Conference materials
UDC 577.344
DOI: https://doi.org/10.18721/JPM.153.252

Physicochemical analysis of bisretinoid A2E photooxidative destruction products

M. A. Yakovleva [1], A. A. Vasin [2]✉, A. E. Dontsov [1], A. A. Gulin [2], A. V. Aybush [2], T. B. Feldman [1, 3], M.A. Ostrovsky [1, 3]

[1] Emanuel Institute of Biochemical Physics, RAS, Moscow, Russia;
[2] N.N. Semenov Federal Research Center for Chemical Physics, RAS, Moscow, Russia;
[3] Lomonosov Moscow State University, Moscow, Russia
✉ a2vasin@yandex.ru

Abstract. Bisretinoid N-retinyl-N-retinylidene ethanolamine (A2E) of the retinal pigment epithelium (RPE) lipofuscin granules is a side product of visual cycle. Its accumulation is associated with degenerative diseases of the retina and retinal pigment epithelium. In this study A2E photooxidation and photodegradation products were studied. Absorption and 2D fluorescence spectra of these substances were detected. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) and Fourier-transform infrared spectroscopy (FTIR) revealed chemical changes during the A2E photooxidation process. Aldehyde accumulation was observed and new structure one of the resulting compounds was proposed.

Keywords: Retinal pigment epithelium, bisretinoid A2E, photooxidation, carbonyls

Funding: Russian Science Foundation Grant 22-24-00549.

Citation: Yakovleva M. A., Vasin A. A., Dontsov A. E., Gulin A. A., Aybush A. V., Feldman T.B., Ostrovsky M.A., Physicochemical analysis of bisretinoid A2E photooxidative destruction products, St. Petersburg State Polytechnical University Journal. Physics and Mathematics. 15 (3.2) (2022) 285–290. DOI: https://doi.org/10.18721/JPM.153.252

Материалы конференции
УДК 577.344
DOI: https://doi.org/10.18721/JPM.153.252

Физико-химический анализ продуктов фотодеструкции бисретиноида A2E

М. А. Яковлева [1], А. А. Васин [2]✉, А. Е. Донцов [1], А. А. Гулин [2], А. В. Айбуш [2], Т. Б. Фельдман [1, 3], М. А. Островский [1, 3]

[1] Институт биохимической физики им. Н.М. Эмануэля РАН, г. Москва, Россия ;
[2] Федеральный исследовательский центр химической физики им. Н.Н. Семёнова РАН, г. Москва, Россия;
[3] Московский государственный университет им. М.В. Ломоносова, г. Москва, Россия
✉ a2vasin@yandex.ru

Аннотация. Бисретиноид A2E, содержащийся в липофусциновых гранул в клетках ретинального пигментного эпителия глаза является побочным продуктом зрительного цикла. Его накопление связано с дегенеративными заболеваниями сетчатки. В данной работе были изучены продукты фотоокисления и фотодеградации A2E, их флуоресцентные свойства, а также химические изменения в процессе фотоокисления. Обнаружено накопление соединений, содержащих альдегидную группу. Предложена новая структура одного из полученных соединений.

Ключевые слова: ретинальный пигментный эпителий, бисретиноид A2E, фотоокисление, карбонилы

Финансирование: Российский Научный Фонд № 22-24-00549.

Ссылка при цитировании: Яковлева М. А., Васин А. А., Донцов А. Е., Гулин А. А., Айбуш А. В., Фельдман Т. Б., Островский М. А. Физико-химический анализ продуктов фотодеструкции бисретиноида A2E // Научно-технические ведомости СПбГПУ. Физико-математические науки. Т. 15. № 3.2. С. 285–290. DOI: https://doi.org/10.18721/JPM.153.252

Introduction

N-retinyl-*N*-retinylidene ethanolamine (A2E) is the main bisretinoid of the lipofuscin granules (LGs) in the retinal pigment epithelium cells. It is the side product of the visual cycle. A2E biogenesis occurs when two molecules of *all*-trans retinal condense with one molecule of phosphatidylethanolamine in the photoreceptor membrane, followed by uptake into RPE and conversion to stable pyridinium bisretinoid [1]. In the RPE, LGs are formed by incomplete lysosomal degradation of photoreceptor outer segment debris following phagocytosis of shed outer segments by RPE cells. LGs accumulate in the RPE of the human eye during aging, particularly in patients with hereditary diseases [2,3] and progressive age-related macular degeneration (AMD) [4].

A2E and its photooxidation and photodegradation products (A2Eox,deg) are major sources of LG fluorescence. The compounds investigated in LGs include A2E [5], A2Eox,deg [6–7] and a series of *all*-trans retinal conjugates [8]. It is known, that A2E is a photoinducible generator of reactive oxygen species (ROS) [9-10] and able to damage cellular structures *in situ* [11–12].

Earlier we demonstrated [13], the LG photooxidation results in the formation of toxic water-soluble thiobarbituric acid (TBA)-reactive products. However, the nature of these products is not fully understood. There is evidence that the source of TBA-reactive products are the lipid peroxidation end-products, i.e., highly reactive electrophilic aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (HNE) in LGs, that suggests a connection between its formation and increased oxidative stress [14]. We assume that the source of highly active aldehydes and ketones may be A2Eox,deg in LGs [13].

Thus, the main goal of this work is to characterize the A2Eox,deg using the 2D fluorescence spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS).

Materials and Methods

A2E synthesis and photooxidation. A2E was synthesized from *all*-trans retinal and ethanolamine in acetic acid and ethanol, as described previously [15]. A2E purity was monitored by high performance liquid chromatography (HPLC) [13]. A2E concentration was determined spectrally using a Shimadzu UV-1700 spectrophotometer (Japan) at a wavelength of 430 nm with = $3.1 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. For photooxidative destruction, 0.4 ml of A2E in methanol (with concentration of 1.8 mM) was irradiated for 120 min at room temperature under constant stirring using a 150 W incandescent lamp with a heat filter (KGM 24-150, 400–700 nm). The luminous flux density irradiating the sample was 80 mWm^{-2} for visible light (400–700 nm), as determined by a photometer (Spectra-Physics 407A, USA).

Spectroscopy. Absorption spectra were recorded on a Shimadzu 3600 UV–vis near-infrared spectrophotometer (Japan). Fluorescence data were recorded on a Horiba Fluoromax. 2D analysis was performed with excitation wavelength step 2 nm. IR spectra were taken with a Fourier IR (FTIR) microscope LUMOS II (Bruker) in ATR mode. The samples were applied dropwise to CaF_2 glass and dried in an argon atmosphere before analysis.

Mass spectrometry. Time-of-flight secondary ion mass spectrometry (ToF–SIMS) (ION-ToF, Germany) with 30 keV Bi_3^+ primary ions were used to detect A2E photooxidation products. 10 spectra were recorded from different regions for each sample.

Results and Discussion

A large number of products of A2E photooxidation and photodegradation are formed when A2E is irradiated with visible light. Previously we have shown using HPLC analysis the formation of these products [7, 16]. Fig. 1a demonstrates absorption spectra of synthesized A2E before and after irradiation by visible light. It is clearly seen that during irradiation there is a decrease in absorption in the region of 430 nm, and an increase in the region of 280 nm. These results are in good agreement with the literature data [7, 13, 17].

2D fluorescence map spectra were collected for non-irradiated (Fig 1,*b*) and irradiated A2E (Fig. 1,*c*). Three fluorescence sites detected for the non-irradiated A2E: excitation at 263 nm with emission at 580 nm; two excitations at 340 and 440 nm with emission at 610 nm (Fig 1b). These excitation maxima are in good accordance with absorption spectra. However, excitation at 263 nm is not clearly expressed in the absorption spectrum. Interestingly, the UV excitation site has a much larger extinction coefficient and a large Stokes shift. Probably, such shift is present due to the existence of pyridinium in A2E. Pyridinium is known to form a fluorescent complex with charge transfer [18].

Blue shift of fluorescence emission sites were observed for A2E irradiated. Two fluorescence sites of A2E irradiated showed up: excitation at 270 nm with emission at 470 nm, excitation at 370 nm with emission at 460 nm (Fig 1c). The fluorescence profile shows that the emission maxima in the area < 500 nm, which are likely associated with the fluorescence of A2E oxidized products. This is probably due to the destruction of the original A2E conjugated structure and the accumulation of oxidation products with a shorter conjugated structure.

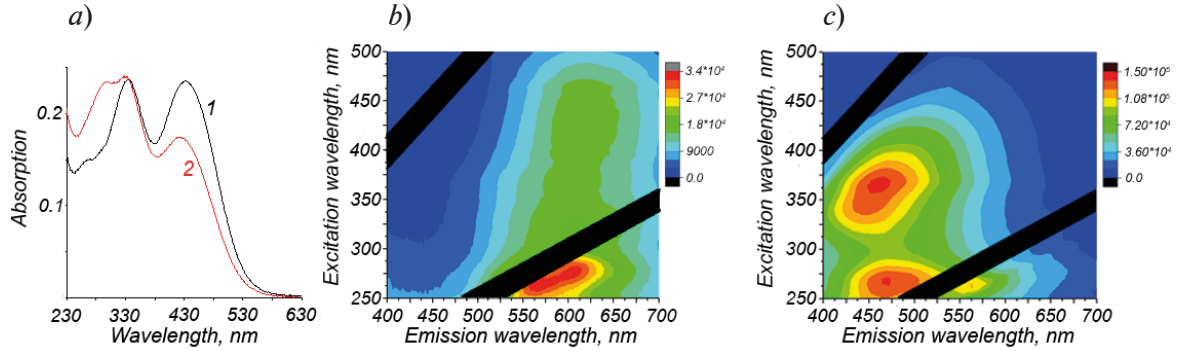

Fig. 1. Absorption spectra before and after irradiation (*a*); fluorescence profiles of unirradiated A2E (*b*); fluorescence profiles of A2E irradiated by visible light (*c*). The color of the area is responsible for the fluorescence intensity from blue (no fluorescence observed) to red (maximum fluorescence signal)

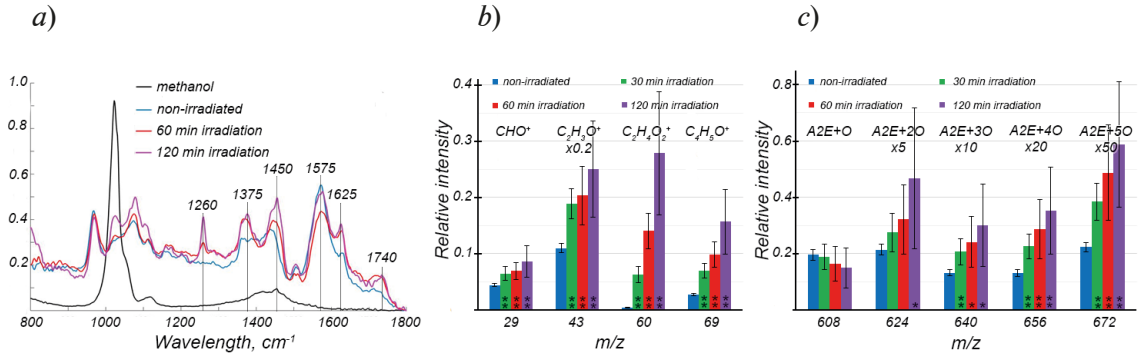

Fig. 2. Chemical changes during oxidation: IR-spectra of unirradiated A2E (blue), A2E after irradiation for 60 min (red) and A2E after irradiation for 120 min (purple) (*a*), the spectrum of the solvent (methanol) is also shown (black); oxidized A2E formation after light exposure revealed by ToF-SIMS (*b*), the intensities of all ions were normalized to the ion intensity with m/z 592 (A2E); formation of ions containing carbonyl groups after light exposure revealed by ToF-SIMS (*c*)

Data are presented as means ±SD from 9 independent experiments. *$p < 0.05$; **$p < 0.005$

A2E was irradiated during 30 min, 60 min and 120 min for chemical changes observation. IR spectrum were collected for irradiated and non-irradiated A2E samples (Fig. 2). IR spectra of methanol were received to exclude solvent bands.

The band at 1740 cm^{-1} could be attributed to the stretching vibrations of the carbonyl group. The bands at 1375 and 1450 cm^{-1} are probably deformational bands of $CH_3C(O)$- and -$CH_2C(O)$-, respectively. The band at 1625 cm^{-1} is probably related to the presence of the pyridine ring. Band 1260 cm^{-1} is expected epoxy group. Growing bands at 1740 cm^{-1} identified the aldehyde accumulation. It was also confirmed by the growth of deformation bands at 1375 cm^{-1} and 1450 cm^{-1}. Wider band at 1740 cm^{-1} for the 60 min irradiated sample indicates presence unsaturated conjugate aldehyde in middle of oxidation act. Significant difference was observed for IR band 1260 cm^{-1}. It could be result in epoxides accumulation during photooxidation process. Thus, formation of both the oxidation products of A2E epoxides and furanoids and the products of their further destruction – aldehydes and ketones were demonstrated.

Using ToF-SIMS relative intensities of A2E oxidized forms were obtained before and after exposure of A2E by light. It can be seen, that during the photooxidation process, the amount of oxidized A2E forms such as A2E+2O, A2E+3O, A2E+4O, A2E+5O A2E increases by about 3 times relative to non-irradiated A2E. These results are the evidence of the accumulation of A2E oxidized forms during irradiation, which contributes to further oxidative degradation. The A2E+O ion intensity (m/z 608) decreases during the oxidation process, because it gradually oxidizes more strongly, turning into compounds with a mass of 624, 656 and 672 (Fig. 2,*b*). The formation of oxygen-containing products such as epoxides, peroxides, ketones, and aldehydes, was revealed by ToF-SIMS analysis of characteristic fragment ions containing carbonyl groups (Fig. 2,*c*). There was a significant increase in carbonyl ions after exposure A2E to light as it can be seen in the diagram. For example, the ion with m/z = 60 ($C_2H_4O_2^+$) increase is particularly significant (about 100 times). Based on the data obtained and the A2E structure, an ion with m/z = 205.07 was identified ($C_{11}H_{11}NO_3^+$), assuming it is an aldehyde. The exact mechanism of oxidative degradation of A2E has not yet been described. However, the A2E tail groups show significant similarity to carotenoids. Therefore, the mechanism of ion formation with m/z 205 may be similar to the mechanism of carotenoid oxidation [19]. Fig. 3 represents the proposed scheme of the product with m/z = 205.07 formation.

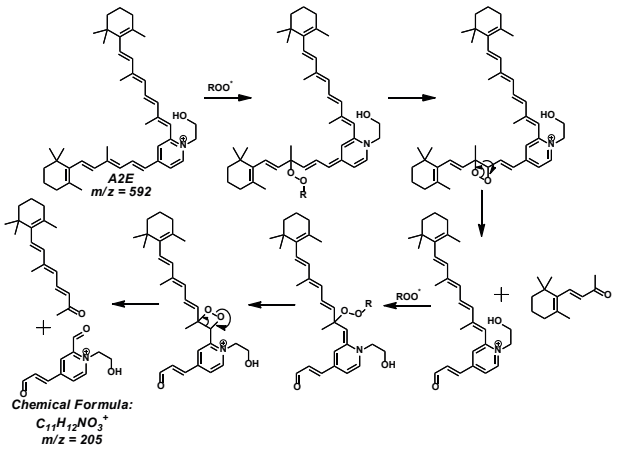

Fig. 3. The proposed scheme of A2E oxidative degradation resulting in formation of aldehyde product with m/z = 205.07

Conclusion

The photooxidation of A2E leads to generation of highly reactive products containing aldehyde groups. We have previously shown that as a result of visible light exposure of LG aldehydes are accumulated in them in a free state and are able to diffuse from the LG into the cytoplasm of the RPE cell [13, 17]. Obtained results allow us to propose other mechanism of cytotoxic action of LGs. A2E, which is the side product of the visual cycle, can also be a source of such compounds in addition to lipid peroxidation products.

REFERENCES

1. **Wolf G.,** Lipofuscin and macular degeneration. Nutrition Reviews. 61 (2003) 342–346.
2. **Sparrow J.R., Boulton M.E.,** RPE lipofuscin and its role in retinal pathobiology. Experimental Eye Research. 80 (2005) 595–606.
3. **Feeney L.,** The phagosomal system of the pigment epithelium: a key to retinal disease, Investigative Ophthalmology & Visual Science. 12 (1973) 635–638.
4. **Holz F.G., Pauleikhoff D., Klein R., Bird A.C.,** Pathogenesis of lesions in late age-related macular disease, American Journal of Ophthalmology. 137 (2004) 504–510.
5. **Lamb L.E., Simon J.D.,** A2E: a component of ocular lipofuscin, Photochemistry and Photobiology. 79 (2004) 127–136.
6. **Ben-Shabat S., Itagaki Y., Jockusch S., Sparrow J.R., Turro N.J., Nakanishi K.,** Formation of a nona-oxirane from A2E, a lipofuscin fluorophore related to macular degeneration, and evidence of singlet oxygen involvement, Angewandte Chemie International Edition. 41 (2002) 814–817.
7. **Yakovleva M.A., Sakina N.L., Kononikhin A.S., Feldman T.B., Nikolaev E.N., Dontsov A.E., Ostrovsky M.A.,** Detection and study of the products of photooxidation of N-Retinylidene-N-retinylethanolamine (A2E), the fluorophore of lipofuscin granules from retinal pigment epithelium of human donor eyes, Doklady Biochemistry and Biophysics. 409 (2006) 223–225.
8. **Kim S.R., Jang Y.P., Jockusch S., Fishkin N.E., Turro N.J., Sparrow J.R.,** The all-trans-retinal dimer series of lipofuscin pigments in retinal pigment epithelial cells in a recessive Stargardt disease model, Proceedings of the National Academy of Sciences. 104 (2007) 19273–19278.
9. **Boulton M., Dontsov A., Jarvis-Evans J., Ostrovsky M., Svistunenko D.,** Lipofuscin is a photoinducible free radical generator, Journal of Photochemistry and Photobiology B. 19 (1993) 201–204.
10. **Sparrow J.R., Boulton M.,** RPE lipofuscin and its role in retinal pathobiology, Experimental Eye Research. 80 (2005) 595–606.
11. **Dontsov A.E., Sakina N.L., Golubkov A.M., Ostrovsky M.A.,** Light-induced release of A2E photooxidation toxic products from lipofuscin granules of human retinal pigment epithelium, Doklady Biochemistry and Biophysics. 425 (2009) 98–101.
12. **Sparrow J.R., Vollmer-Snarr H.R., Zhou J., Jang Y.P., Jockusch S., Itagaki Y., Nakanishi K.,** A2E-epoxides damage DNA in retinal pigment epithelial cells. Vitamin E and other antioxidants inhibit A2E-epoxide formation, Journal of Biological Chemistry. 278 (2003) 18207–18213.
13. **Yakovleva M., Dontsov A., Trofimova N., Sakina N., Kononikhin A., Aybush A., Gulin A., Feldman T., Ostrovsky M.,** Lipofuscin granule bisretinoid oxidation in the human retinal pigment epithelium forms cytotoxic carbonyls, International Journal of Molecular Sciences. 23 (2022) 222.
14. **Schutt F., Bergmann M., Holz F.G., Kopitz J.,** Proteins modified by malondialdehyde,4-hydroxynonenal, or advanced glycation end products in lipofuscin of human retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 44 (2003) 3663–3668.
15. **Adler L. 4th., Boyer N.P., Anderson D.M., Spraggins J.M., Schey K.L., Hanneken A., Ablonczy Z., Crouch R.K., Koutalos Y.,** Determination of N-retinylidene-N-retinylethanolamine (A2E) levels in central and peripheral areas of human retinal pigment epithelium, Photochemical & Photobiological Sciences. 14 (11) (2015) 1983–1990.
16. **Feldman T.B., Yakovleva M.A., Arbukhanova P.M., Borzenok S.A., Kononikhin A.S., Popov I.A., Nikolaev E.N., Ostrovsky M.A.,** Changes in spectral properties and composition of lipofuscin fluorophores from human retinal pigment epithelium with age and pathology, Analytical and Bioanalytical Chemistry. 407 (2015) 1075–1088.
17. **Dontsov A., Yakovleva M., Trofimova N., Sakina N., Gulin A., Aybush A., Gostev F., Vasin A., Feldman T., Ostrovsky M.,** Water-soluble products of photooxidative destruction of the bisretinoid A2E cause proteins modification in the dark. International Journal of Molecular Sciences. 23 (2022) 1534.
18. **Kosower E. M., Skorcz, J. A.,** Pyridinium Complexes. III. Charge-Transfer Bands of Polyalkylpyridinium Iodides, Journal of the American Chemical Society. 82 (9) (1960) 2195–2203.
19. **Mordi R. C., Walton J. C.,** Identification of products from canthaxanthin oxidation, Food Chemistry. 197 (2016) 836–840.

THE AUTHORS

YAKOVLEVA Marina
lina.invers@gmail.com
ORCID: 0000-0003-4243-2787

AYBUSH Arseny
aiboosh@gmail.com
ORCID: 0000-0002-0496-9105

VASIN Alexander
a2vasin@yandex.ru
ORCID: 0000-0003-0152-3391

FELDMAN Tatiana
feldmantb@mail.ru
ORCID: 0000-0003-2613-056X

DONTSOV Alexander
adontsovnick@yahoo.com
ORCID: 0000-0002-8367-5008

OSTROVSKY Mikhail
ostrovsky3535@mail.ru
ORCID: 0000-0003-4350-2812

GULIN Alexander
aleksandr.gulin@phystech.edu
ORCID: 0000-0001-7117-4276

Received 15.07.2022. Approved after reviewing 17.07.2022. Accepted 19.07.2022.