Ph.D. thesis-booklet

Functional dynamics of AppA revealed by fluorescence and ultrafast spectroscopic methods

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Pécs, 2020

UNIVERSITY OF PÉCS
MEDICAL SCHOOL

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2020

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Program: Investigating functional protein dynamics using biophysical methods (B-130/1993)

Head of the Program: Prof. Dr. Miklós Nyitrai

Funding:

EFOP-3.6.1-16-2016-00004

EFOP-3.6.2-16-2017-00005

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Introduction

Photoreceptors using flavin

Based on our present knowledge hundreds of enzymes [1] contains flavin in some form, though only some of these are photoactive [2,3]. The function of photoactive flavoproteins are strongly tied to the structural changes induced by the light. Based on their response to light, these proteins can be grouped into three main families: photolyases and cryptochromes being part of the same family, LOV (Light Oxygen Voltage) proteins and BLUF (Blue light sensing using FAD) domain proteins [4]. In this work we studied the functional dynamics of a BLUF protein by the means of fluorescence and ultrafast spectroscopy.

Blue light sensing using FAD (BLUF domain) proteins

BLUF-domain proteins can be found around ten percent of algaes and sequenated bacterias [5,6]. BLUF-domain proteins can have many different biological functions: they have a role in the biosynthesis of photosynthetic genes, in biofilm formation and producing the photo-avoidance response too [7,8]. This family is special because during light-sensing they undergo only slight structural changes compared to other photoreceptors which undergo significant structural changes during light-sensing (e.g.: forming covalent bonds). [5]. In 2002 two research group found them independently, at the same time. One of the groups studied the regulation of photosynthetic gene expression in *Rhodobacter sphaeroides* [7,9], while the other group studied the photo-avoidance of *Euglena gracilis* algae [10]. In other photosynthetic purple bacterias that resemble *Rhodobacter sphaeroides* the regulation of the photosynthetic system changes upon intensive light illumination. [11,12] In the case of *R. sphaeroides* upon blue light illumination the photosynthesis is reduced by a great amount. From this they concluded that the bacteria must have a blue-light sensing protein which they later found is AppA that we will discuss in the next part. [5,13].

AppA

They discovered AppA in 1995 [14] in a *R. sphaeroides* mutant in which the puc operon didn't activate because of the mutation. The protein got the name AppA based on this: activation of photopigment and puc expression A [5]. During the study of the protein they found that the protein bounds a flavin at it's N-terminal. From this they presumed it has a role in redox-reactions. [15]. In

2002 they found that [7,9] after absorbing blue light significant conformation changes happen in AppA. Because of these changes the AppA-PpsR complex dissociates and the biosynthesis of the photosynthetic genes stops as PpsR inhibits their transcription. [5].

Of course in bacteria other BLUF-domain proteins have a role too for example the PixD/Slr1694 which first they described in cyanobacterium *Synechocystis.* It produces positive phototaxis upon illumination of white, blue and red light [16,17]. In PixD mutant bacterias upon intensive light illumination the PixD PixE complex which is responsible for positive phototaxis weakens. [5,18–23].

Structure of the A BLUF-domain

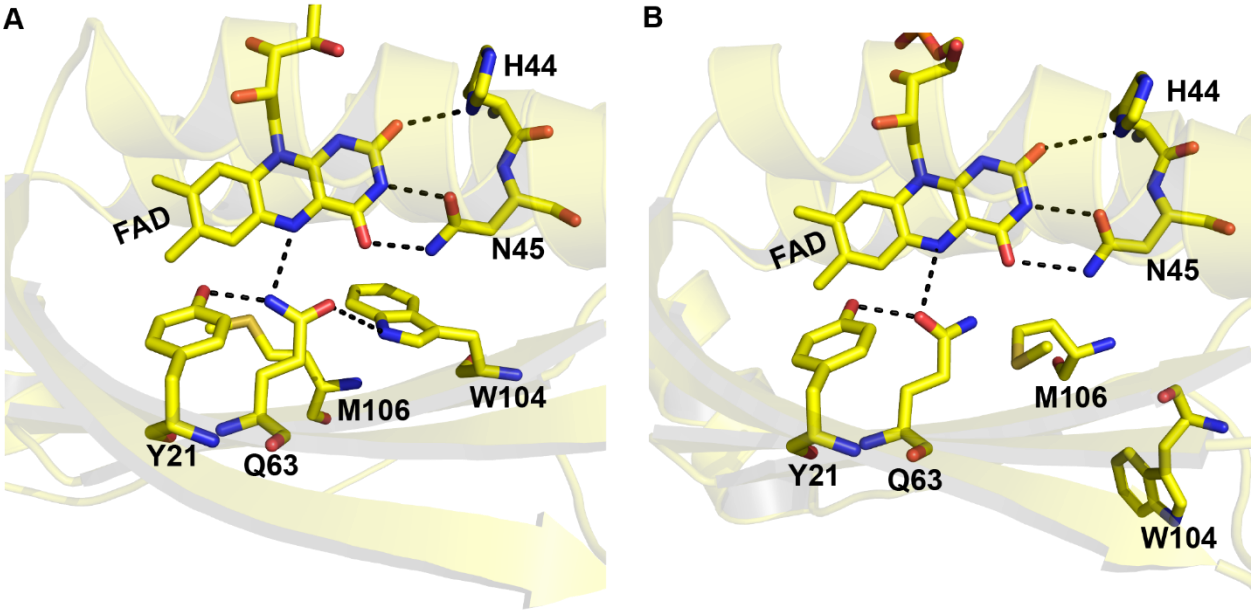

Figure 1 Ribbon diagrams of the BLUF domain crystal structures from WT (A, pdb:1yrx) and C20S (B,pdb:2iyg) AppABLUF domains showing W104 in the vicinity of the flavin in the Trp_{in} conformation (**A**) and away from the flavin in the Trp_{out} conformation (**B**). Important residues involved in an H-bond network around the flavin (Y21, H44, N45, Q63) are shown. Dashed lines represent H-bonds between the flavin and the residues.

In BLUF domain the FAD forms a tetrade with the amino acids in it's vicinity which are a tyrosine, a glutamine and a tryptophane. [4] Upon blue-light illumination the hydrogene bond of the tetrade is changed and creates the light state of the BLUF-domain. During the dark to light activation the absorbtion maximum of the FAD S0-S1 transition shifts 10-15 nm.

The change in the tetrade's hydrogene bond sytem is noticable in the protein's IR spectrum: the wavenumber value connected to the carbonil vibration of the $C_4 = O_4$ of FAD [24] downshifts by a value of $20\ cm^{-1}$ [25,26]. The key role of the tetrade's Y21 and Q63 is demonstrated by that

replacement of Y21 or Q63 with other amino acids the aforementioned red-shift cannot be observed. [4,25–29].

BLUF-domain W104

The W104 we studied also has an important role as it's mutation produces significant change of the photoactivation process [4,30]. In the mutant where this tryptophane was replaced by analanine in case of $AppA_{BLUF}$, time-resolved infrared experiments showed only restricted structural changes after photoactivation compared to the wild-type protein. They also observed that the relaxation of the photoactivated state happens eighty-times faster in the case of the $AppA_{BLUF}$ mutant having alanine in position 104. [8, 31]. In another study where the mentioned tryptophan was exhanged to phenylalanine in $AppA_{BLUF}$ the quantum-efficiency of photoactivation had a 1.5 fold increase. Also the relaxation speed connected to changing back from light state to dark state had a slight incerase [32]. In case of the full-length protein the W104A mutation blocks the red-shift [33], thus making the protein insensitive to blue light and blocking it's normal function [31].

As the studies of recent years produced contradictions about the exact position of the 104 trytophan during photoactivation in AppA and other BLUF domain proteins it is an exciting topic. Based on it's first crystal structure in the dark state the W104 is near the flavin. [34]. Opposing this Ilme Schlichting's group found [35], that it points away from the flavine in both the dark and light states. The same thing was observed in the crystal structures of other BLUF domain proteins like PixD [36,37], the BlrB [38] and the OaPAC [39] What's more in the case of Slr1694 protein if you take ten crystallographic substructure only one of them is in the direction of the flavin. [37].

Udvarhelyi et al [40], and Collette at all [41] independently presumed that in the dark state the tryptophan points away from the flavin, while Goyal and Hammes-Schiffer [42] based on their independent calculations concerning energetics stated that both conformation is valid though the inward one is more stable. NMR structure experiments [43], and UV resonance Raman experiments also favored the inward position for the dark state. [44]. Based on fluorescence experiments the trytophane is not exposed to the solvent on the other hand it's position still remained debated. [4,33,45].

Aim of our studies

With the evolution of optogenetics it becomes more and more urgent to understand accurately the light induced mechanisms of proteins. Precise information regarding this could help us regulate certain physiological processes with the help of light. The photoactivation of AppA is a process like this which so far has many controversions. Elucidating these controversions could help us understand other processes as well.

The aim of our work is to observe the behavior of AppA's 104 tryptophan during photoactivation and to elucidate the mentioned controversions regarding this in the literature. Our further aim to study the position of W104 regarding it's interaction with FAD. In order to get answers based on strong evidence we examined these processes with a very wide range of spectroscopic methods like fluorescence lifetime-decay, anisotropy-decay, quenching, transient infra-red and FRET experiments. In these experiments it is very important to achieve that the gathered data is only produced by the W104 so we tried to rule out other disturbing factors as much as we could.

Materials and Methods

Materials Glycogen (Type III: from rabbit liver) was purchased from Sigma-Aldrich. 7AW was purchased from Aldrich.

Expression and purification of W64F, W64F/W104A, 7aza-W104/W64F, Y21F/Y56F/W64F and Y21F/Y56F/W64F/W104F AppABLUF proteins.

The BLUF domain of AppA ($AppA_{BLUF}$: residues 5-125) was expressed in BL21(DE3) *Escherichia coli* cells. Protein expression and purification were performed in the dark as described previously [46]. The constructs for the site-directed mutants W64F, W64F/W104A and Y21F/Y56F/W64F were generated by PCR amplification (overlap extension method) from wild-type pET15b_$AppA_{BLUF}$ using the NdeI and BamHI restriction enzymes. The DNA sequence for expressing the Y21F/Y56F/W64F/W104F $AppA_{BLUF}$ mutant was purchased from Thermo Fisher Scientific.

All constructs were verified by DNA sequencing.

The 7aza-W104/W64F construct was expressed in a Tryptophan auxotroph M5219 *Escherichia coli* strain, purchased from the Belgian Coordinated Collection of Micro-Organisms (http://bccm.belspo.be/). The W64F $AppA_{BLUF}$ contained construct was transformed by heat shock into M5219 *E.coli* strain. A single colony was inoculated into 25mL Luria Broth (LB) media incubated overnight at 200 rpm and 37 °C. The overnight culture was used to inoculate 1 L M9 medium in a 4L flask and cells were allowed to grow at 30°C until OD600nm reached ~0.6. At this point, the cells were washed three times with washing buffer (M9 mineral salts solution and 20% glucose) to remove all Trp from the medium harvested, resuspended in 1L M9 media and cultured for additional 30 minutes at 30°C to ensure full Trp depletion [47]. 7AW was then added and incubated for 15 minutes to allow the cells to uptake it after which the temperature was decreased to 18°C for 30 min followed by addition of 0.8 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to induce protein expression. Cells were harvested, lysed and purified using Ni-NTA affinity resin as previously described [46]. The apoprotein is produced during protein expression and was isolated by size exclusion chromatography.

Steady state optical and fluorescence measurements

Absorption spectra were measured on a Perkin Elmer Lambda XLS+ and Jasco V-660 spectrophotometer. Fluorescence emission spectra were obtained with a Horiba Jobin Yvon Fluorolog spectrofluorometer using three excitation wavelengths (λ_{exc}=295nm, λ_{exc}=350 nm,

λ_{exc}=455 nm). The applied slit width was set at 5 nm for both the excitation side and the emission side. Unless otherwise indicated all fluorescence spectra were measured in the dark at 22°C in a 10 mm x 1 mm quartz cuvette.

Nanosecond time-resolved fluorescence measurements: Time-resolved fluorescence measurements in the nanosecond time range were performed on a Horiba Jobin Yvon Nanolog FL3-2Ihr spectrofluorometer operating in the time-correlated single photon counting (TCSPC) mode and coupled to a R928P Hamamatsu photomultiplier. The applied detector voltage was set at 950 V. The measurement range of the TCSPC system was 200 ns, the repetition rate of the excitation pulses was 1 MHz, and the sync delay was 50 ns. Decay curves were collected in 4096 channels of a multi-channel analyser using a channel width of 55 ps. For the deconvolution procedure, the dynamic instrument response function (IRF) was determined using a freshly made solution of glycogen in water (half-bandwidth ~1 ns).

To measure the fluorescence lifetimes, the excitation wavelength was set at 321 nm (7aza-tryptophan excitation) and 455 nm (FAD excitation) using pulsed nanoLEDs (Horiba) with pulse duration <1ns and 1.2 ns, respectively. The fluorescence emission was collected at 380 nm (λ_{exc}=321 nm) and at 520 nm (λ_{exc}=455 nm). The appropriate emission wavelength was set by a built-in the system monochromator. All measurements were performed using the Data Station v 2.6 software and the lifetime and anisotropy data were analysed using the Jobin Yvon DAS6 v6.6 and FluoFit softwares.

To generate and maintain the light-adapted state of the BLUF proteins, a Thorlabs M385 L2-C1 UV Led (λ_{exc}=385 nm) was placed above the sample compartment and covered by a black cloth. The light-adapted state was reached after 10 minutes of illumination. Sample concentration was in the 5-10 µM range. All samples were illuminated continuously during the fluorescence measurements. To eliminate the 385 nm scattered light, an UG11 filter was placed between the sample and the detector. 10 mm x 1 mm quartz cuvettes were used for the measurements. UV-vis absorption spectra were obtained before and after the fluorescence measurements to monitor the integrity of the light-adapted state, using a Thermo Scientific Evolution 600 UV-vis spectrophotometer.

Picosecond time-resolved fluorescence measurements: Time-resolved fluorescence experiments in the ps time range were performed using a spectrally resolved Kerr-Gate femtosecond fluorometer. The setup employs a Kerr shutter [48,49] and allows measuring

fluorescence spectra with a resolution of ~100 fs and up to the nanoseconds timescale [50]. Briefly, the excitation pulse centered at 390 nm is obtained by frequency-doubling, using a BBO crystal, part of the 780 nm pulse from the Ti:sapphire laser/amplifier system (Quantronix Integra-C) operating at 1 kHz. The remaining 780 nm beam is led through a motorized delay-line and focused into the Kerr medium where it spatially overlapped the fluorescence from the sample. The Kerr medium used was CS2 (response function width ~1.2 ps). The sample was flowed through the 1mm pathlength optical cell using a peristaltic pump. To generate and maintain the light-adapted state the sample reservoir was illuminated using a ThorLabs M450LP1 LED (λexc=450 nm). Transient fluorescence spectra were measured on time windows up to 1500 ps for all samples. Global analysis of the time and spectrally resolved data sets in terms of a linear combination of a discrete number of components, each with a distinct exponential rate constant and decay-associated spectrum [51], was completed using Glotaran [52].

Transient infrared spectroscopy

All transient infrared absorption (TRIR) measurements reported here used the 10 kHz ULTRA facility developed at the Central Laser Facility of the Rutherford Appleton Laboratories which are described in detail elsewhere. [53] ULTRA offers wide tunability in the visible region, a broad bandwidth in the IR probe, sub 100 fs rime resolution and excellent stability. In the present experiments it was used in the visible pump – IR probe geometry. The excitation (pump) wavelength was 450 nm with a pulse energy of a few hundred nJ focussed to a 100 micron spot size. It was checked that the spectra and kinetics were independent of the pump wavelength and pulse energy. IR transmission was measured sequentially for pump-on and pump-off using a 5 kHz mechanical chopper, and the data were processed to provide the TRIR difference spectra.

Results

FRET measurements

To provide quantitative information on the position of Trp104 during the photoactivation process in AppA, we used fluorescence resonance energy transfer (FRET) measurements. FRET efficiency between W104 and FAD has been obtained by two methods: measuring the fluorescence lifetime of the donor (7AW in position 104) in the absence and in the presence of the acceptor (FAD) and by using the method of acceptor enhancement. In this latter case the

increase of the acceptor fluorescence intensity is monitored when the donor is present: if the donor transfers energy to the acceptor, enhancement of the fluorescence intensity of the acceptor is observed.

The 7-aza-Trp analogue (7AW) enables FRET measurements between residue 104 and flavin in the presence of tyrosines

Besides W104, $AppA_{BLUF}$ contains one more tryptophan residue, W64. To eliminate a FRET contribution arising from W64, we have used the W64F mutant in our studies. In addition, to avoid fluorescence emission from the two tyrosine residues (Y21 and Y56) in $AppA_{BLUF}$, we used 295 nm as the excitation wavelength, where tyrosines do not absorb. However, the emission spectra of W64F and of the tryptophanless mutant W64F/W104A display a substantial fluorescence emission around 345 nm, suggesting the existence of a fluorophore (other than of a tryptophan residue) and hence the presence of another component that may contribute to FRET in addition to W104. The 345 nm emission component is shown below to arise from tyrosinates.

Tyrosine residues are well known to deprotonate at high pH to form tyrosinates which have very similar fluorescent features to tryptophan. In particular, an increase of the pH results to an increase of the absorption at 295 nm with concomitant decrease at 270 nm and a fluorescence emission shift from 303 nm to 340 nm [54,55]. This generally occurs at pH>10.5 which is the pKa of free tyrosine [56]. However, as it has been reported for a series of proteins [57–60] tyrosine residues can have lower pKa values which results in the formation of tyrosinates and hence fluorescence emission appears around 340 nm, even at pH<10.5.

To test whether tyrosinates are also formed in the AppA W64F/W104A mutant at pH=8.0 we performed pH dependent measurements of the absorbance at 295 nm and determined a pKa value of 8.0. This finding suggests that at pH=8.0, half of the tyrosine residues exists as tyrosinates giving rise to the strong fluorescence emission at ~ 345 nm. This will necessarily result in a distorted picture for any fluorescence spectroscopy measurement where tryptophan fluorescence is monitored. To eliminate any contribution from tyrosinates, we used 7AW and exploited its spectroscopic properties.

7AW was first incorporated in bacterial proteins 50 years ago [61]. It is an ideal non-invasive *in situ* probe of the structure and dynamics of proteins, as it introduces minimal structural and functional modifications to the protein and in addition it has favourable spectral properties

compared to canonical Trp and other Trp analogues [62,63]. In particular, the absorption and fluorescence maxima of 7AW are red-shifted by 10 nm and 50 nm, respectively compared to that of canonical Trp. This red shift in the absorbance allows us to excite selectively the tryptophan analogue 7AW, using λ_{exc}=310nm (canonical Trp has no absorbance at 310 nm) and to avoid the tyrosinates' absorbance at 295 nm.

Using tryptophan auxotroph cell lines, we incorporated the 7AW analogue in the place of W104 in the W64F mutant of AppABLUF.

FRET: Fluorescence lifetime measurements of 7aza-W104/W64F AppABLUF

To determine the FRET efficiency (E) between 7AW and FAD we performed time correlated single photon counting (TCSPC) measurements on the apoprotein and on the protein in the dark-adapted and light-adapted states (Fig. 3E). FRET efficiency was calculated using equation :

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \qquad (1)$$

where τ_{DA} is the fluorescence lifetime of the donor (7AW) in the presence of the acceptor (FAD) and $\tau_D$is the fluorescence lifetime of the donor alone. The lifetime of the tryptophan analogue is shorter when FAD is present, suggesting the existence of Förster-type energy transfer. Calculating the FRET efficiency – using the average lifetimes – we obtained 23% efficiency in the dark-adapted case and 40 % in the light-adapted case. An estimate of the average distance between the tryptophan analogue and the flavin can be obtained from this. Hence in the dark-adapted state, the distance between 7AW and the flavin is estimated to be 20.5 Å. The slower rotational correlation time observed in the case of the light-adapted state of the protein suggests that the tryptophan is not able to freely rotate as it is hydrogen-bonded in the Trp_{in} position. Based on this it's not surprising that the distance calculated between tryptophan and flavin in the light-adapted state is only 9.5 Å.

FRET: acceptor enhancement method

FRET efficiency between 7azaW104 and the flavin can also be calculated by measuring the intensity loss of the emission of the tryptophan or the intensity enhancement of the emission of the flavin due to the resonance energy transfer. In the latter case, known as the acceptor enhancement approach [64,65] the FRET efficiency is calculated by

$$E = \frac{\varepsilon_A(\lambda_D^{ex})}{\varepsilon_D(\lambda_D^{ex})}\left[\frac{I_{AD}(\lambda_A^{em})}{I_A(\lambda_A^{em})} - 1\right] \quad (2)$$

where $\varepsilon_A(\lambda_D^{ex})$ and $\varepsilon_D(\lambda_D^{ex})$are the extinction coefficients of the acceptor and donor at the donor excitation wavelength (λ_D^{ex}), and $I_{AD}(\lambda_A^{em})$ and $I_A(\lambda_A^{em})$ are the acceptor intensity in the presence and the absence of the donor, respectively. Inspection of the data reveals a significant increase of the emission of FAD in the presence of the donor both in the dark-adapted and light-adapted states indicating efficient FRET from 7AW to FAD. This fluorescence enhancement is even more pronounced in the light-adapted state. We calculate a FRET efficiency of E_{dark}=42% and E_{light}=59%, which corresponds to a distance R_{dark}=17.7 Å and R_{light}=8.3 Å for the dark- and light-adapted states, respectively[25,66].

Quenching experiments

In order to measure the accessibility of W104 we performed acrylamid quenching measurements based on former literarue [33]. In our case we used the 7-azaTrp-W64F mutant using 310 nm excitation to excite only the W104. The quenching curves that we obtained show that in the dark state W104 is more accessible than in the light state which is in union with our other type of measurements.

7-aza W104 has restricted movement in the light-adapted state as revealed by fluorescence anisotropy decay measurements.

To probe the conformational dynamics of W104 and steric restrictions, we have applied time-resolved fluorescence anisotropy decay measurements, which has been used for decades to characterize protein dynamics. The relaxation of anisotropy induced by a polarized excitation pulse reflects both the dynamics of the fluorophore itself and the protein or segment to which is attached to.

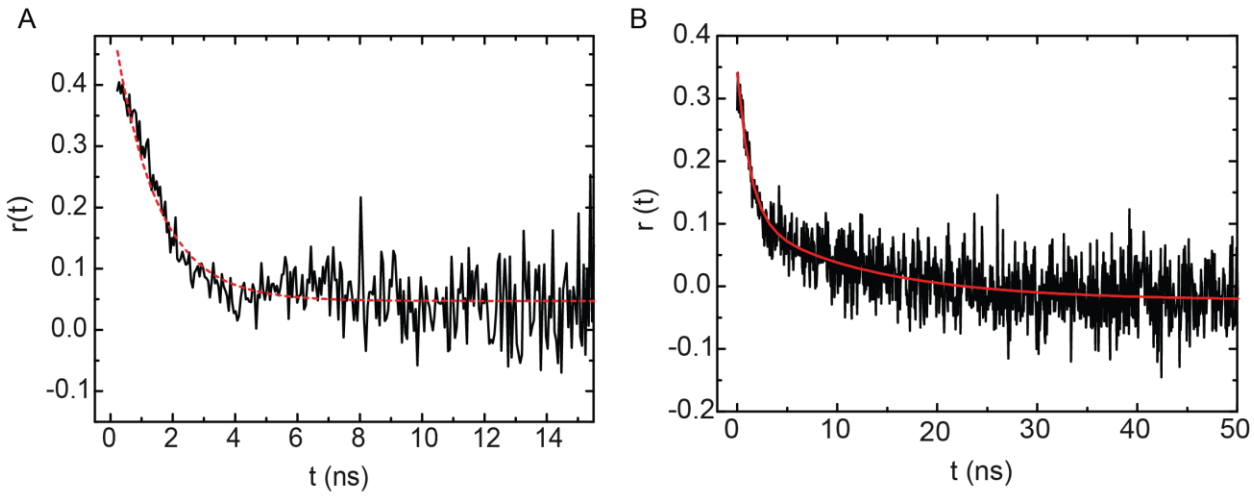

Figure 2 Decay of the fluorescence anisotropy of 7aza-W104/W64F AppABLUF in the dark-(A) and light- adapted states (B).

Single exponential fitting reveals a decay constant for the dark-adapted state, θ*dark*=1.5 ± 0.06 ns and for the light-adapted state, θ*light*=11.1 ± 0.5 ns. The short rotational correlation time in the dark-adapted state reflects the free rotation of the tryptophan residue. The observed 11 ns long rotational correlation time in the light adapted state corresponds to the rotation of the whole protein and agrees well with the value expected based on the empirical formula proposed by Visser[67] This observation suggests that upon blue light absorption, the W104 tryptophan residue moves closer to the flavin and gets hydrogen bonded to an adjacent amino acid residue, most probably Q63.

Fluorescence lifetimes of the flavin in the dark- and light-adapted state

Ultrafast electron transfer from neighbouring aromatic amino acid residues (Trp) to the electronically excited flavin chromophore results in substantial shortening of the fluorescence lifetime of the flavin. Measurements of the fluorescence lifetime of the flavin can therefore provide significant information on the flavin environment. Here, we applied TCSPC and Kerr-gated fluorescence spectroscopy[50] to determine the fluorescence lifetimes of the flavin in the dark- and light-adapted state in order to obtain further information on the position of W104 during photoactivation and its influence on the quenching mechanism. In the dark-adapted state of the 7aza-W104/W64F, there is a dominant phase with a decay constant τ*dark*=500 ps that is around fifty times slower than the dominant phase of the light-adapted state, τ*light*=11ps. The faster decay in the light-adapted state, suggests that the excited state of the

flavin is quenched by electron transfer from close-by aromatic residues that should be positioned closer to the flavin compared to their position in the dark-adapted state. We therefore assign the drastic acceleration of the fluorescence decay in the light-adapted state of AppA W64F to the movement of the W104 towards the flavin and its acting as the main quencher in the light-adapted state. In addition, the emission spectra of the W64F AppABLUF lose the characteristic vibrational shoulder at ~500 nm in the light-adapted state. These changes are also accompanied by a decrease in the fluorescence and are indicative of an enhanced electron transfer in the case of the light-adapted state. Using the simple empirical expression known as the Dutton ruler [68] we roughly estimated the distances between flavin and the tryptophan for the dark-adapted state (R_{dark}=9.5 Å) and the light-adapted state (R_{light}=6.7 Å).

Transient infrared measurements

To study the protein dynamics in the $AppA_{BLUF}$ domain after blue light absorption we performed transient infrared measurements on the dark- and light-adapted states of $AppA_{BLUF}$ W64F. Comparison of the dark- and light adapted spectra of the W64F AppA mutant reveals a ~ 9 cm^{-1} downshift for light adapted state, that is similar to the shift observed for the WT AppA[46] and attributed to the formation of new hydrogen bond to C4=O. That result demonstrates the arrangement of a rigid environment for W104 in the light-adapted state due to the formation of a H-bond to the flavin, and supports our FRET measurements that reveal a change of the orientation of W104 close to the flavin in that state. It is also in line with our anisotropy decay measurements that demonstrate a slower rotation (~11 ns) for W104 in the light-adapted state , reflecting rotation of W104 together with the whole protein due to the more restricted environment of W104.

Conclusions

A wide range of fluorescence techniques, time-resolved infrared spectroscopy and unnatural amino acid incorporation have been used to probe the functional dynamics of W104 during the photoactivation process in AppABLUF. Fluorescence resonance transfer (FRET) measurements and replacement of W104 with the unnatural amino acid 7AW have provided quantitative information on the position of W104 in the dark- and light-adapted states. In the light-adapted state, W104 is in a restricted environment with an enhanced H-bond network compared to that in the dark-adapted state, as revealed by the long rotational correlation time (~11 ns) for W104 from fluorescence anisotropy decay measurements and the downshift of the C4=O carbonyl band from infrared measurements. In addition, in the light-adapted state the short fluorescence lifetime of the flavin suggests that efficient electron transfer takes place to the excited flavin from the closely placed W104. Overall, our study addresses previous discrepancies on the position of W104 during the photocycle of AppA and supports a conformation of W104 close to the flavin in the light-adapted state whereas in the dark-adapted state W104 is present in a less restricted environment pointing away from it.

In the following I highlight the main findings of my work:

1) Steady state fluorescence measurements performed during FRET measurements between $AppA_{BLUF}$ FAD and W104 revealed that the W64F mutation is not enough to study W104 as there are other not expected contributing factors to fluorescence.

2) Absorbtion dependent pH measurements showed that the measured extra fluorescence mentioned in the 1st point comes from tyrosinate. Our measurements showed that the tyrosine on protein has lower pK value and that's why it becomes tyrosinate.

3) We solved the problem stated in the 1st point by using 7-aza-tryptophan analogue. Using the 7-aza-tryptophan in the 104 position we can measure it independently.

4) Based on the aforementioned points we showed with the measurements in the following points that the W104 is turned towards the FAD in the photoactivated state compared to the dark state where it points away.

5) Using lifetime measurements for FRET calculations we found that the distance between FAD and W104 is 20.5 Å in the dark state and 9.5 Å in the light state.

6) Using acceptor enhancement method for steady state measurements we found that the distance between FAD and the W104 is 17.7 Å in the dark state and 8.3 Å in the light state.

7) Performing acrylamide quenching measurements we concluded that in the light state the W104 is in a less accessible more rigid environment.

8) Using anisotropy measurements we found that in the light state the W104 is in a more rigid environment compared to the dark state.

9) Ultrafast lifetime measurements supported that the photoactivated FAD relaxation is faster in the light state as there is electron transfer from the closely spaced W104 compared to the dark state.

10) Transient infratred measurements showed that during photoactivation W104 is closely spaced to FAD forming hydrogen bonding.

Publications

The PhD thesis is based on the following article:

1.

Karadi, Kristof ; M. Kapetanaki, Sofia ; Raics, Katalin ; Pecsi, Ildiko ; Kapronczai, Robert ; Fekete, Zsuzsanna ; Iuliano, James ; Tolentino Collado, Jinnette ; Gil, Agnieszka ; Orban, Jozsef et al.
Functional dynamics of a single tryptophan residue in a BLUF protein revealed by fluorescence spectroscopy
SCIENTIFIC REPORTS 10 Paper: 2061 , 15 p. (2020)
IF: 3,998

Posters and presentations related to the PhD thesis:

1.

Karádi, Kristóf Kálmán ; Kapronczai, Róbert ; Pirisi, Katalin ; Lukács, András
Fotoindukált elektron transzfer vizsgálata flavinokban fluoreszcencia és tranziens abszorpciós spetroszkópiai módszerekkel
In: 46. Membrán-Transzport Konferencia Sümeg
(2016) pp. 69-69. , 1 p.

2.

Kapronczai, Róbert ; Szekeres, Gábor ; Karádi, Kristóf ; Grama, László ; Lukács, András
Az AppA fotociklusának vizsgálata fluoreszcencia spektroszkópiai módszerekkel
In: 46. Membrán-Transzport Konferencia Sümeg
(2016) p. 68

3.

Kapronczai, Róbert ; Karádi, Kristóf ; Pirisi, Katalin ; Lukács, András
Functional dynamics of the key tryptophan in AppA revealed by fluorescence spectroscopy
In: Ács, Kamilla; Bencze, Noémi; Bódog, Ferenc; Ha□ner, Tamás; Hegyi, Dávid; Horváth, Orsolya Melinda; Hüber, Gabriella Margit; Kovács, Áron; Kis Kelemen, Bence; Lajkó, Adrienn; Schilli, Gabriella Krisztina; Szendi, Anna; Szilágyi, Tamás Gábor; Varga, Zoltán (szerk.) Book of Abstracts = Absztraktkötet : V. Interdiszciplináris Doktorandusz Konferencia
Pécs, Magyarország : Pécsi Tudományegyetem Doktorandusz Önkormányzat, (2016) p. 173

4.

KAPRONCZAI, RÓBERT ; PASITKA, JONATÁN ; KARÁDI, KRISTÓF ; SZEKERES, GÁBOR ; GRAMA, LÁSZLÓ ; NYITRAI, MIKLÓS ; LUKÁCS, ANDRÁS

Tirozinát fluoreszcencia spektroszkópiai vizsgálata az AppA nevű fotoaktív fehérjében

In: Zimányi, László (szerk.) A Magyar Biofizikai Társaság XXVI. Kongresszusa : Szeged, 2017. augusztus 22-25.

Szeged, Magyarország : Magyar Biofizikai Társaság, (2017) pp. 81-81. Paper: P27 , 1 p.

5.

Kapronczai, Róbert ; Karádi, Kristóf ; Pécsi, Ildikó ; Grama, László ; Lukács, András

AppA funkcionális dinamikájának vizsgálata fluoreszcencia rezonancia energia transzfer (FRET) segítségével

In: 48. Membrán-Transzport Konferencia

(2018) Paper: 31 , 1 p.

6.

Karádi, Kristóf ; Kapronczai, Róbert ; Sofia, Kapetanaki ; Pécsi, Ildikó ; Fekete, Zsuzsanna ; Lukács,András

AppA funkcionális dinamikájának vizsgálata triptofán-analógot tartalmazó mutánson végzett fluoreszcencia spektroszkópiai eljárások segítségével

In: 49. Membrán-transzport Konferencia, Sümeg, 2019.05.14-17

(2019) Paper: 30 , 1 p.

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