Novel UV-Releaser of Arachidonic Acid Based on Nitrophenyl-type Photoremovable Protecting Group

Darya N. Litunenko, Tatyana Yu. Karogodina, Alexey Yu. Vorob'ev, and Alexander E. Moskalensky*

Nososibirsk State University, 2 Pirogova str., Novosibirsk 630090, Russia

*** e-mail:** a.mosk@nsu.ru

Abstract. Arachidonic acid (AA) is one of the most important polyunsaturated fatty acids. It is the precursor to a wide range of eicosanoids, which are involved in many signaling cascades in the body. The conversion of arachidonic acid to eicosanoids starts with its liberation from membrane phospholipids by a dedicated enzyme. Here we present photolabile "caged" analog of AA, capable of liberation of the free acid under ultraviolet (UV) light with ~340–365 nm wavelength. This can be used in cell culture studies to mimic the first step in the biosynthesis of eicosanoids. We directly confirm the production of AA using high-performance liquid chromatography (HPLC). Furthermore, we found that novel compound yields 4-times more AA in comparison with previously published alternative. Our results demonstrate that the use of the caged compounds can be used to control the release of AA with UV light. The described caged analogs of AA are likely to be anchored in the membrane and enable light-triggered liberation of the free acid to the cytoplasm. This would be superior to the usual approach using the external addition of AA to cells, which suffers from poor solubility of the acid in culture medium and uncontrolled passage through membrane. © 2022 Journal of Biomedical Photonics & Engineering.

Keywords: arachidonic acid; photoremovable protecting group; caged compounds.

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1 Introduction

Arachidonic acid (AA) is one of the most important polyunsaturated fatty acid with many physiological roles [1]. It is a component of the phospholipids which make up cellular membranes. AA is the precursor to a wide range of eicosanoids, including prostaglandins, thromboxanes, and leukotrienes [2]. Eicosanoids are small membrane-permeable lipid messengers, which have a lot of biological functions, including the regulation of inflammation and platelet activation. In particular, arachidonic cascade plays the main role in the developing of hyperinflammatory state during COVID-19 infection [3]. The key feature of lipid signaling is free diffusion through membranes. Thus, lipid messengers cannot be stored in vesicles and are biosynthesized locally. They usually have short lifetime, which ensures their local action. The study of lipid messengers biosynthesis and their spatial distribution is interesting both from fundamental point of view and for drug development.

The conversion of arachidonic acid to eicosanoids occurs in the cytoplasm and starts with its liberation from membrane phospholipids by phospholipase A2. The study of biosynthesis of eicosanoids *in vitro*, using the external addition of AA to cells, suffers from poor solubility of the acid in culture medium and uncontrolled passage through membrane. This problem can be solved by the use of photolabile "caged" analogs of arachidonic acid conjugated with photoremovable protecting groups (PPGs). Application of PPGs to control the activity of biological molecules has been widely used and extensively studied [4]. It allows one to use light to "uncage" the molecule and rapidly induce the desired effect. Importantly, the "uncaging" can be done independently of other processes associated with mixing, diffusion, and interaction with membranes, after equilibration of these processes [5]. Another important

feature is that light can be focused to induce local effect. The process can be triggered both outside cells and in the cytoplasm because cells are transparent to light.

Photolabile "caged" derivatives of AA were reported previously [6], including that with water-soluble protective group [7]. In this paper, we present and study two photolabile "caged" derivatives of AA, one previously described bearing 4,5-dimethoxy-2-nitrobenzyl protecting group (DMNB) [8] and novel bearing 1-(2-nitro-4,5-dimethoxyphenyl)ethyl protecting group (DMNPE). The goal of this paper is to compare their ability to release AA under UV light in terms of speed and total yield. To achieve this goal, we measure the dynamics of AA release during illumination using the HPLC technique.

2 Materials and Methods

2.1 Synthesis of the Compounds

The first compound, **Caged AA1 (**DMNB-caged AA, Fig. 1b**)** was synthesized as described in our previous paper [8]. A solution of 39.5 mg (0.13 mmol) of arachidonic acid in 2 ml of CH_2Cl_2 was added to 26.3 mg (0.12 mmol) of (4,5-dimethoxy-2-nitrophenyl)methanol. Then solutions of 1.5 mg 4-*N,N*-dimethylaminopyridine in 0.5 ml of CH_2Cl_2 and 30.0 mg (0.14 mmol) of DCC in 2 ml of CH_2Cl_2 were added with stirring. Resulted mixture was stirred for 2 hours, during this time white solid precipitate was formed. Solid was filtered off and filtrate was evaporated *in vacuo*. Oilish residue was chromatographed on SiO_2 with EtOAc-hexane (1:1) mixture as eluent. Fraction with $R_f \sim 0.35$ was collected. After removal of eluent yellowish oil was obtained, 39.0 mg (65%).

^{1}H NMR ($CDCl_3$, 400 MHz, J Hz): 0.83 (m, 3H); 1.18–1.33 (m, 6H); 1.63–1.76 (m, 3H); 1.84–1.94 (m, 1H); 2.05–2.13 (m, 2H); 2.36–2.42 (m, 2H); 2.72–2.81 (m, 6H); 3.91 (s, 3H); 3.93 (s, 3H); 5.24–5.39 (m, 8H); 5.46 (s, 2H); 6.96 (s, 1H); 7.67 (s, 1H).

HRMS (m/z): 499.2936 (exp), 499.2934 (calc).

Caged AA2 (DMNPE-caged AA, Fig. 1c**)** was synthesized analogously using 1-(4,5-dimethoxy-2-nitrophenyl)ethan-1-ol instead of (4,5-dimethoxy-2-nitrophenyl)methanol.

^{1}H NMR ($CDCl_3$, 400 MHz, J Hz): 0.86 (m, 3H); 1.18-1.36 (m, 6H); 1.58 (d, 3H, J = 6.5 Hz); 1.63-1.76 (m, 3H); 1.86-1.94 (m, 1H); 1.99-2.13 (m, 2H); 2.28-2. 36 (m, 2H); 2.72-2.81 (m, 6H); 3.91 (s, 3H); 3.93 (s, 3H); 5.24-5.41 (m, 8H); 6.45 (q, 1H, J = 6.5 Hz); 6.97 (s, 1H); 7.55 (s, 1H).

HRMS (m/z): 513.6659 (exp), 513.6655 (calc).

Photolysis mechanism of compounds conjugated with nitrobenzyl-based protecting groups has been described [9, 10]. Although it may depend on the solvent and pH, generally two products are formed: the "uncaged" compound and the "byproduct", or protecting group residual. The byproduct then degrades and form many different products (Fig. 1d).

2.2 Absorption Spectra and Photolysis

UV-VIS absorption spectra were measured in quartz cuvette with 10 mm light path using Shimadzu UV-1900 spectrophotometer. The photolysis ("uncaging") experiments were carried out in the same cuvette as follows. Compounds were dissolved in 3 mL of DMSO to the final concentration of 100 µM. We prepared a setup consisting of two UV LEDs with 365 nm central wavelength and 480 mW optical power. A microcontroller was used to set the illumination time. After each session of illumination, we measured UV-VIS absorption spectrum.

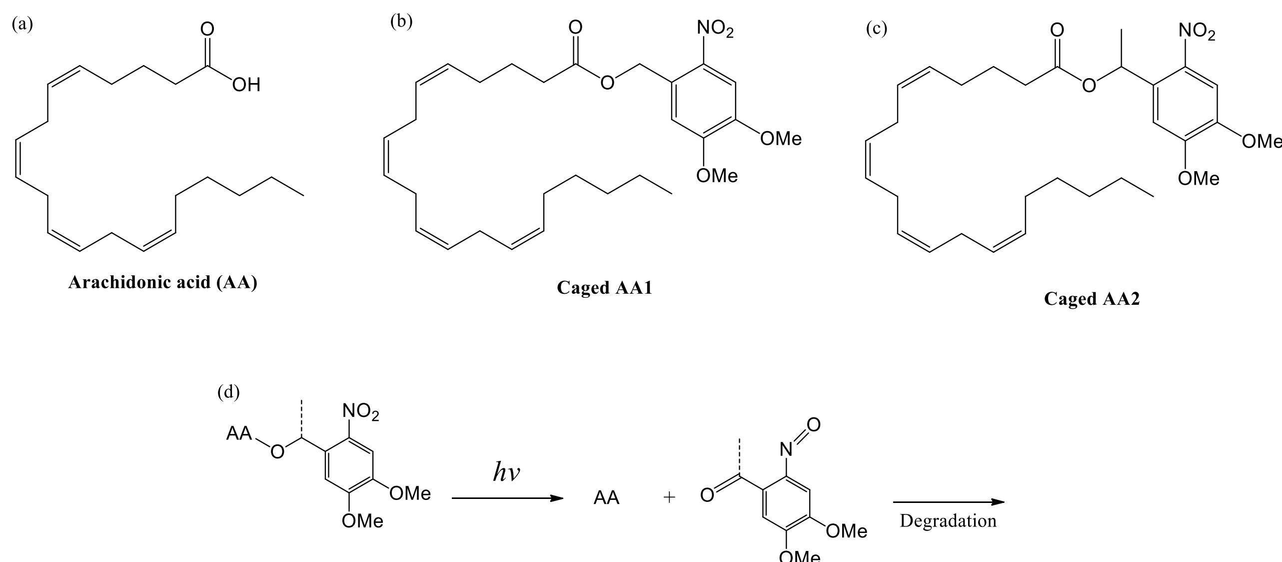

Fig. 1 (a) Arachidonic acid (AA); (b, c) two different analogs of AA conjugated with dimethoxynitrophenyl-type photoremovable protective groups; (d) the scheme of photolysis (photodecomposition).

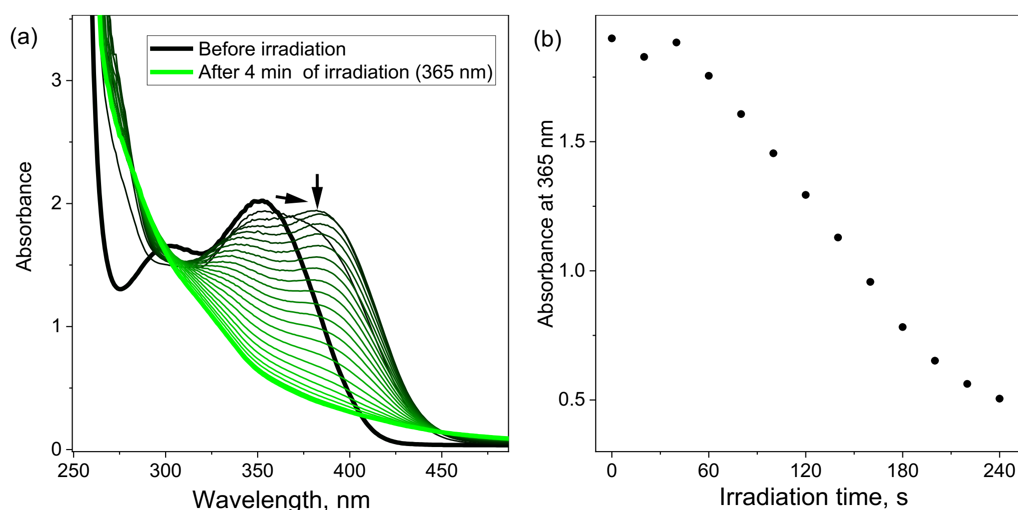

Fig. 2 (a) Absorption spectrum of **Caged AA2** during photolysis (irradiation by 365 nm LED); (b) the decrease of absorbance at 365 nm.

2.2 HPLC Experiments

HPLC experiments were done with Agilent 1260 Infinity II device equipped with Poroshell column (120 EC-C18, 3×150mm, 2.7µm) and diode-array detector. The separation conditions were as follows: 98% water / 2% ACN from 0 to 3 min; linear gradient to 100% ACN from 3 to 10 min; 100% ACN from 10 to 20 min. With these conditions, the retention time of AA was 14.3 min.

3 Results and Discussion

3.1 Absorption Spectra

While the unmodified AA does not absorb above 220 nm, the absorption spectra of both caged compounds have strong absorption band at 350 nm. The absorption spectrum for **Caged AA2** is shown in the Fig. 2a (black line). We measured extinction coefficients and obtained the following values: 7.9×10^4 $M^{-1}cm^{-1}$ for **Caged AA1** and 3.5×10^4 $M^{-1}cm^{-1}$ for **Caged AA2**.

Next, we performed the photolysis experiments as described in Section 0. The experimental results for **Caged AA2** are shown in the Fig. 2, and we published similar data for **Caged AA1** earlier [8]. The main absorption band first shifts to longer wavelength, indicating the formation of primary photoproduct of the PPG, and decays during further photolysis. Same shift to longer wavelength was observed in Ref. [11], and it was shown to be due the absorption of 2-nitrosobenzaldehyde. In our case, the primary photoproducts are 4,5-dimethoxy-2-nitrosobenzaldehyde and 1-(4,5-dimethoxy-2-nitrosophenyl)ethenone for **Caged AA1** and **Caged AA2**, respectively. Further decay in the absorption spectra is related to the formation of many different products from PPG residuals, which are hard to identify. The changes in absorption spectra indicate the conversion of compounds, but do not directly prove the release of AA. Therefore, we performed another set of experiments using HPLC approach.

3.2 Photorelease of Arachidonic Acid

To directly evaluate the photorelease of AA, we used the HPLC as described in the Section 0. First, we performed a calibration for pure AA dissolved in DMSO. Fig. 3 shows the increase of HPLC peak at 14.3 min. with the increasing concertation of AA (inset) and the linear calibration curve. This calibration curve was used in further experiments to determine the concentration of "uncaged" AA.

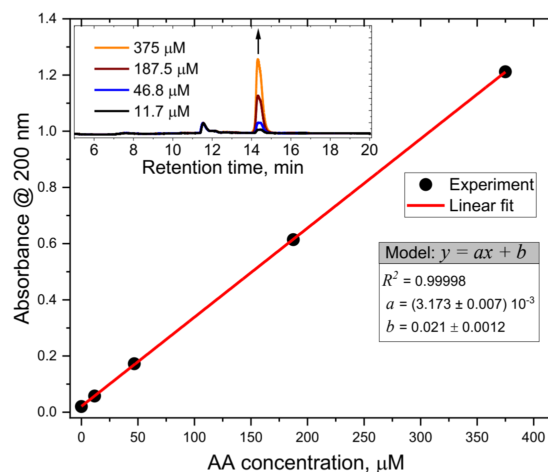

Fig. 3 Calibration curve (the height of HPLC peak at 14.3 min *versus* the concentration of AA in the sample). Inset shows the HPLC results.

The sample containing 250 µM of **Caged AA1** in 0.5 mL of DMSO was prepared in a transparent chromatographic vial. After recording the initial HPLC profile, we illuminated the sample using two UV LEDs with 340 nm central wavelength for 2 min and run the analysis again. This step was repeated several times until the release

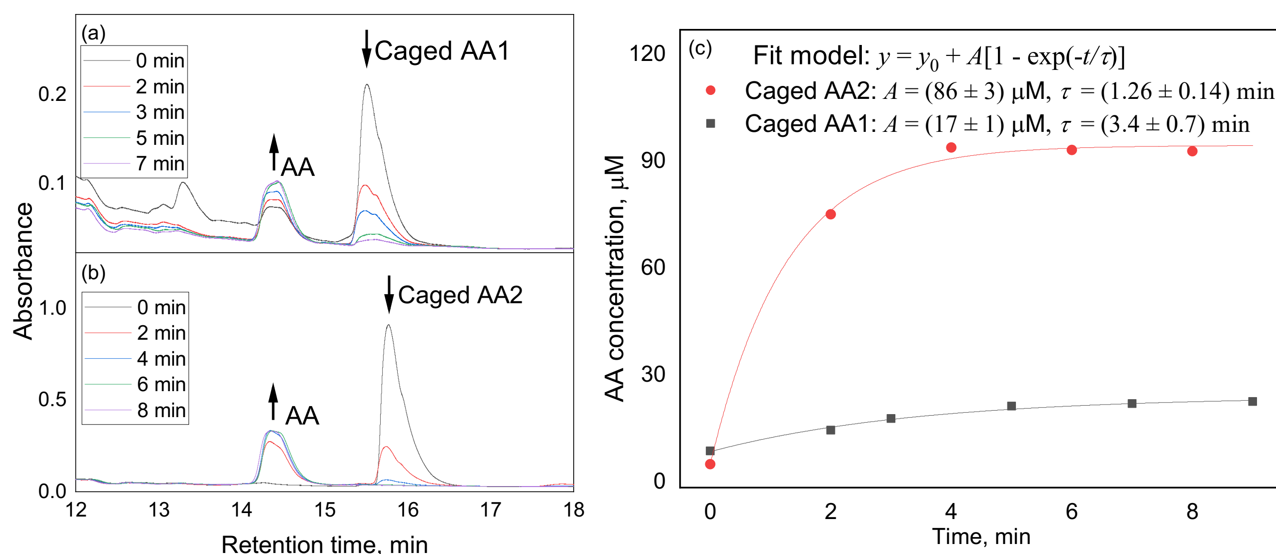

Fig. 4 (a) HPLC profiles during the illumination of **Caged AA1** by UV light; (b) the same for **Caged AA2**; (c) Production of the free AA in the course of illumination.

of AA stopped. Fig. 4a shows the obtained results. The same experiment was performed for the sample of **Caged AA2** with the concertation of 300 μM (Fig. 4b). HPLC peaks corresponding to caged analogs of AA decreased with illumination, in agreement with the photolysis experiments described in the previous section. However, the experiments cannot be compared directly because different LEDs were used and glass vials were used instead of quartz cuvettes. In contrast, the peak at 14.3 min retention time monotonically increased with illumination, indicating the photorelease of AA. According to the obtained kinetics, the photorelease of AA is faster for **Caged AA2** ($\tau = 1.26$ min *versus* 3.4 min). The total yield of AA is also much higher for **Caged AA2** despite lower extinction coefficient. Using the calibration curve, we determined the final concentration of AA, which was 17 μM for **Caged AA1** and 86 μM for **Caged AA2** (Fig. 4c), corresponding to 6.8% and 28.8% of the parent compound, respectively.

The obtained total yield is within the previously reported range for DMNB group [12]. The efficiency of the uncaging reaction varies greatly, depending on the functional group that has been caged and on the caging group itself. It is known that substitution at the benzylic site affects the photorelease quantum yields [13]. Methyl group in this site typically increases the efficiency, as it was first shown in Ref. [14], where the quantum yield increased from 13% to 64% in solution. Stronger electron-withdrawing group, such as CF_3, work even better, but they are not widely used because the synthesis is more difficult [15]. The study of the effect of substituents at the benzylic site for 4,5-dimethoxy nitrobenzene derivatives was performed in Ref. [16]. Previously there were no comparison between different groups for uncaging of AA, which is special carboxylic acid in terms of polyunsaturation. The fact that DMNPE counterpart provides more efficient uncaging of AA (in comparison with DMNB group) is in line with known data, but the total efficiency of 29% is quite high. For instance, DMNPE-caged ATP has the photorelease quantum yield of 7%.

Similar approach using HPLC technique was described recently [17] for AA derivatives based on coumarin-based photocaging group. The authors have not performed the calibration procedure and determination of total yield, but they estimated the time of total photolysis and obtained similar results as in the present papers (3–5 min). Coumarin-based groups have longer-wavelength absorption band (~380 nm) and the photorelease quantum yield in range of 4–22% [12]. Moreover, the groups were modified for specific subcellular localization, such as mitochondria, endoplasmic reticulum, lysosomes, and the plasma membrane. This enables photorelease of lipid messengers in distinct organelles, which is benefitial for biological studies because their action is usually highly localized. Further development of the present work would be also related with the modification of the caging group for specific subcellular localization, primarily the plasma membrane as it is the natural source of AA in cells.

Another promising direction in the development of “caged compounds” (not necessarily related to AA) is the use of visible-light PPGs. Visible (or near-infrared) light is capable of deep penetration into tissue, making it possible to accurately control the action of the drug in space and time [18]. This approach is promising for reducing the necessary dose of the drug and the accumulation of its active form in the environment [19, 20]. For *in vivo* applications, the PPGs operating in the single photon mode in the IR spectrum are required, and two major research directions exist in this area. The first is the use of BODIPY-based protecting groups [21, 22]. The second is the use of self-sensitizing molecules that generate singlet oxygen upon irradiation, which destroys the molecule itself and releases the active compound [23, 24]. However, this approach can be less compatible with AA because the unsaturated acid is easily oxidated by the reactive oxygen species.

4 Conclusion

In this paper, we present two photolabile "caged" analogs of AA, capable of liberation of the free acid under ultraviolet (UV) light with ~340–365 nm wavelength. We directly confirm the production of AA using HPLC method. Furthermore, we found that novel compound releases AA faster and has 4-times higher total yield in comparison with previously published alternative. Our results demonstrate that the use of the caged compounds can be used to control the release of AA with UV light. The described caged analogs of AA are likely to be anchored in the membrane and enable light-triggered liberation of the free acid to the cytoplasm. We anticipate that this approach will be used in cell culture studies to mimic the first step in the biosynthesis of eicosanoids.

Disclosures

The authors declare that they have no conflict of interest.

Acknowledgments

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