Absorption Spectra of FAD Embedded in Cryptochromes

Nielsen, Claus; Nørby, Morten S.; Kongsted, Jacob; Solov'Yov, Ilia A.

Published in:
Journal of Physical Chemistry Letters

DOI:
10.1021/acs.jpclett.8b01528

Publication date:
2018

Document version
Accepted manuscript

Citation for published version (APA):

Terms of use
This work is brought to you by the University of Southern Denmark through the SDU Research Portal. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:
- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 03. Oct. 2020
Absorption Spectra of FAD Embedded in Cryptochromes

Claus Nielsen, Morten Steen Nørby, Jacob Kongsted, and Ilia A. Solov'yov

J. Phys. Chem. Lett., Just Accepted Manuscript • DOI: 10.1021/acs.jpclett.8b01528 • Publication Date (Web): 15 Jun 2018

Downloaded from http://pubs.acs.org on June 18, 2018
Absorption Spectra of FAD Embedded in

Cryptochromes

Claus Nielsen,* Morten S. Nørby, Jacob Kongsted, and Ilia A. Solov’yov*

Department of Physics, Chemistry and Pharmacy, University of Southern Denmark,

DK-5230 Odense M, Denmark

E-mail: clausnielsen@sdu.dk; ilia@sdu.dk
Abstract

The magnetic compass sense utilized by migratory birds for long-distance navigation functions only once light of a certain wavelength is present. This piece of evidence fits partially with the popular hypothesis of chemical magnetoreception in cryptochrome proteins, located in the bird retina. According to this hypothesis a magnetosensitive radical pair is produced after photoexcitation of an FAD co-factor inside cryptochrome, and as such the absorption properties of FAD is of crucial importance for cryptochrome activation. In this letter we reveal that, however, absorption spectra of FAD show very little variation between six different cryptochromes, suggesting that the electronic transitions are barely affected by the chemical differences in the proteins. This conclusion hints on the presence of a secondary photoreceptor or cofactor, that could be necessary to explain green-light activated magnetoreception in birds.
Introduction

Migratory birds have been shown to possess a magnetic compass sense (1), that could possibly function through a photoinduced radical pair allegedly residing within the flavoprotein cryptochrome (1–6). According to a widely accepted mechanism (1, 3–6), prior to formation of this radical pair, a flavin adenine dinucleotide (FAD) co-factor within cryptochrome is photoexcited, such that it may accept an electron from a nearby tryptophan residue (1, 6–10). It is, thus, evident that the postulated radical pair hypothesis of magnetoreception relies on the photoabsorption properties of FAD which in turn may deliver special properties of the magnetic sensor. Since it is believed that formation of the magnetosensitive radical pair is preceded by photoabsorption of FAD, the absorption spectrum of FAD is expected to be pivotal in determining the range of light conditions at which the magnetic compass sense is operational.

Experiments with migratory birds have shown that the wavelength of the ambient light is crucial for the magnetic compass sense: the birds could utilize the magnetic compass when exposed to blue or green light, but not when only red light was available (1, 11, 12). This is in a partial agreement with the absorption spectrum of FAD, which only absorbs in the blue and UV regions of the spectrum (5, 13), but as FAD is bound within cryptochrome, its absorption spectrum could differ significantly from the spectrum of FAD in isolation, as is already seen e.g. in the experimentally obtained spectrum of Arabidopsis thaliana cryptochrome 1 where an absorption shoulder at 470 nm appears in the bound FAD (14). It would, therefore, be interesting to know whether a significant redshift of the absorption spectrum of FAD could be caused by its interactions with a specific cryptochrome matrix such as for example cryptochrome 4 from the European robin (15), increasing the range of light conditions where the magnetic compass would be operational.

In the present study we have investigated by computational methods the absorption
spectra of FAD within six different cryptochromes, namely cryptochromes from *Drosophila melanogaster* (DmCry), *Arabidopsis thaliana* (AtCry1), *Mus musculus* (MmCry) and *Xenopus laevis* (XlCry), as well as cryptochromes 1 and 4 from the European robin, *Erithacus rubecula* (ErCry1 and ErCry4); note that magnetic field effects have been reported for all of these species (3, 16–20). The structures of the studied cryptochromes were obtained from crystallographic data when available (21–24) and otherwise taken from homology modelling, after extensive molecular dynamics (MD) equilibration (15, 25, 26). The important aspect of the present study is the accurate and systematic consideration of the protein environment on the absorption properties of the flavin chromophore inside the six selected cryptochromes. Such an investigation has never been accomplished before and only some early data is available for AtCry1, where most of the environment was effectively neglected (27).

**Results and discussion**

The studied systems are summarized in Table 1. Calculations of the absorption spectra was carried out in five steps: (i) obtaining the equilibrated structure of cryptochrome, (ii) performing a quantum mechanics/molecular mechanics (QM/MM) geometry optimization

<table>
<thead>
<tr>
<th>System</th>
<th>Organism</th>
<th>Gene</th>
<th>PDB ID</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DmCry</td>
<td><em>Drosophila melanogaster</em></td>
<td>Cry-1</td>
<td>4GU5</td>
<td>(21, 22, 25)</td>
</tr>
<tr>
<td>AtCry1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Cry-1a</td>
<td>1U3C</td>
<td>(6, 9, 23)</td>
</tr>
<tr>
<td>ErCry1</td>
<td><em>Erithacus rubecula</em></td>
<td>Cry-1a</td>
<td>-</td>
<td>(25)</td>
</tr>
<tr>
<td>ErCry4</td>
<td><em>Erithacus rubecula</em></td>
<td>Cry-4</td>
<td>-</td>
<td>(15)</td>
</tr>
<tr>
<td>MmCry</td>
<td><em>Mus musculus</em></td>
<td>Cry-1</td>
<td>4KoR</td>
<td>(24)</td>
</tr>
<tr>
<td>XlCry</td>
<td><em>Xenopus laevis</em></td>
<td>Cry-DASH</td>
<td>-</td>
<td>(26, 28)</td>
</tr>
</tbody>
</table>
Figure 1: **FAD within cryptochrome with the quantum (QM) region highlighted.** Cryptochrome matrix is shown in the background, and the flavin part of FAD is highlighted. This highlighted part consists of 30 atoms and is used as the core quantum region in the calculations, while the rest of FAD and the protein, along with water and ions not shown here, are considered to be the environment, and is represented by a polarizable embedding potential in the spectrum calculations. The N5 nitrogen atom is labeled and possesses an additional hydrogen in the case of the FADH spectrum calculation.

of the core quantum region of the protein shown in Fig. 1, (iii) calculating the polarizable embedding (PE) potential to describe the surroundings of the core quantum region, (iv) performing the spectrum calculation, and (v) shifting the spectrum to correct for a systematic computational error. Steps (i)-(ii) and (v) are described extensively in the supporting information (SI), while steps (iii)-(iv) are described in more detail below.

The six computed absorption spectra of FAD embedded in different cryptochromes were each averaged over 10 structures, in order to account for the thermal motion in the proteins. The 10 structures were obtained from the extended molecular dynamics (MD) simulations, as explained in the SI, and correspond to statistically independent configurations of...
proteins sampled at a 10 ns interval of the production simulation, see Figs. S2 and S3. The resulting spectra are shown in Fig. 2 with the positions of the peaks summarized in Table 2; the individual spectra for the 10 structures are presented in Fig. 3. All the six studied cryptochromes show very similar absorption properties for the flavin part of the FAD cofactor. One may note that the spectrum from ErCry4 is slightly blue-shifted relative to AtCry1 and DmCry for the two peaks at wavelengths longer than 350 nm, but the shift is less than 40 nm. The secondary peak at 384 nm in the calculated AtCry1 spectrum deviates by about 20 nm from the corresponding peak at 360 nm in the experimental spectrum, but such a discrepancy is expected when a different transition was used for aligning the calculated and experimental spectra; the spectra were shifted by 60 nm in order to make the primary peak directly comparable with experiment (see the Method details and Fig. S1 in the SI), but at the cost of a less accurate secondary peak.

The choice of using 10 structures per cryptochrome to produce the final spectra seems reasonable when considering Fig. 3; Fig. 3 shows no clear outliers for each of the studied cryptochromes, and it seems reasonable to assume that additional samples would make no significant impact on the averaged spectra shown in Fig. 2. This rationale was further checked by considering the spectra of 10 additional structures for ErCry4, and then deriving the averaged spectrum of all 20 structures, see Fig. S5 in the SI. The comparison shows

Table 2: **Position of the absorption peaks in six studied cryptochromes.** The peak positions are calculated as the mean value over the peak positions of the 10 structures. The “± values” indicate the corresponding standard deviation.

<table>
<thead>
<tr>
<th>System</th>
<th>Primary peak (nm)</th>
<th>Secondary peak (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DmCry</td>
<td>450 ± 3</td>
<td>389 ± 7</td>
</tr>
<tr>
<td>AtCry1</td>
<td>450 ± 4</td>
<td>385 ± 6</td>
</tr>
<tr>
<td>ErCry1</td>
<td>441 ± 5</td>
<td>383 ± 9</td>
</tr>
<tr>
<td>ErCry4</td>
<td>434 ± 4</td>
<td>369 ± 9</td>
</tr>
<tr>
<td>MmCry</td>
<td>443 ± 4</td>
<td>392 ± 5</td>
</tr>
<tr>
<td>XlCry</td>
<td>442 ± 4</td>
<td>379 ± 4</td>
</tr>
</tbody>
</table>
Figure 2: Absorption spectra for the investigated cryptochromes. Shown are the averaged spectra for the flavin part of the FAD moiety embedded in 6 different cryptochromes, see Table 1, and the experimental AtCry1 spectrum (14) for comparison. Experimental data is in mM$^{-1}$cm$^{-1}$ while the computed spectra are in atomic units. Note that experimental data was not available below 300 nm. Each calculated spectrum is averaged over 10 independent computations in order to take the thermal motion of the protein systems into account. The individual computed spectra can be seen in Fig. 3. The calculations were carried out up to 1300 nm, but the absorbance was zero for all wavelengths higher than 540 nm.

that a spectrum averaged over 20 structures does not differ significantly from the spectrum averaged over the 10 original structures.
Figure 3: **Individual spectra for the investigated cryptochromes.** Each panel contains 10 spectra, and each spectra per panel corresponds to a specific molecular configuration of cryptochrome taken from atomistic MD simulations.

It should be stressed that the model employed for the present investigation relies on some assumptions. The chosen quantum (QM) region consisted of 30 atoms of FAD, i.e. the isoalloxazine moiety as illustrated in Fig. 1, and was isolated from the rest of FAD (contained in the polarizable embedding) by cutting a covalent bond. This choice of QM region thus assumed that the electronic density affected by the studied transitions are localized on the isoalloxazine moiety, since they are confined to the QM region during the absorption spectrum calculations. This assumption was tested by a calculation of the AtCry1 spectrum.
with the full FAD co-factor in the QM region, performed on a single AtCry1 structure, and the calculation showed no significant change in the absorption spectrum by inclusion of the remainder of FAD in the QM region, see Fig. S4.

Secondly, only the electrostatic interactions between the protein matrices and the isoalloxazine moiety were considered. The embedding potential used in the polarizable embedding strategy accounts for electrostatic and induction effects. Thereby other effects of purely quantum mechanical origin such as dispersion, charge-transfer and non-electrostatic repulsion are not included. However, by enlarging the part of the system described using QM these effects may be included depending of course on the ability of the chosen electronic structure method to capture e.g. dispersion effects. The validity of this assumption, i.e. excluding the protein matrix from the QM region, was investigated for a similar system with the conclusion that the environment need not be contained in the QM region (13).

An important feature is, however, missing in the computed spectra. The peak at 450 nm is known to have a shoulder at slightly higher wavelengths when FAD is bound inside a protein (14, 29), see Fig. S1 in the SI. This peculiarity, however, is not seen in the computations. The reason for this could be that the shoulder appears due to vibronic structure, or that it is a separate electronic transition not captured by the conventional TDDFT method. Accounting for e.g. vibronic structure in the absorption bands of chromophores bound in a protein environment is highly non-trivial and thus beyond the scope of the present investigation. In fact, it is highly non-trivial to account for potential vibrational structures for chromophores when embedded into discrete environments. When using a continuum solvation model like the Polarizable Continuum Model (PCM) the vibrational structure can be computed in very much the same way as for a molecule in the gas-phase, but such calculations build on the fact that the complete system (represented discretely only by the chromophore) has been fully geometry optimized. Moving to complex environments leads to calculations of the central quantities needed for the inclusion of the vibrational structure becoming less well-defined,
and we believe that today no single method has been published which combined accurate embedding strategies with the ability to account for vibrational structures in absorption (or emission) bands.

Although the calculated spectra have no absorbance beyond 550 nm, this is not a shortcoming of the method but simply means that no electronic excitations occur at higher wavelengths. In order to prove this, the absorption spectrum of FADH from AtCry1 was also calculated and compared with an experimental result; Fig. 4 illustrates that FADH has a non-zero absorbance up to about 650 nm. Note that only a single structure of cryptochrome with FADH was used for this proof-of-principle calculation, such that the thermal motions in the system were not accounted for by an average over multiple configurations as for the FAD case in Fig. 2 and Fig. 3. The experimental and calculated spectra of FADH are in good agreement, except for small discrepancies around 330 nm marked by the two sets of vertical lines in Fig. 4. Once again such minor discrepancies are to be expected for the peaks not used for aligning the calculated and experimental spectra. The other differences between the spectra can be attributed to the neglected thermal motion, and in particular the vibronic transitions not included in the calculations. For more details on the FADH spectrum calculations, please see the SI.

**Conclusion**

Overall, the studied cryprochrome structures do not seem to have any significant effect on the absorption spectrum of the embedded FAD co-factor. Since the performed additional calculations on FADH illustrate that electronic transitions at wavelengths above 550 nm can be obtained by the applied computational methods, we conclude that FAD embedded in cryptochromes cannot absorb green light of 550 nm or above, and that a secondary photoreceptor co-factor, or a ligand, must be involved in order to explain the experimental
Figure 4: Calculated and experimental AtCry1 FADH spectra. The calculated spectrum was obtained for a single configuration of AtCry1. The four electronic transitions marked by vertical lines are in good agreement with those observed experimentally, and the additional absorbance seen in the experimental spectrum especially at about 600 nm and 380 nm could be caused by vibronic transitions. Experimental data was adapted from (14) and is only available down to 300 nm. Experimental data is in mM$^{-1}$cm$^{-1}$ while the computed spectra are in atomic units.

finding that green light should be sufficient for activation of the avian magnetic compass (12). Note that some members of the cryptochrome/photolyase protein family have already been demonstrated to possess secondary photoreceptor co-factors (30–32). The results obtained and presented in this paper points to the fact that an additional chromophore is needed in order to fully describe the activation mechanism of the protein. It is thus our plan in the future to investigate which other chromophore(s) would be needed in order to explain the green-light activation of the magnetic compass. This could be studied by performing multi-chromophore calculations within a Frenkel Hamiltonian picture as discussed in relation to the polarizable embedding method in (33).
Theoretical methods

We base our calculation of absorption spectra on the unification of the PE model (34, 35) with an efficient multi-frequency formulation and implementation of the linear complex polarization propagator (CPP) response function (36) to result in the PE-CPP method (37). The PE method is an advanced QM/MM procedure where the atoms defining the environment are given a classical description in terms of an embedding potential. The embedding potential consists of terms describing the permanent charge distribution utilizing a set of localized multipoles. Furthermore, the induced charge distribution is accounted for by a set of localized polarizabilities. Based on this embedding potential a ground state DFT calculations is performed using a double self-consistent-field model (34, 38), i.e. both the polarization of the QM density and the classical polarization of the environment is fully iterated. The absorption is calculated directly from a linear response formalism (as used in the PE-CPP method) in terms of the imaginary part of the frequency-dependent electric-dipole polarizability derived from the part of the system defined as the QM region (37, 39, 40). The imaginary part of the frequency-dependent polarizability is obtained as the solution to a number of response equations, where the environmental effects are introduced both explicitly and indirectly through the change of one-electron orbital energies due to the ground state embedding potential (37).

All absorption spectra were computed using the DALTON program (41) interfaced with the PE library (42) and Gen1Int (43, 44). The embedding potential representing the environment consists of localized multipole moments up to the second order (quadrupoles) and localized dipole-dipole polarizabilities. All localized multipoles and polarizabilities were computed utilizing the LoProp approach (45) employing the Polarizable Embedding Assistant Script (PEAS) interfaced with FragIt (46) together with Molcas (47). The expansion centers for the localized properties (multipoles and polarizabilities) are taken as the atomic
centers of the atoms defining the environment. To derive the multipole moments we used the 6-31+G* basis set. The basis set was initially recontrated in order to fit with the requirements of the LoProp method (45). Parameters for a polarizable embedding potential obtained in this way have been validated by earlier studies (48, 49).

For the quantum region we utilized the CAM-B3LYP functional (50) and Dunning's correlation-consistent basis sets cc-pVDZ to expand the electronic wavefunctions for hydrogens and aug-cc-pVDZ for heavier atoms (51).

In order to obtain the spectra, the extinction coefficient was calculated for many different wavelengths, yielding a set of data points. Interpolation with splines were used to connect the points in order to get smooth curves as the resulting spectra. The points of the spectra were sampled with a constant step in energy of 0.068 eV, such that at 400 nm the points are about 9 nm apart, and about 5 nm apart at 300 nm. This sampling rate limits the precision with which absorption peaks can be determined, but should be sufficient to provide reliable absorption spectra due to the broadness of the absorption peaks in cryptochromes (14).

**Acknowledgements**

We want to thank Emil Sjulstok Rasmussen for providing many of the investigated structures, and Peter Hore for the original suggestion of the study. IAS and CN are grateful for financial support from the Lundbeck Foundation, the Danish Councils for Independent Research, the Russian Science Foundation (grant no. 17-72-20201), and the DeiC National HPC Center (SDU) for providing computational resources necessary for the calculations.
References


(43) Gao, B. Gen1Int Version 0.3.0. 2014; http://repo.ctcc.no/projects/gen1int.


