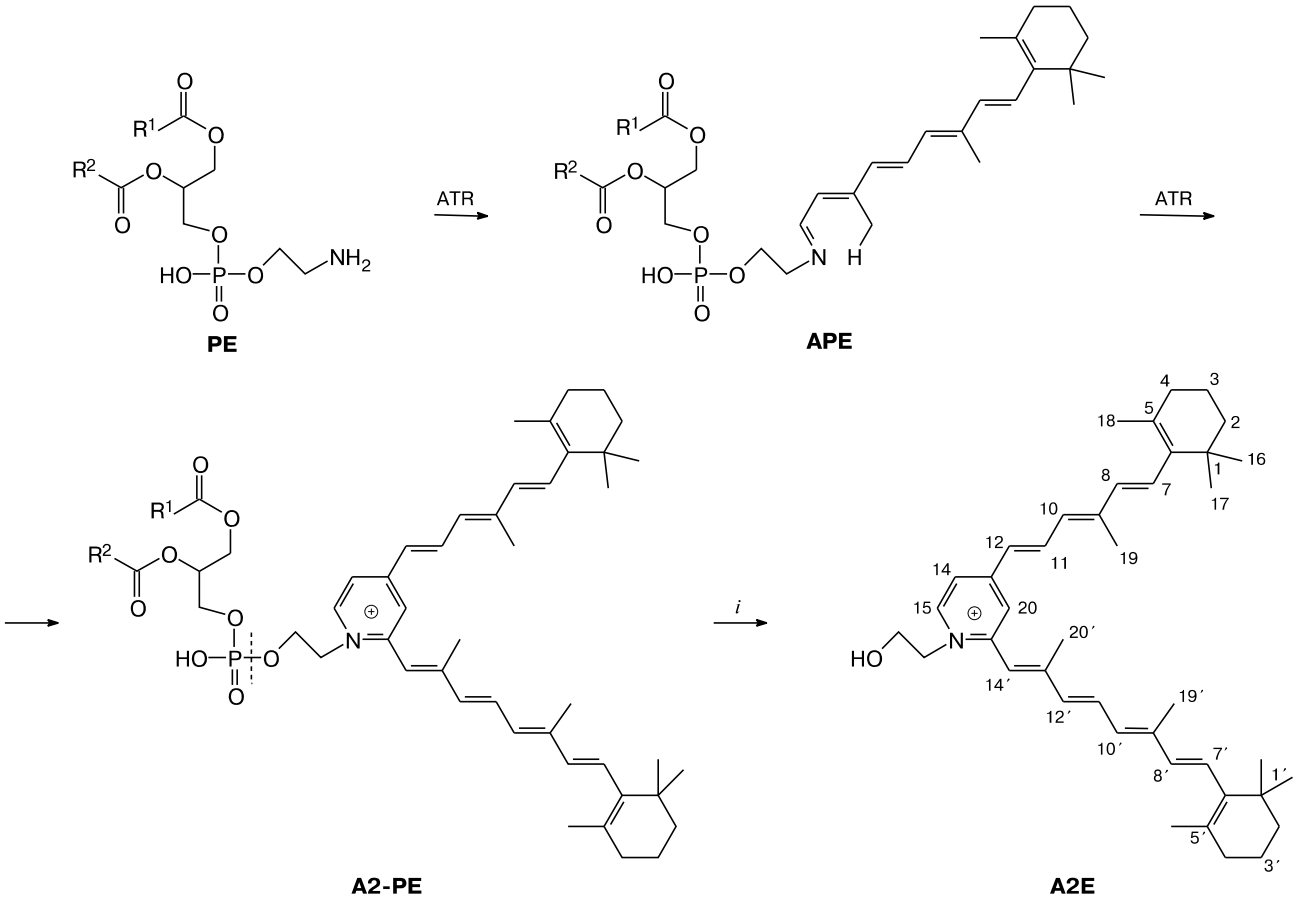
of photoreceptor outer segment membrane, phosphatidylethanolamine (PE) (Scheme 1).[8,9] The precursors of A2E, *N*-retinylidenephosphatidylethanolamine (APE) and *N*-retinyl-*N*-retinylidenephosphatidylethanolamine (A2PE) are formed in rod outer segments, A2E is formed in RPE lysosomes by enzymatic hydrolysis of the phosphate ester of phosphatidyl-pyridinium bisretinoid, A2PE.[8,9] Besides A2E, LG include about ten other retinal-containing fluorophores.[10]

In the presence of oxygen, A2E undergoes photooxidation to produce a mixture of products[11,12] (Scheme 2) which are cytotoxic agents themselves, their reactivity, conversely, not being light-mediated.[13] These oxidation products are more hydrophilic than the parent A2E and are capable of leaving LG[14] and diffusing through cytoplasm to other RPE intracellular organelles.[15] The oxidized A2E derivatives were detected by HPLC in LG isolated from senile human eyes as well as from RPE of mice with ABCR mutation modeling Stargardt disease.[16,17] Both human and mice RPE were found to contain A2E-ep-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 269—276, January, 2010.

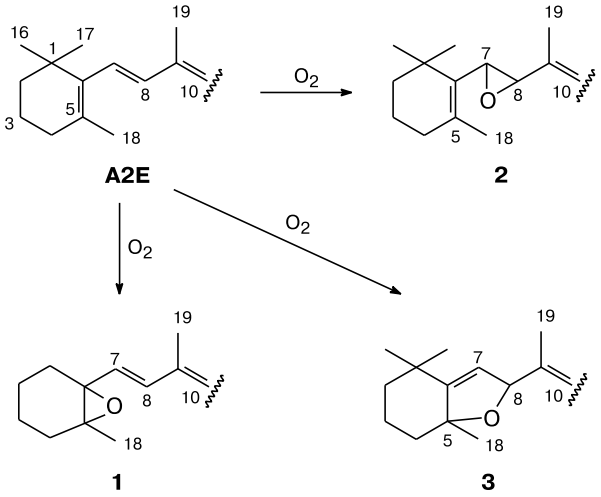
1066-5285/10/5901-0276

Scheme 1



i. Phospholipase D, hydrolysis

Scheme 2



oxides, 5,6-epoxy- (**1**) and 7,8-epoxyderivatives (**2**), and also 5,8-furanoderivatives (**3**).[18] It is quite possible that degenerative processes developed in retina and RPE are mediated by A2E with other present in LG fluorophores, and by their photooxidized derivatives as well.

Importantly, the formation and accumulation of oxidized products is reflected in the fundus autofluorescence spectral pattern. As known,[15,19,20] irradiation with visible light alters the absorbance and fluorescence spectra of A2E, and also of LG. Thus, to make the new noninvasive diagnostic technique (fundus autofluorescence imaging) more informative, it is essential to know how exactly visible light illumination and aging processes alter fluorescence pattern of LG and their main fluorophore, A2E. This work was undertaken to solve the aforesaid task.

Experimental

The reagents used were obtained from Sigma, Sigma—Aldrich, Fluka, Komponent-Reactiv; 1 mL plastic disposable tubes and disposable pipettes were purchased from Eppendorf. The Sigma and Fluka solvents for HPLC analysis were HPLC grade.

Isolation of LG. LG were isolated from human RPE cells removed from donors aged 17—70 years. Donor eyes lacking any ocular pathology, after isolating retina for transplantation, were allowed for experimentation from the Eye Tissue Bank of Sv. Fyodorov Eye Microsurgery Complex, Moscow. All isolation procedure steps were performed under dimmed light conditions.

LG were isolated and purified as described in Ref. 10. The samples contained ~$2 \cdot 10^7$ granules per 1 mL of 0.1 *M* phosphate buffer (pH 7.3).

LG samples for evaluating age-dependent relative content of oxidized fluorophores were isolated and pooled from 53 eyes of donors aged 17—40 and 47 eyes of donors aged 40—70. In each age bracket examined, LG were pooled from three independent isolations from three independent groups of donors.

LG samples for evaluating the effect of LG irradiation with visible light on the relative content of oxidized fluorophores were isolated from 60—65 donor eyes, non-age-matched.

Synthesis of *N*-retinyl-*N*-retinylideneethanolamine (A2E). A2E was synthesized and purified as described previously.[8] The purity of A2E was HPLC-controlled using the Knauer (Germany) chromatograph.

Preparation of chloroform extract of LG. After adding a twofold excess of $CHCl_3$—MeOH (2 : 1) mixture to LG suspension in the phosphate buffer, the suspension was stirred by an electrical mixer for 2 min, then incubated for 10 min at 4 °C and centrifuged (680 *g*, 10 min, 4 °C, MLW K 26 D). The lower chloroform phase was collected with syringe and transferred to the flask. The resultant chloroform extract was concentrated with a water-jet injector. After addition of 200 μL of MeOH, the precipitate obtained was chromatographed.

Irradiation of samples. Irradiation was performed using 150 W incandescent lamp KGM 24-150 as a source and the optical system of a slide projector with a heat filter. The samples were exposed to whole-spectrum (390—700 nm) visible light under constant stirring.

Absorption and fluorescence spectra. The absorption spectra were recorded on Shimadzu UV-1700 spectrophotometer (Japan); fluorescence spectra — on Shimadzu RF-5301PC fluorometer (Japan). Since the concentration dependence of photooxidized products could not be precisely correlated with the initial reaction conditions, all fluorescence spectra were normalized to the maximum fluorescence intensity. The analysis was based on assessment of changes in emission maxima positions.

HPLC separation of ATR derivatives. ATR derivatives and other polyenes were eluted (Knauer, Diaspher 120 C18 4×120 mm, particle size 5 μm) with the following linear gradient of acetonitrile in water (+ 0.05% TFA): 80% MeCN (20 min), 100% MeCN (20 min) and a flow rate of 1.5 mL min^{-1} with monitoring at 430 nm (Knauer K-2501 spectrophotometric detector).

HPLC quantitation was done using EuroChrom 5.05 program package. To determine the error for each sample, 3 independent chromatograms were measured and averaged. The component percentages in the mixture were based on the summed area of all peaks, thus providing the estimates of relative component content and irradiation-induced or age-dependent changes in the latter. Data processing yielded the linear distribution, so, for further significance estimation Student´s test was applied.

Results and Discussion

Visible light-induced changes in absorption spectra of LG and synthetic fluorophore A2E

The LG suspension in phosphate buffer and the methanol solution of synthetic fluorophore A2E were subjected to intense irradiation at 390—700 nm for 2 h. We have also analyzed the spectral patterns from LG chloroform extracts before and after their irradiation with visible light.

Fig. 1 shows the absorption spectra of LG suspension, chloroform extract of LG, and synthetic fluorophore A2E solution before and after irradiation.

Although the absorption spectrum of LG suspension (see Fig. 1, *a*) has no pronounced maxima because of the strong light scattering, it is seen, nonetheless, that LG absorbance at 430 nm under irradiation with visible light is decreased with the simultaneous increase in absorbance intensity at 290 nm.

The absorption spectrum of the chloroform extract prepared from the suspension of nonirradiated LG (Fig. 1, *b*) exhibits more distinct (compared to the parent LG suspension) maxima (shoulders) at 270, 330 and 430 nm. After irradiation of LG, their chloroform extract demonstrates virtually no absorption at 340 and 430 nm, whereas the short-wavelength maximum is shifted to the wavelength of 260 nm.

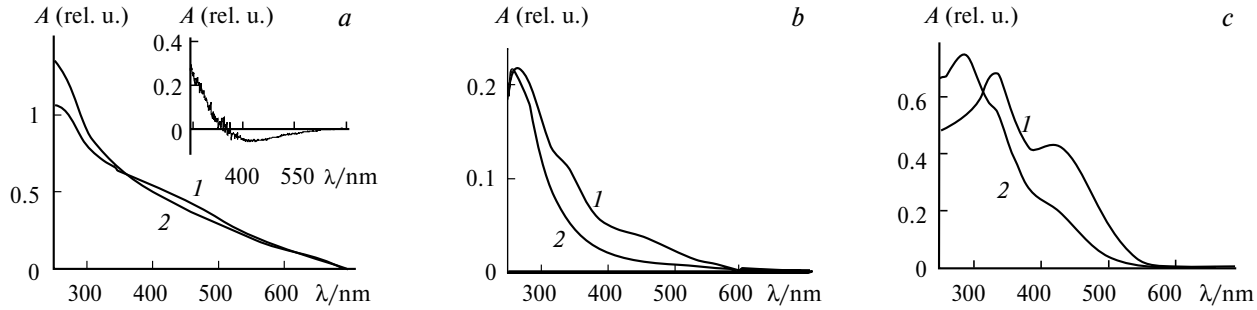


Fig. 1. Absorption spectra of LG suspension in phosphate buffer (*a*), chloroform extracts from LG (*b*), synthetic A2E in MeOH (*c*) before (*1*) and after irradiation for 2 h (*2*). The inset shows differential absorption spectrum obtained by subtracting spectrum (*1*) from spectrum (*2*).

The absorption spectrum of the synthetic fluorophore A2E (Fig. 1, *c*) peaks at 340 and 430 nm. The spectral pattern of irradiated (for 2 h) A2E is altered: absorbance at 340 and 430 nm is markedly decreased, distinct maxima disappear, and a new peak in UV region (280 nm) appears.

Thus, irradiation with visible light of both LG suspension in phosphate buffer and the methanol solution of synthetic A2E results in their blue-shifted absorbance maxima. In other words, the species absorbing at 340—430 nm vanish upon irradiation, while the new species absorbing in UV region are formed. These results allow considering the chloroform extract of LG and the synthetic fluorophore A2E as the model systems for studying visible light-induced or age-related changes in LG.

Visible light-induced changes in fluorescence spectra of LG and synthetic fluorophore A2E

Fluorescence in all samples was excited by 280-, 340- and 430-nm irradiation corresponding to the absorbance maxima of A2E, main LG fluorophore.

Fig. 2 shows the emission spectra of LG suspension in phosphate buffer, chloroform extract of LG, and methanol solution of synthetic fluorophore A2E before and after irradiation.

Fluorescence emission spectra of all irradiated samples were altered (compared to nonirradiated samples) much stronger than the absorption spectra. All maxima are blue-shifted by 25—40 nm (see Fig. 2 and Table 1). Table 1 lists the distribution of emission maxima for all samples before and after irradiation. The observed shifted maxima can be probably attributed to the oxidized fluorophore derivatives formed upon irradiation.

It should be noted that the fluorophore producing maximum at 540 nm contributes inconsiderably to the total emission in the model systems (chloroform extract of LG and synthetic A2E), being at the same time well seen in LG (see Fig. 2, *b*—*i*). Most likely, this is a fluorescence from one or several fluorophores which are not extracted from LG into chloroform.

Thus, fluorescence emission spectra of all irradiated samples are shifted 25—40 nm to short wavelengths.

Excitation spectra of LG suspension

Fluorescence excitation spectra of LG suspension were measured at emission wavelengths of 335, 450 and 500 nm

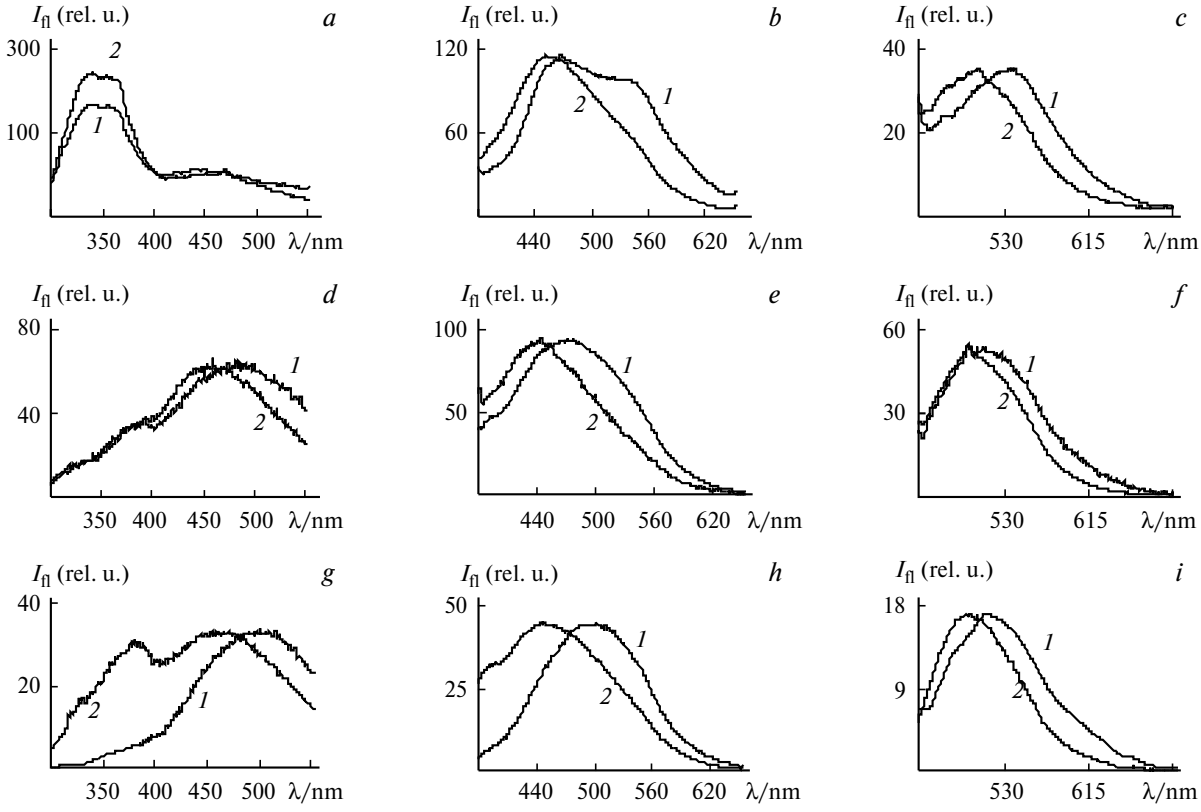


Fig. 2. Fluorescence emission spectra of LG suspension in phosphate buffer (*a*—*c*), chloroform extracts from LG (*d*—*f*) and synthetic A2E in MeOH (*g*—*i*) before (*1*) and after irradiation for 2 h (*2*). Fluorescnce was excited by 280-nm (*a*, *d*, *g*), 340-nm (*b*, *e*, *h*) and 430-nm irradiation (*c*, *f*, *i*).

Table 1. Fluorescence emission maxima in the spectra of LG suspension in phosphate buffer, chloroform extract of LG, and synthetic A2E in MeOH before and after visible light irradiation for 2 h

Sample	λ_{ex} /nm[a]	Fluorescence emission maxima/nm[b]	
		NI	I
Lipofuscin granules	280	335, 360, 470	335, 360, 444
	340	470, 540	444, 540 (shoulder)
	430	540	540 (shoulder), 500
Chloroform extract of LG	280	380 (shoulder), 490	380, 450
	340	475	440
	430	500, 540 (shoulder)	500
Synthetic A2E	280	380, 500	384, 458
	340	500	458
	430	500	494

[a] Excitation wavelength.
[b] NI is "nonirradiated"; I is "visible light irradiated, 2 h".

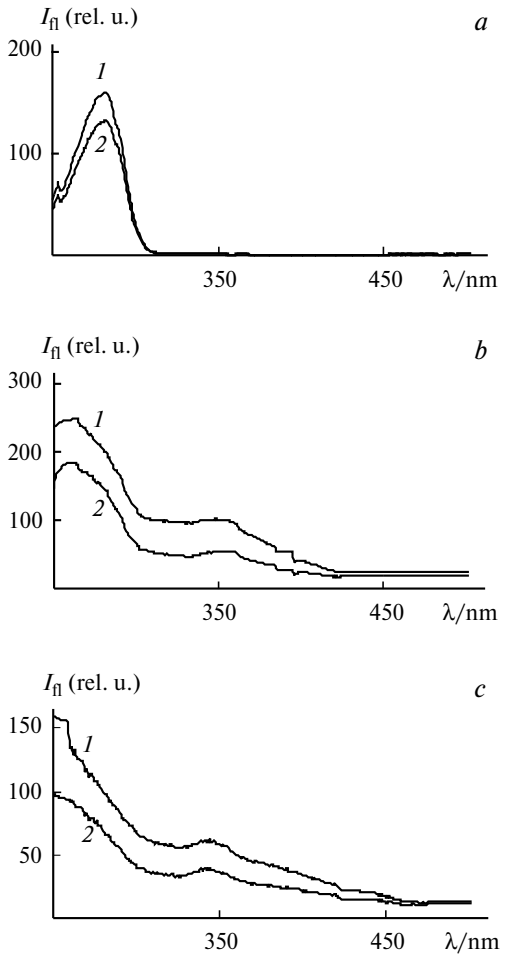


Fig. 3. Fluorescence excitation spectra of LG suspension with emission at 335 (*a*), 450 (*b*) and 500 nm (*c*) before (*1*) and after irradiation for 2 h (*2*).

(Fig. 3) for the qualitative analysis of LG fluorophores. The excitation spectrum with fluorescence at 335 nm (Fig. 3, *a*) has the maximum at 280 nm. It is most probably due to LG protein component (tryptophan fluorescence).[21]

The excitation spectra with fluorescence at 450 and 500 nm show maxima at 270, 350 and 450 nm. The short-wavelength maximum at 270 nm can be tentatively attributed to oxidized ATR derivatives, and the maxima at 350 and 450 nm — to nonoxidized ATR derivatives, *e.g.*, A2E and ATR dimer.[12,18]

From Figs 2 and 3 it is well seen that a considerable contribution to total emission is made by species absorbing in the UV region. Consequently, the proper fundus autofluorescence pattern interpretation would require accounting the contribution from not only A2E but also the components absorbing at short wavelengths.

Excitation and fluorescence of such species cannot be practically studied *in vivo* because of limitations imposed by spectral characteristics of lens and other intraocular light filters. However, their *in vivo* examination would be enabled in the case when a colorless UV-absorbing lens is implanted to a patient eye instead of a cataract lens removed at surgery, provided this patient shows signs of degenerative retinopathy. Such cases do occur in ophthalmologic clinical practice. In the fundus autofluorescence diagnostics of such patients, estimation of the contribution from the species absorbing at short wavelengths and emitting at long wavelengths could supply an important information about macular degeneration pathogenesis.

HPLC analysis of the chloroform extract of LG and methanol solution of A2E before and after visible-light irradiation

In our and other works[11,12,17,18] it was shown that A2E undergoes photooxidation when subjected to visible light, to give epoxide and furanoid derivatives.

Here we present a comparative HPLC analysis of the chloroform extract of LG and methanol solution of A2E before and after irradiation (Fig. 4).

The chromatogram of irradiated A2E disclosed two peaks arising from the oxidized A2E derivatives (Fig. 4, *b*,*c*, peaks of oxy-A2E).[13] Alongside with the new-formed products, the decrease in A2E content is observed. Note that apart from these identified A2E derivatives, the chromatogram indicates the presence of new products with the shorter retention times compared to A2E and oxy-A2E (Fig. 4, *c*, assembled peaks *1* and *2*).

HPLC analysis of the chloroform extracts of LG before and after visible-light illumination gave the similar results (see Fig. 4, *d*—*f*). As in the case of A2E, the formation of oxidized A2E derivatives by LG irradiation is indicated by the presence of two additional peaks (see. Fig. 4, *e*, peaks of oxy-A2E) whose appearing is again accompanied

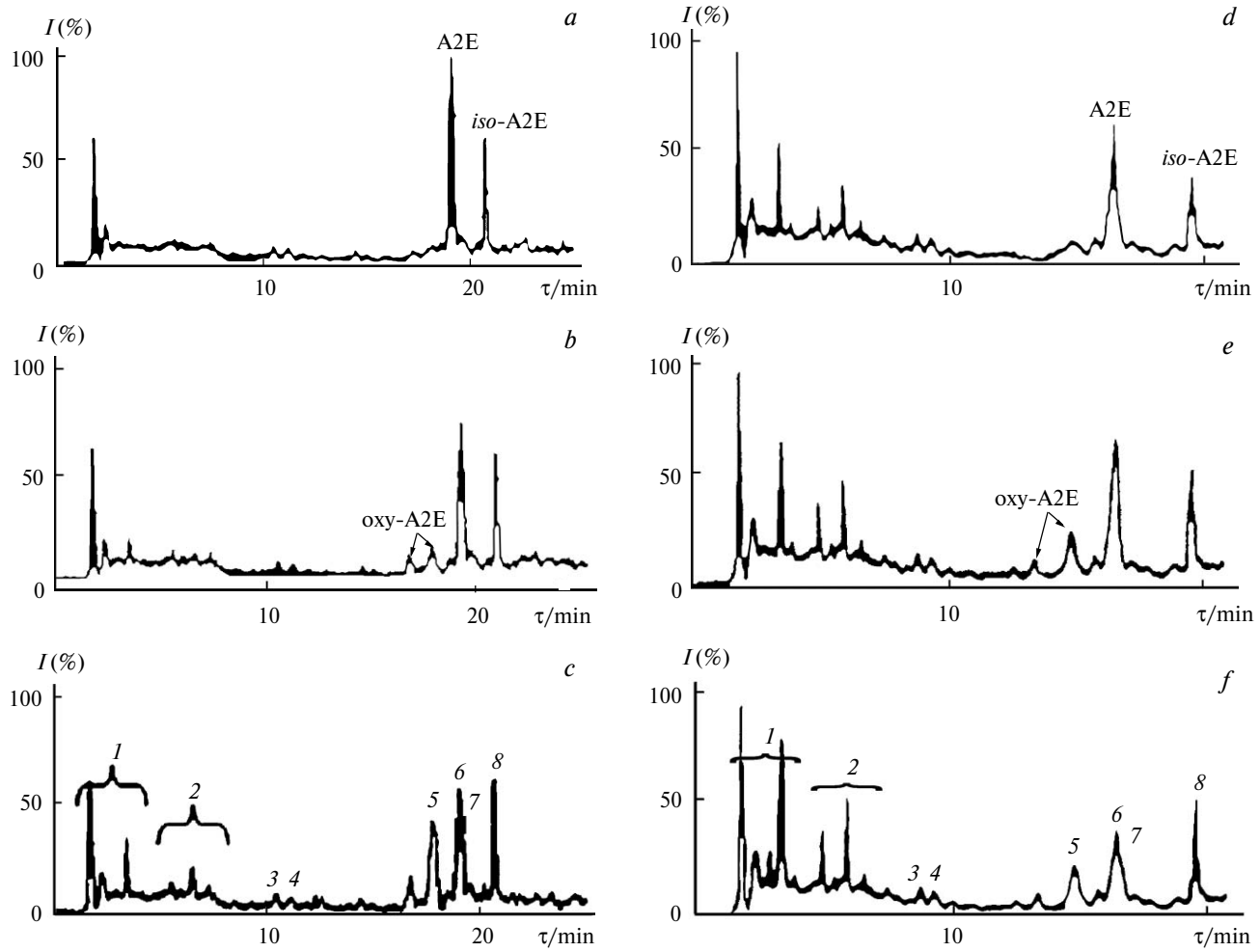


Fig. 4. HPLC analysis of synthetic A2E in MeOH (*a*—*c*) and chloroform extracts from LG (*d*—*f*) before (*a*, *d*) and after (*b, c, e, f*) irradiation with visible light for 1 (*b*, *e*) and 2 h (*c*, *f*).

by A2E consumption (see Fig. 4, *f*, peaks *6*—*8*). Along with the identified oxy-A2E products, the chromatogram shows the increased intensity of the bands corresponding to the species having shorter retention times as compared with A2E and its oxy-derivatives (see Fig. 4, *f*, assembled peaks *1* and *2*). It is noteworthy that the retention times of new-formed oxidized derivatives assayed in irradiated A2E in methanol and in chloroform extracts of irradiated LG are in good agreement. This agreement indicates that both in methanol solution and as LG constituent, A2E is photooxidized with formation of similar (in particular, in spectral characteristics) products.

The HPLC data for nonirradiated and irradiated chloroform extract of LG (see Fig. 4, *d*—*f*, Table 2) demonstrate that the total percentage of major *iso*-A2E isomeres (see Fig. 4, *f*, peaks *6*—*8*) before irradiation was ~47% (see Fig. 4, *d*), after irradiation (2 h) — ~41% (see Fig. 4, *f*). Thus, irradiation increases the relative content of oxidized derivatives of A2E and, possibly, other fluorophores.

Age-dependent changes in relative content of fluorophores in LG from donors without visible ocular pathology

As follows from HPLC data for the chloroform extract of LG, the relative content of oxidized derivatives of A2E and, possibly, other fluorophores increased after LG irradiation. There are also the data[16] providing evidence for an increase in oxy-A2E content in LG with aging.

Accordingly, it appeared interesting to perform a comparative HPLC analysis of different fluorophores or groups of fluorophores in LG isolated from donor eyes within specific age brackets (age groups 1 and 2). Lacking of visible ocular pathology or marks from ophthalmosurgical operations was established by ophthalmologists of the Eye Tissue Bank of Sv. Fyodorov Eye Microsurgery Complex, Moscow.

The age range of the group 1 was 17—40 years, and of the group 2 — 40—70 years.

The revealed age dependence of LG fluorophore composition correlated well with the relevant changes induced

Table 2. Relative content of different fluorophores in the chloroform extract of LG within specific age groups of donors without visible ocular pathologies (M±σ)

Peak[a]	Fluorophore content (%)				
	Group 1[b] (17—40 years)	Group 2[b] (40—70 years)	LG		
			nonirradiated[c]	irradiated	
				t = 1 h[d]	t = 2 h[e]
1	14.91±1.76	17.39±0.22	17.83±2.21	18.87±2.31	19.92±1.32
2	19.68±1.23	23.63±1.76	18.87±1.76	22.97±2.25	24.98±2.27
3	3.36±0.56	3.94±0.24	3.72±0.56	3.76±0.76	3.64±0.52
4	4.03±0.78	1.14±0.11	4.65±0.78	3.99±0.35	3.73±0.73
5 (oxy-A2E)	6.58±0.25	7.05±0.44	7.56±0.23	6.98±0.28	6.32±0.54
6 (A2E)	29.52±1.03	33.32±1.05	29.67±1.56	27.91±1.72	26.81±1.65
7	2.03±0.56	1.39±0.23	1.93±0.32	1.31±0.12	1.03±0.34
8 (*iso*-A2E)	19.89±1.12	12.14±1.12	15.77±1.27	14.21±1.31	13.57±1.43

[a] Peak or group of peaks in the chromatogram (Fig. 4, *d—f*).
[b] Means were compared by the Student´s test. The level of significance $p < 0.05$.
[c] See Fig. 4, *d*.
[d] See Fig. 4, *e*.
[e] See Fig. 4, *f*.

by irradiation. In the group 1, the relative content of A2E isomers (see Fig. 4, peaks *6*, *7* and *8*) was 52% on average, while in group 2 — ~47% ($p < 0.05$) (see Table 2). A decrease in the content of A2E isomers in LG isolated from the older donors could result from partial age-related oxidation of A2E and *iso*-A2E. This means the demonstrated in our experiments visible light-induced A2E photooxidation in LG can be considered as a model of age-associated alteration of LG fluorophore composition.

Thus, LG fluorophores undergo photooxidation during irradiation with visible light. As a result, the maxima in their fluorescence spectra are markedly (25—40 nm) blue-shifted, and the total relative content of oxidized derivatives (peaks *1—5*) increases (from 48 to 53% ($p < 0.05$), see Table 2).

Since the derivatives generated by photooxidation of fluorophores exhibit cytotoxicity independent of irradiation,[13] it seems important to evaluate the ratio of nonoxidized and oxidized derivatives of LG fluorophores in dependence on age and pathology, especially such as age-related macular degeneration.

The estimate of contributions from nonoxidized and oxidized LG fluorophores would be most helpful in interpreting fundus autofluorescence patterns as allowing better diagnostic accuracy at early and advanced stages of degenerative disorders.

The modern and expensive instrument for reliable fundus autofluorescence imaging, the confocal scanning laser ophthalmoscope, affords recording fluorescence emitted at 500—600 nm and excited by 488-nm irradiation with Ar laser (see Ref. 7). Specially modified ophthalmoscope would allow separate recording of fluorescence in the ranges of 500—540 nm and 540—600 nm. This design makes possible the assessment of "short wavelength" and "long wavelength" fundus autofluorescence excited at the same wavelength of 488 nm, thus enabling to determine the ratio of nonoxidized and oxidized derivatives of LG fluorophores. Such componentization of autofluorescence could provide useful diagnostic information.

The component analysis of fundus autofluorescence can be performed with a quite common and available in many ophthalmologic clinics fundus-camera. In this case there are more possibilities for varying excitation wavelength and for differential fluorescence recording but, at the same time, assessment of weak fundus autofluorescence is less reliable. Apparently, these problems will be overcome in the next generation analyzers.

However, the need in comparative analysis of age-dependent fluorescence characteristics of LG in a normal eye and, in particular, at different pathological conditions, is quite obvious. Our research in this direction will be continued.

This work was financially supported by the Russian Academy of Sciences (the basic research program of the Presidium of the Russian Academy of Sciences "*Basic Science Applications to Medicine*", the basic research program of the Branch of Chemistry and Material Science of the Russian Academy of Sciences (*OX-01*) "*Theoretical and Experimental Investigation of Chemical Bond Nature and the Mechanisms of Basic Chemical Reactions and Processes*") and by the Russian Foundation for Basic Research (Project No. 08-04-12132).

References

1. C. J. Kennedy, P. E. Rakoczy, I. J. Constable, *Eye*, 1995, **9**, 763.
2. M. Rozanowska, T. Sarna, E. J. Land, T. G.Truscott, *Free Radic. Biol. Med.*, 1999, **26**, 518.
3. M. A. Ostrovsky, A. E. Dontsov, N. L. Sakina, M. Boulton, J. Jarvis-Evans, *Sensornye Sistemy* [*Sensor Systems*], 1992, **6**, 51 (in Russian).
4. M. Boulton, A. Dontsov, J. Jarvis-Evans, M. A. Ostrovsky, D. Svistunenko, *J. Photochem. Photobiol. B: Biol.,* 1993, **19**, 201.
5. M. Boulton, M. Rozanowska, B. Rozanowski, T. Wess, *Photochem. Photobiol. Sci.,* 2004, **8**, 759.
6. M. A. Ostrovsky, *Uspekhi Biologicheskoi Khimii* [*Advances in Biological Chemistry*], 2005, **45**, 173 (in Russian).
7. S. Schmitz-Valckenberg, F. G. Holz, A. C. Bird, R. F. Spaide*, Retina,* 2008, **28**, 385.
8. C. A. Parish, M. Hashimoto, K. Nakanishi, J. Dillon, J. Sparrow, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 14609.
9. S. Ben-Shabat, C. A. Parish, H. R. Vollmer, Y. Itagaki, N. Fishkin, K. Nakanishi, J. R. Sparrow, *J. Biol. Chem.,* 2002, **277**, 7183.
10. G. E. Eldred, M. L. Katz, *Exp. Eye Res.*, 1988, **47**, 71.
11. J. P. Dillon, Z. Wang, L. B. Avalle, E. R. Gaillard, *Exp. Eye Res.,* 2004, **79**, 537.
12. M. A. Yakovleva, N. L. Sakina, A. S. Kononikhin, T. B. Feldman, E. N. Nikolaev, A. E. Dontsov, M. A. Ostrovsky, *Dokl. AN*, 2006, **409**, 411 [*Dokl. Biochem. Biophys.* (*Engl. Transl.*), 2006, **409**, 223].
13. J. R. Sparrow, H. R. Vollmer-Snarr, J. Zhou, Y. P. Jang, S. Jockusch, Y. Itagaki, K. Nakanishi, *J. Biol. Chem.,* 2003, **278**, 18207.
14. V. S. Sokolov, E. A. Sokolenko, A. V. Sokolov, Yu. A. Ermakov, O. A. Finogenova, A. E. Dontsov, M. A. Ostrovsky, *Biologicheskie Membrany* [*Biological Membranes*], 2005, **22**, 336 (in Russian).
15. A. E. Dontsov, N. L. Sakina, A. M. Golubkov, M. A. Ostrovsky, *Dokl. AN*, 2009, **425**, 683 [*Dokl. Biochem. Biophys.* (*Engl. Transl.*), 2009, **425**, 98].
16. L. B. Avalle, Z. Wang, J. P. Dillon, E. R. Gaillard, *Exp. Eye Res.,* 2004, **78**, 895.
17. R. A. Radu, N. L. Mata, A. Bagla, G. H. Travis, *Proc. Natl. Acad. Sci. U.S.A.,* 2004, **101**, 5928.
18. Y. P. Jang, H. Matsuda, Y. Itagaki, K. Nakanishi, J. R. Sparrow, *J. Biol. Chem.,* 2005, **280**, 39732.
19. A. E. Dontsov, N. L. Sakina, M. A. Ostrovsky, *Sensornye Sistemy* [*Sensor Systems*], 2006, **20**, 265 (in Russian).
20. A. E. Dontsov, N. L. Sakina, B. Bilinska, L. Krzhizhanovsky, T. B. Feldman, M. A. Ostrovsky, *Dokl. AN,* 2005, **405**, 458 [*Dokl. Biochem. Biophys.* (*Engl. Transl.*), 2005, **405**, 458].
21. A. White, *Biochem J.,* 1959, **71**, 217.

Received July 6, 2009;
in revised form October 28, 2009