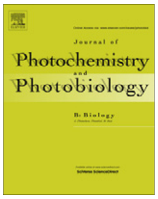
Journal of Photochemistry and Photobiology B: Biology 109 (2012) 12–19

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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



Modeling of a violaxanthin–chlorophyll b chromophore pair in its LHCII environment using CAM-B3LYP

Dominik Kröner [a], Jan Philipp Götze [b,*]

[a] Theoretische Chemie, Institut für Chemie, Universität Potsdam, Karl-Liebknecht-Straße 24-25, D-14476 Potsdam, Germany
[b] Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, D-45470 Mülheim an der Ruhr, Germany

ARTICLE INFO

Article history:
Received 4 October 2011
Received in revised form 25 November 2011
Accepted 18 December 2011
Available online 8 January 2012

Keywords:
CAM-B3LYP
Density functional theory
Light-harvesting complex II
Photosynthesis
Violaxanthin
Chlorophyll b

ABSTRACT

Collecting energy for photosystem II is facilitated by several pigments, xanthophylls and chlorophylls, embedded in the light harvesting complex II (LHCII). One xanthophyll, violaxanthin (Vio), is loosely bound at a site close to a chlorophyll b (Chl). No final answer has yet been found for the role of this specific xanthophyll. We study the electronic structure of Vio in the presence of Chl and under the influence of the LHCII environment, represented by a point charge field (PCF).

We compare the capability of the long range corrected density functional theory (DFT) functional CAM-B3LYP to B3LYP for the modeling of the UV/vis spectrum of the Vio + Chl pair. CAM-B3LYP was reported to allow for a very realistic reproduction of bond length alternation of linear polyenes, which has considerable impact on the carotenoid structure and spectrum. To account for the influence of the LHCII environment, the chromophore geometries are optimized using an ONIOM(DFT/6-31G(d):PM6) scheme.

Our calculations show that the energies of the locally excited states are almost unaffected by the presence of the partner chromophore or the PCF. There are, however, indications for excitonic coupling of the Chl Soret band and Vio. We propose that Vio may accept energy from blue-light excited Chl.

1. Introduction

The light reactions of photosynthesis sustain the energetic needs for almost all processes in life on earth. However, they require more energy than can be obtained by the reaction center complex alone. Antenna protein complexes have evolved which transfer photonic energy to these reaction centers. One part of these arrays is the light harvesting complex II (LHCII), which is a family of several different proteins [1–3]. Major LHCII antenna proteins form trimers which are located within the thylakoid membrane, and contain xanthophylls and chlorophylls, which are the photoactive compounds. Chlorophylls are the key players, receiving the required wavelengths and transmitting energy to the reaction centers. Xanthophylls have been shown to control the ability of LHCII to react to high or low light intensities (for extended reviews, see Refs. [3–5]). The actual control and interaction mechanisms are still a hot topic [6–8].

It is clear that discussing the role of chlorophylls and xanthophylls on the basis of quantum theory strongly depends on the computational approach. Recent developments in theory inspire hope that the large amount of coupled chromophores of LHCII might be addressed by *e.g.*, frozen density embedding [9,10]. There is, however, a very basic problem of quantum chemistry when it comes to the discussion of chlorophylls and carotenoids: The choice of quantum chemical method. Standard quantum chemistry approaches may not be fast or accurate enough when it comes to medium-sized systems with extended conjugated π-systems. This problem has been addressed in the past by the investigations of Dreuw and coworkers [11–14]. In these studies the authors combined time-dependent density functional theory (TD-DFT, [15]) with configuration interactions singles (CIS [16]) to account for long-range charge-transfer states. Another study by Martins et al. is based on semi-empirical approaches [17]. Recently, it was also shown that there is a strong dependence of the excited state energies on the chromophore geometry [18]. Another issue is the presence of strong double excitation character in some excited states of carotenoids, for which several approaches have been tested in the past (*e.g.*, Refs. [19–21]).

Novel key aspects of our theoretical study are the application of CAM-B3LYP, the long range-corrected version of the DFT functional B3LYP using the Coulomb-attenuated method [22], and the explicit inclusion of the immediate protein/membrane environment. It has been shown earlier that CAM-B3LYP allows for a more realistic reproduction of bond length alternation (BLA) compared to several widely used DFT functionals (among them B3LYP) [23–25], which may become important, for instance, for the simulation of UV/vis spectrum of linear polyenes [26]. With the insight that X-ray data

---

* Corresponding author.
E-mail address: goetze@mpi-muelheim.mpg.de (J.P. Götze).

1011-1344/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2011.12.007

are lacking especially such structural details [18], re-optimization of the structures with CAM-B3LYP in particular is, therefore, of special interest for our study.

Eventually, we aim to elucidate a potential role for violaxanthin (Vio), which is closely associated with a chlorophyll b molecule (Chl). Violaxanthin was initially supposed to be a xanthophyll supporting the light harvesting process [27]. This was contradicted by absorption/emission spectra of Vio in LHCII [28]. The search for a possible Vio purpose went on, spawning numerous experimental and theoretical studies (*e.g.*, Refs. [12,13,29–32]).

Regarding the spectroscopic basics, Vio can be ideally treated as having a C_{2h} symmetry like a linear polyene, if the terminal rings are neglected [27]. Within this analogy, both S_0^{Vio} and S_1^{Vio} are of A_g^- symmetry, and S_2^{Vio} has $B_u{}^+$ symmetry. As there is no change in symmetry for $S_{0\rightarrow1}^{Vio}$, it is clear that this transition is optically forbidden. In contrast, $S_{0\rightarrow2}^{Vio}$ has a very strong optical band.

For Chl, we can assume a similarly idealized view, considering only the metal-porphyrine. This would yield D_{4h} as point group and the $S_{0\rightarrow1}^{Chl}$ transition would then be of E_u symmetry, which is optically allowed (albeit weaker than $S_{0\rightarrow2}^{Vio}$) [33].

The setup of our calculations will be described in Section 2. Vio and Chl possess different electronic structures, namely a linear conjugated π-system versus a magnesium complex porphyrine system, and represent, therefore, different challenges for quantum chemical methods. Although addressing all these challenges is beyond the scope of this report, we will show that using CAM-B3LYP has some advantages over B3LYP (Section 3.1), primarily by delivering a comparable quality of results for both molecules.

Yet, it is known that time-dependent (TD-)DFT can only properly describe the second excited state of Vio ($1B_u^+$), as other energetically low states like $2A_g^-$ or $1\ B_u^-$ contain significant double excitation character [34,35]. However, $1B_u^+$ should be the spectrally important state when it comes to the initial absorption event in Vio. Although we do not expect our setup to deliver insights which go beyond the initial absorption process (such as the energies of the elusive xanthophyll dark states [6,8,36]), we are still able to get substantial information on how the two chromophores interact electronically.

In addition to the comparison of (TD-)CAM-B3LYP and (TD-)B3LYP results (to demonstrate the differences resulting from the advanced inclusion of long range interactions), the effect of embedding the chromophores in the protein environment is investigated. This is necessary to understand whether (and to what extent) already distortions of the molecular geometry, caused by optimization inside the protein, result in changes of the UV/vis spectrum or influence the excitonic coupling between the two chromophores (Section 3.2). Eventually, the explicit presence of the protein and membrane environment in the form of a point charge field (PCF) is studied to check its direct impact on spectra and electronic structure (Section 3.3). The article will finish with a summary and outlook in Section 4.

2. Model construction and methods

For all calculations, the Gaussian09 program was used [37].

2.1. Geometry optimization and UV/vis spectra

Initial method testing was done by optimizing Vio and Chl separately in acetone (using a polarizable continuum model, PCM [38]). The structures of our model compounds can be seen in Fig. 1. Both the CAM-B3LYP [22] and B3LYP [23–25] methods were used, with a 6-31G(d) basis [39]. The corresponding UV/vis spectra were calculated using time-dependent density functional theory (TD-DFT [15]).

When embedding the chromophores in a protein environment, we used pea LHCII (*Pisum sativum*, PDB data set 2BHW [2]) as structural framework for our study. In the crystal structure, Vio (residue 504) is present once per monomer within the LHCII trimer. Closely associated, we find a Chl (residue 609) molecule, its phytyl group in close contact to Vio. If applicable, results will be presented as the average over all three chromophore pairs (Chl + Vio) of the LHCII trimer. We employed an ONIOM(DFT/6-31G(d):PM6) scheme [40,41] to roughly mimic the closest shell of protein environment for optimization in the protein. This way, we aim at a reasonable mechanical embedding of the chromophores in the LHCII complex. The DFT method was either CAM-B3LYP or B3LYP.

The environment shell (the low layer, treated with PM6 [40,41]) for Chl + Vio of subunit A covers molecules within about 4 Å radius of the chromophore pair. It consisted of Val22-Phe28, Tyr44-Trp46, Phe189, Ala221-Phe228, three chlorophyll a molecules (residues 603, 604 and 608) and a phosphatidyl-glycerole (LHG, residue 801) of chain A. The shell was completed by some elements from chain C, which were Trp128-Gln131, Gly136, two chlorophyll b molecules (residues 610 and 612) and a diacyl glycerole (DGD, residue 802). Note that we changed Lys23 and Ser223 of chain A, as well as Ala129 and Thr130 of chain C, to glycine residues by cutting their functional groups. This was done for the sake of computational effort, as the functional groups were not in any way in direct contact to the chromophores. N-terminal cut-off positions were saturated with aldehyde groups, using the carbon and oxygen backbone positions from the preceding amino acid and a manually added hydrogen; C-termini were saturated with an uncharged amino group using the nitrogen atom position from the amino acid following in sequence and two manually added hydrogens. Note that the crystal geometry of the DGD 802 is missing one ester chain and the chlorophyll a 608 is missing part of the alkyl moiety of its phytyl side chain. These side chains are close to the border of the LHCII complex and should, therefore, have no significant influence for the optimization. Missing hydrogens were added manually.

The pockets for subunit B and C were constructed in the same manner, an example is shown in Fig. 2. Note that the shell is partially open due to the location of the chromophores close to the surface, which could not be addressed without employing a molecular dynamics approach including a membrane segment. As no severe distortions were observed in the region of the gap, we consider the shell appropriately complete regardless of the missing membrane.

During the optimization, only the non-hydrogen atoms of the shell were frozen, high layer chromophores and low layer hydrogens were allowed to relax.

Gas phase TD-DFT spectra on the basis of the ONIOM geometries were augmented by a neutral Tyr24 point charge group (Amber99, see below), coordinating Mg^{2+}. Test calculations have shown that significant UV/vis differences between the coordinated and uncoordinated Chl (data not shown) occur. As we did not want to explore the effect of Mg^{2+} coordination, but of the environment as a whole, we kept the Tyr24 point charges for a proper Mg^{2+} coordination sphere in all our calculations containing Chl. We will refer to these systems as *in vacuo* regardless.

2.2. Embedding into a point charge field

To study the long-range Coulomb effect of the whole protein and the thylakoid membrane on the chromophores, we embedded these into an environment in form of a point charge field (PCF). The exact procedure of the PCF construction can be found in Supplementary material. The final PCF consisted of: