Journal of Photochemistry & Photobiology, B: Biology 165 (2016) 133–140

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Light induced structural changes of the photoprotein mnemiopsin: Characterization and contribution in photoinactivation

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ARTICLE INFO

Article history:
Received 30 August 2016
Accepted 14 October 2016
Available online 18 October 2016

Keywords:
Photoprotein
Mnemiopsin
Aequorin
Photoinactivation
Light induced structural changes

ABSTRACT

Mnemiopsin, an EF-hand Ca^{2+} binding photoprotein isolated from luminous ctenophore *Mnemiopsis leidyi*, emits blue light from its chromophore, coelenterazine, which is non-covalently bond in its central hydrophobic core. Previous studies have revealed unique biochemical properties for ctenophore photoproteins such as inactivation by light, but only few have focused on photoinactivation process. To understand the nature of photoinactivation process we have investigated the impact of light alone and in the presence of Ca^{2+} ion on the structure of this photoprotein. We used UV–Vis, circular dichroism (CD) and fluorescence spectroscopy following Ca^{2+} binding assay to analyze the light effects on mnemiopsin conformation in comparison with aequorin at both apo and holo form. Our results showed light induced structural changes which resulted into photoinactivation. These changes include significant modification on secondary structure of mnemiopsin in comparison with aequorin. Our data also revealed that light could influence structure of apo protein regardless of presence of coelenterazine. The comparative studies of Ca^{2+} ion binding affinity following light exposure, also showed that light induced structural changes could presumably affect coelenterazine binding or its conformation in binding site in such a way that causes photoinactivation. In conclusion, we have proposed that structural rearrangement of helix 5 and C-terminal motif could be responsible for light induced structural changes.

1. Introduction

Photoprotein mnemiopsin which is extracted from *Mnemiopsis leidyi* is a calcium binding protein from the ctenophore family [1,2]. Ctenophore's photoproteins, in spite of their significant structural similarity, contain very low sequence similarity with well-known coelenterate's photoproteins (*e.g.* obelin and aequorin) [3]. These differences in sequence lead to different functional properties among these families such as different sensitivity to Ca^{2+} ion, different pH dependent activity and specifically photosensitivity [2,4]. Crystal structure of coelenterate photoproteins and recently Ca^{2+} loaded apo berovin which belongs to the ctenophore family has revealed that generally photoproteins contain four EF-hand motifs so that three of them, loop I, loop III and loop IV, are Ca^{2+} binding sites [5–7]. According to the suggested mechanism based on coelenterate photoproteins, in coelenterazine binding cavity reacts with O_2 during incubation and yields 2-hydroproxide coelenterazine. Binding of Ca^{2+} ion to their sites induces conformational changes in the protein which catalyzes the oxidative decarboxylation of coelenterazine, resulting in the excited state of the product, coelenteramide, which relaxes to its ground state by emitting blue light [8,9]. Certain conditions like persistence of Ca^{2+} ion or other divalent cations, narrow pH range and light exposing can affect the light emission intensity or even inactivation of ctenophore photoproteins [5,10]. The inactivation due to the light exposing in this family is more interesting since its mechanism is not unfolded yet and is taken into consideration only by a few studies so far.

Ward and Seliger had studied action spectrum, quantum yield and the kinetic of photoinactivation of mnemiopsin. They observed the first order kinetic for five different wavelengths, 253.7, 279, 295, 374, and 453 nm for photoinactivation process [11]. By analysis of the action spectrum, they suggested that absorption by aromatic residues could leads to extremely efficient photoinactivation. Finally, they concluded absorption of light by the protein bound coelenterazine could sensitize the inactivation of another labile group on the mnemiopsin which may be the site at which oxygen is bound. Anctil and Shimomura also studied the mechanism of photoinactivation and reactivation of mnemiopsin [12]. They found that photoinactivated mnemiopsin can be reactivated in the dark following incubation with coelenterazine and O_2 only in solutions having a pH very close to 9.0. By this observation, they proposed that light splits both coelenterazine and oxygen from the photoprotein, inactivating coelenterazine in the process, but they did not detect coelenterazine in the photoinactivated samples of

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http://dx.doi.org/10.1016/j.jphotobiol.2016.10.018
1011-1344/

mnemiopsin. Recently report by Powers et al. on photoprotein BfosPP also has proved the results suggested by the Ward and Seliger about the influence of UV absorption and photoinactivation kinetic as well [13]. They found that activity of light exposed BfosPP is not diminished if it subsequently regenerated with coelenterazine in dark. Accordingly, they have suggested that the apo protein itself is not changed by light exposure, but rather that light exposure alters the conformation of coelenterazine in the binding pocket in such a way that the protein is unable to emit light. Alternatively, excited state energy from UV photons absorbed by aromatic residues proximal to the bound coelenterazine may participate Förster resonance energy transfer (FRET) to the coelenterazine, leading to photo-oxidation. Here, like report by Anctil and Shimomura, coelenterazine absorbance peak is not observed in the UV–Vis spectrum of photoinactivated BfosPP. Also, similar observation is briefly reported by Markova et al. in the case of photoinactivated berovin [5]. However, neither of them discussed about details of the lack of coelenterazine and its derivatives bound with the photoinactivated protein.

To understand the structural features of mnemiopsin responsible on its photosensitivity, we have designed an experiment to investigate the effects of light on mnemiopsin 1 structure in comparison with aequorin. We applied spectroscopic methods to characterize the light effect alone and in the presence of Ca^{2+} ion as a secondary effector on protein structure which could trigger the bioluminescence reaction. Here we have tried to study possible structural changes induced by light on apo or holo protein in detail and its consequence on ligand binding.

2. Materials and Methods

2.1. Materials

cp-Coelenterazine was purchased from Resem BV (The Netherlands). Kanamycin were obtained from Invitrogen (Carlsbad, CA, USA). The Ni-NTA agarose resin was provided by Qiagen (Qiagen, Hilden, Germany). Plasmid extraction kit and polymerase chain reaction (PCR) purification kit were purchased from Bioneer (Bioneer, South Korea). Isopropyl D-thiogalactopyranoside (IPTG) was obtained from Fermentas (Fermentas, Vilnius, Lithuania). *E. coli* BL21 (DE3) was purchased from Novagen (Madison, WI, USA). The 1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) was purchased from EMD Millipore (Merck Millipore, Merck, Germany). Tris, $CaCl_2$ and all other chemicals were obtained from Merck (Darmstadt, Germany). Reproducibility of the data presented in this manuscript was confirmed by repeating the experiments at least three times. The data presented here are typical experimental data.

2.2. Protein Expression, Purification and Bioluminescence Activity Determination

Expression and purification of His-tagged recombinant mnemiopsin 1 (GenBank accession no. GQ231544) and its luminescence activity was carried out as described previously [4].

2.3. Experimental Design: Sample Preparation

Apo protein, holo protein and coelenterazine were prepared in the presence and absence of Ca^{2+} according to Table 1. All samples were incubated in 4 °C for 16 h in dark. Then half of them were exposed to sunlight for 1 h on ice, and immediately used for spectroscopic studies (Table 1).

2.4. UV–Visible Measurements

Absorption spectra on UV–Vis region (200–800 nm) were conducted on Nanodrop 2000c (Thermo Scientific, USA). All measurements were done using 8 μM mnemiopsin and 8 μM coelenterazine in buffer 1 (50 mM Tris and 1 mM EDTA (pH 9.0)) for Ca^{2+} free samples and buffer 2 (50 mM Tris, 1 mM EDTA and 1.5 mM Ca^{2+} (pH 9.0)) for Ca^{2+} loaded samples. Accordingly, aequorin absorbance was measured at 8 μM protein and 8 μM coelenterazine in the prepared buffers as well.

Table 1
Photoprotein samples (aequorin and mnemiopsin) prepared and used in this study.

Conditions	Control samples	Protein samples	
Light[a]	Coelenterazine	Apo photoprotein	Holo photoprotein
	Coelenterazine + Ca^{2+}	Apo photoprotein + Ca^{2+}	Holo photoprotein + Ca^{2+}
Dark[b]	Coelenterazine	Apo photoprotein	Holo photoprotein
	Coelenterazine + Ca^{2+}	Apo photoprotein + Ca^{2+}	Holo photoprotein + Ca^{2+}

[a] Ca^{2+} was added immediately after light exposing and before spectroscopic analysis.
[b] Ca^{2+} was added immediately before spectroscopic analysis.

2.5. Fluorescence Measurements

Intrinsic fluorescence was measured on Perkin–Elmer luminescence spectrophotometer LS 55 apparatus, (Perkin–Elmer, USA). The excitation wavelength was set at 280 nm and the emission spectra were obtained between 300 and 550 nm (both slits of excitation and emission were set to 5 nm). Both protein and coelenterazine concentrations were 0.6 μM in buffer 1 and buffer 2 (with 0.11 mM Ca^{2+}).

2.6. Far-UV CD Measurements

Far-UV CD spectra were recorded on a JASCO J-715 spectrophotometer (Tokyo, Japan). All measurements were done using 8 μM protein and 8 μM coelenterazine in buffer 1 and buffer 2 containing 1.5 mM Ca^{2+} for both mnemiopsin and aequorin. The results were presented as molar ellipticity, [Θ] (degree cm^2 dmo^{-1}) using the equation $[\Theta]_\lambda = (\Theta \times 100\ MRW / Cl)$ by Jasco software. In this equation "MRW is a Mean amino acid Residue Weight, C is the protein concentration in mg/ml and l and Θ represent the length of the light path in cm and the measured ellipticity in degree, respectively".

2.7. Ca^{2+} Binding Measurement

The Ca^{2+} binding assay was done by using the ultrafiltration procedure and all glassware, tube and filters were treated as described elsewhere [14,15]. The Ultracel-3k (YM-3, 3 kDa cut-off, yellow top, Millipore) was used for separating unbound Ca^{2+} ions. 500 μl of 40 μM mnemiopsin or aequorin samples were mixed with 1 μl Ca^{2+} ion resulting the final concentrations of Ca^{2+} ion ranging from 0.1 μM to 1 mM. Following overnight incubation, the sample was centrifuged at 4000*g* for 1 min, forming 50–100 μl filtrate. Light treated samples were exposed to sunlight for 1 h before centrifuge. The free Ca^{2+} ion concentration was determined using calcium chelator, BAPTA, as described elsewhere [16,17]. A standard curve was plotted using UV spectrum at 254 nm of BAPTA reagent with final concentration of 1 mM, against prepared standard calcium solutions, then the filtrated Ca^{2+} ion concentration was determined by standard curve.

2.8. Bioinformatics Studies

Since the three dimensional structure of mnemiopsin is not determined yet, berovin structural information, a ctenophore photosensitive photoprotein with about 90% sequence identity to mnemiopsin, was used for structural analysis. The crystal structure of Ca^{2+} loaded protein of both aequorin (pdb ID: 1SL8) and berovin (pdb ID: 4MN0) are used for this study. The StrucTools (https://hpcwebapps.cit.nih.gov/structbio/), the computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov), was used to calculation of B-factor. The accessible molecular surface area and salt bridges were calculated using WHAT