



Explicit solvent effects on the visible absorption spectrum of a photosynthetic pigment: Chlorophyll- c_2 in methanol

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ABSTRACT

The explicit solvent effects on the light absorption properties of a photosynthetic pigment are analyzed from a combined study using Monte Carlo simulation and quantum mechanical Density-Functional Theory calculations. The case considered is chlorophyll- c_2 in methanol and excellent results are obtained for both position and intensities in the entire visible region. Explicit solvent molecules are essential for describing the absorption spectrum. Analysis is also made of the coordination of the Mg atom, the influence of solute-solvent hydrogen bonds, the existence and location of dark states for internal conversion mechanisms and the adequacy of the four-state model for classifying the transitions.

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1. Introduction

Very few systems deserve more investigation of the visible part of the absorption spectrum than photosynthetic pigments. The light absorption in this spectral region triggers one of the most important reactions in nature, the photosynthesis process [1]. Thus it is quite understandable the considerable amount of experimental and theoretical works dedicated to the study of their absorption spectra. Theoretical studies essentially began with the Gouterman [2] consideration of the 4-orbital model and several calculations of the absorption spectrum using semi-empirical methods have been reported. More recently time-dependent density functional theory (TD-DFT) [3] has been the method of choice. Considerable effort has been directed to the description of the ground and excited states of the isolated molecule [4–13] with solvent effects included either as micro-solvation or using a continuum model, or both. These studies have advanced our knowledge of their electronic properties but the position of the absorption maxima are misplaced by as much as 30–40 nm. Thus a theoretical investigation of the explicit solvent effect on the light absorption of a protein-free photosynthetic pigments is a relevant step in the direction of understanding these important systems. This timely study is the subject of this Letter. Using a combination of Monte Carlo (MC) simulation and quantum mechanical (QM) calculations we address to the important questions of the metal coordination [5,6,10], solute-solvent hydrogen bonds, the adequacy of the 4-orbital model, the existence and location of states of low-intensity transition from the ground state (dark states) [5,13,14] and the

environment contribution to the location of all absorption bands. The present study focus on the analysis of the absorption spectrum of chlorophyll- c_2 ($Chl-c_2$), shown in Figure 1, in methanol. This choice is driven by the existence of experimental data for the spectral properties of chlorophyll- c_2 in methanol and aqueous mixtures [15] and by the specific interest in investigating also the influence of hydrogen bonding on the spectral properties.

2. Calculation methods

Metropolis MC simulation were carried out using one molecule of $Chl-c_2$ and 1500 methanol molecules in the NPT ensemble at room condition in a parallelogram box of initial lengths $54 \times 54 \times 37$ (Å) and average lengths of $53.6 \times 53.6 \times 36.6$ (Å). The average calculated density is 0.765 g/cm^3 , in agreement with the experimental density of methanol (0.787 g/cm^3). The intermolecular interactions are described by the Lennard-Jones plus Coulomb potentials with the conventional three parameters for each site i (ϵ_i , σ_i and q_i). For methanol and $Chl-c_2$, the ϵ_i , and σ_i parameters of the potential were obtained from the optimized parameters for liquid simulation (OPLS) of Jorgensen and co-workers [16,17]. For Mg we used the parameters suggested by Aqvist [18]. The inclusion of the solute polarization is made using PCM, as before [19]: we determine the atomic charges for the classical MC simulation from an electrostatic fit (CHELPG) [20] in a PCM-B3LYP/6-31G(d,p) calculation. The dipole moment obtained for the isolated $Chl-c_2$ is 11.61 D and in-methanol the resulting dipole moment is 15.20 D. All the MC simulations were performed with the DICE program [21] and consisted of a thermalization of 7.5×10^7 MC steps, followed by an averaging stage of 1.5×10^8 MC steps. After the MC simulation, we selected configurations composed of one $Chl-c_2$ and the nearest 99 methanol molecules

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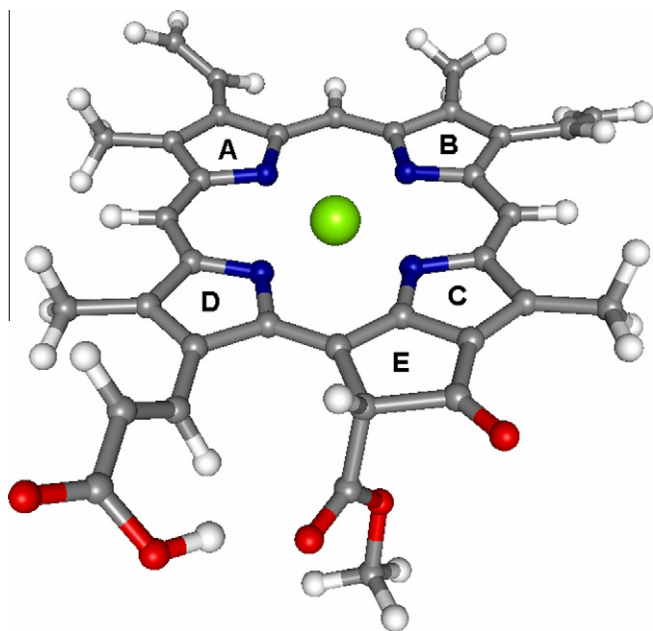


Figure 1. Chlorophyll-*c*₂ and the ring definitions with the atoms representation: C (gray), N (blue), O (red), H (white) and Mg (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for the subsequent QM calculations. In addition, for taking the statistical averages we sampled 100 configurations with less than 12% of statistical correlation. Therefore, the calculated absorption spectrum of *Chl-c*₂ in methanol were performed in 100 MC configurations composed by 100 molecules (1 solute + 99 solvent).

3. Results and discussion

Before discussing the results with the explicit solvent molecules included we consider the simplest cases of the isolated molecule and the influence of the PCM method on the calculated spectrum. Table 1 shows the calculated spectrum of isolated *Chl-c*₂ using the TD-B3LYP/6-31G(d,p) [3] level and also using the polarizable continuum model (PCM) [22,23] to represent the solvent (both implemented in the GAUSSIAN-03 program [24]). The geometry of *Chl-c*₂ was determined from a minimum-energy structure obtained from a B3LYP/6-31G(d,p) calculation. The results for the isolated molecule give transition wavelengths that are too small with all excitations on the blue side compared to experiment in solution. The peak of the Soret (B) band (414 nm) is to the blue side by ca. 0.25 eV. Similar results are also obtained with the B3PW91 functional with the same basis set. This picture is similar to that obtained in the interesting work of Vokáčová and Burda [8]. All transitions are characterized as $\pi-\pi^*$ and are thus expected to red-shift in the solvent environment. The inclusion of the solvent effects by using the continuum

Table 1
Calculated transition wavelength (nm) and intensities of the chlorophyll-*c*₂ in vacuum and in methanol with the continuum (PCM) and the discrete (ASEC) models. Calculations with the B3LYP/6-31G(d,p) model. See text.

Vacuum		PCM		ASEC		Experiment [15]
λ	f	λ	f	λ	f	λ
566	0.03	575	0.01	584	0.01	633 (Q _y)
564	0.00	574	0.04	578	0.03	586 (Q _x)
419	0.29	440	0.69	439	0.38	
414	0.45	433	0.69	434	0.19	451(B)
410	0.51	431	0.32	430	0.48	
399	0.06	412	0.11	414	0.08	

model (PCM) corrects the transitions in the right direction but the calculated red-shifts are essentially half of the shift necessary (for the Soret band, for instance) to obtain agreement with experiment. The results named ASEC (average solvent electrostatic configuration) [25] correspond to a discrete model of the solvent described as an electrostatic approximation, i.e. the solvent molecules are treated only as point charges. These solvent molecules are placed according to the MC simulation, described below, and thus include the Mg-coordinated and hydrogen-bonded solvent molecules, but treated only as point charges. We note that these results essentially reproduce the PCM predictions, except for a slight improve in the Q_y band thus increasing the separation between the two Q bands. Overall, there is still an error in the ASEC prediction for the peak position of the Soret band relative to experiment that amounts to as much as ~20 nm. We will then consider this using the sequential QM/MC methodology [26,27].

We now discuss and present the results with explicit consideration of the solvent. First, we focus on the coordination of the Mg atom. It is normally expected that the magnesium atoms of chlorophylls and bacteriochlorophylls in solution should be penta- or hexa-coordinated [5,10,12,28]. Micro-solvation calculations have considered both cases with 1:1 and 1:2 solvent complexes in minimum-energy structures optimized using different semi-empirical or DFT methods [5,12,28]. Figure 2 shows the radial distribution function between the Mg atom and the oxygen atom of methanol. The first solvation shell defines the coordination number and it can be seen to start at 2.0 Å, end at 2.8 Å with a maximum at 2.25 Å. Integration of the RDF up to 2.8 Å gives the coordination number of two methanol molecules. The peak is very sharp suggesting that all configurations are hexa-coordinated, and the possible existence of penta-coordination is verified to be statistically insignificant. Figure 3 shows one configuration with a clear positioning of two methanol molecules coordinating with the Mg atom. The average distance between the Mg atom and the oxygen atom of methanol is 2.25 Å. In addition, we also find methanol molecules that are hydrogen bonded to *Chl-c*₂. From the statistical analysis of the configurations, we find an average of 2.15 hydrogen bonds between *Chl-c*₂ and methanol. Figure 3 also shows the most probable hydrogen-bonded methanol found in several configurations.

The calculated spectrum of *Chl-c*₂ in methanol uses 100 configurations composed by the chlorophyll surrounded by 12 explicit methanol molecules and embedded in the electrostatic background of the remaining 87 methanol solvent molecules. This corresponds to one-hundred B3LYP/6-31G(d,p) calculations on a

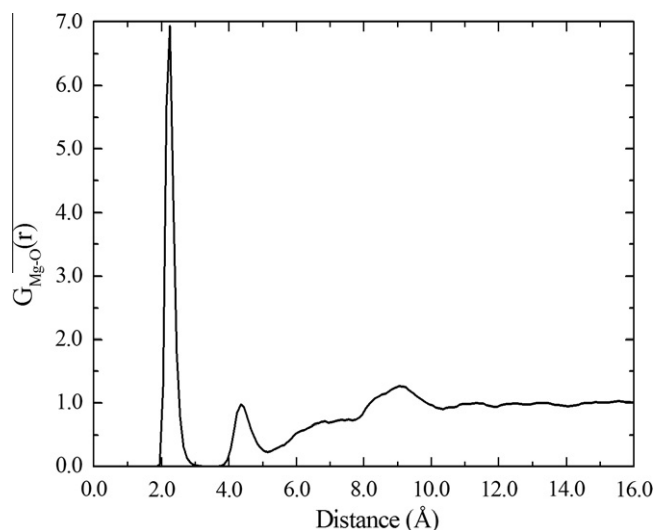


Figure 2. Radial distribution function between the Mg atom of chlorophyll-*c*₂ and the O atom of methanol.

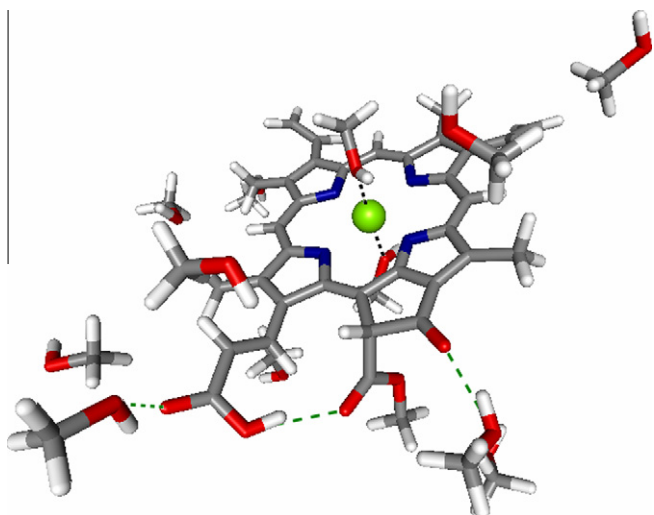


Figure 3. One configuration showing the coordination in the Mg atom and some hydrogen bonded methanol molecules.

system with 145 atoms and a total of 534 electrons and naturally includes all solvent molecules that are coordinated with Mg or hydrogen bonded. These solvent molecules have their positions in conformity with the thermodynamic condition. The situation is illustrated in Figure 4 where one of these configurations is shown. Because of the large amount of data, it is clear that (considering the different transitions composing the bands and the different oscillator strength) taking the average is more difficult, and in fact, it is unnecessary. Instead, we have convoluted all the transition energies to obtain a calculated spectrum in the region between 400 and 700 nm. The result is shown in Figure 5(top) and shows now an excellent agreement with the experimental spectrum. The Soret band is obtained at 449 nm, compared with the experimental maximum at 451 nm. The calculated spectrum also shows a shoulder at ca. 400 nm that can be seen in the experiment. The different colors in Figure 5 are used to characterize the different calculated transitions that compose the calculated band. This is particularly important for the Q bands that are overlapping in the total spectrum, but separate convolutions allow to obtain the reported values of 590 and 608 nm. The largest discrepancy is ob-

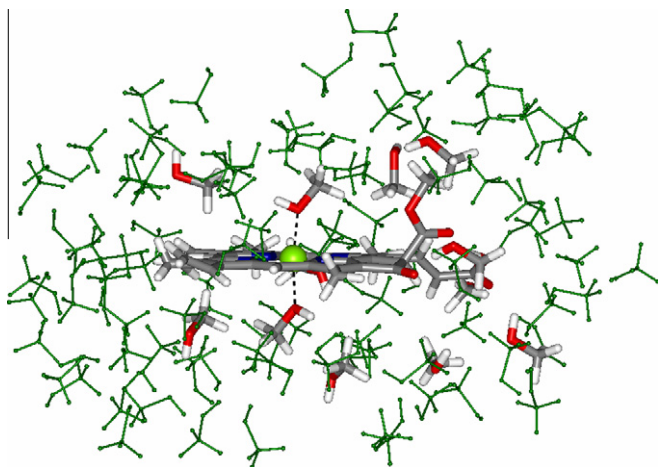


Figure 4. Illustration of one of the configurations composed by the chlorophyll-c₂, the twelve nearest explicit methanol molecules and the electrostatic background composed of additional eighty-seven solvent molecules.

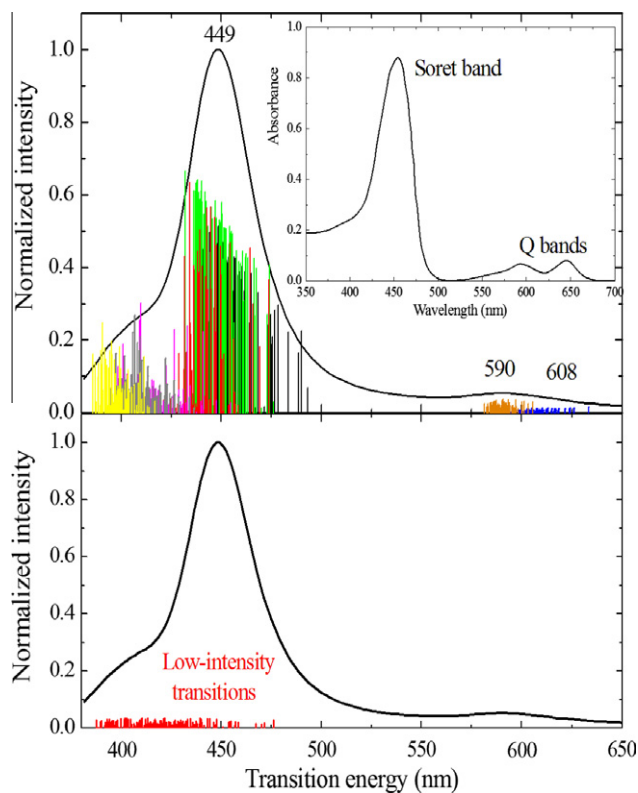


Figure 5. The theoretical spectrum in the visible region with the experimental absorption spectrum in the inset obtained from the experimental data [15] (top); location of the calculated low-intensity (dark) states (bottom).

tained for the Q_y band, but note that in this region of the spectrum the difference between the calculated and experimental values, corresponds to only 0.08 eV (645 cm⁻¹). The excellent agreement for the Soret band is obtained after a very large solvatochromic red-shift, from the in-vacuum condition, of nearly 0.25 eV, corresponding to a non-electrostatic contribution (compare with results of Table 1) of ca. 0.12 eV. Nearly 1/3 of this value is obtained by using explicitly only the hydrogen bonded methanol molecules. Also the Q_y band shifts by as much as 0.15 eV, of which 0.08 eV is of non-electrostatic nature. The conclusion is that the influence of the solvent on the light absorption properties of photosynthetic pigments is dramatically large but also that it can be obtained by a realistic theoretical model that incorporates the liquid statistics and the explicit intermolecular interactions. The Soret band is not composed by one transition, instead there are several transitions calculated with varying intensities. The composition of this band is an outcome of the contributions of the different configurations necessary for treating the thermodynamic condition. Thus, it is a complex band and the present model includes also the inhomogeneous contribution to the band broadening, as seen in Figure 5. We find that the two Q bands are in conformity with the Gouterman 4-orbital picture involving transitions from the HOMO and HOMO-1 to the LUMO and LUMO+1 orbitals. However, the equivalent description for the intense Soret band is more complex, as noted before [4]. Inspecting the configurations, we can note that transitions to the LUMO+2 and LUMO+3 orbitals also appear. This latter orbital is more localized in the carboxylic group and the lower D and E rings. Similarly, some transitions originate in the HOMO-2 orbital, which is localized in the A and B rings. This will lead to low-intensity charge transfer transitions. Calculated states with very small oscillator strengths are not seen in the spectrum but can be important in energy transfer processes [1,7,13,14,

29,30]. These calculated dark states are shown in Figure 5(bottom) where all calculated transitions with oscillator strengths smaller than 0.03 are shown. In total, there are two of such states in each configuration and they are mostly located in the region between 400 and 450 nm. Some are found in the region between the Q_x and the Soret bands, as obtained before for optimized complexes [5], but they are also found below and above the Soret band. In both free base and Zinc tetrapoly-porphyrins these dark states were experimentally obtained [30] by near-infrared excitations from the Q band and their localizations with respect to the Soret transition are in conformity with our calculations for the *Chl-c₂*.

4. Conclusions

Solvent effects are found to be very important in the location of the absorption bands of photosynthetic pigments being responsible for sizable red shifts in chlorophyll-*c₂* compared to the spectrum in vacuum. The sequential QM/MM method includes the important intermolecular interactions associated to the explicit use of the solvent. Excellent results are then obtained in the entire visible region giving the two Q bands at 608 and 590 nm and the Soret band at 449 nm, in excellent agreement with experiment (633, 586 and 451 nm). The Mg atom was found to prevail as six-coordinated in the protein-free chlorophyll-*c₂* and hydrogen bonds in the carboxylic group were also determined to be important contributor to the spectral shifts. Our results strongly support the presence of low-intensity transitions (dark states) for chlorophyll-*c₂* in liquid methanol. This finding is in agreement with recent experimental data for related photosynthetic chromophores (Zinc tetrapoly-porphyrins) in solution [30] and also with a recent theoretical study suggesting the existence of a number of dark electronic states of bacteriochlorophylls below the main Soret transition [5]. The dark states for chlorophyll-*c₂* in liquid methanol were located in the 400–500 nm range. The importance of taking into account specific solute–solvent interactions for understanding the photophysics of complex species of interest in photosynthesis has been pointed out by several recent works [31–34]. This Letter thus emphasizes the importance of the non-electrostatic interaction with the solvent for understanding the light absorption properties of photosynthetic pigments and thus the initial step of the light reaction in photosynthesis.

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