QM/MM dynamics of a Peridinin model in triplet state in three prototypical solvents

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Abstract

Peridinin (Per) is a carbonyl-containing carotenoid playing a key role in light harvesting and photoprotection in dinoflagellates. This carotenoid plays its photoprotective role by quenching the potentially dangerous ^{3}Chl-*a* triplet state through the formation of the non-reactive ^{3}Per triplet state through Dexter energy transfer mechanism. We have investigated by means of Quantum Mechanics / Molecular Mechanics ab initio molecular dynamics simulations at room temperature the structural and dynamical properties of a Peridinin model system (PMS) in triplet state in three different solvents: cyclohexane, apolar/aprotic; acetonitrile, polar/aprotic; and methanol (MeOH), polar/protic. Our results of ^{3}PMS in MeOH show that the lactonic carbonyl has a stronger tendency to accept hydrogen bonds compared to the corresponding singlet ground state (^{1}PMS). This effect may play some so far overlooked role in Per-containing proteins (notably the water soluble Peridinin-Chlorophyll-Proteins - PCPs).

The vibrational properties of the ^{3}PMS dynamics in the three solvents have been analyzed by means of decomposition of the vibrational density of states in effective normal modes. The results show that the solute-solvent interactions can influence some vibrational bands of ^{3}PMS; in particular, they are able to modulate the position of the lactonic C=O stretching band. The situation is particularly evident in the case of MeOH , where the dynamics of the MeOH----O=C hydrogen bond interactions can strongly influence the band position and shape. As vibrational spectroscopy (notably step-scan FTIR difference spectroscopy) has been largely used to investigate ^{3}Per in PCPs, especially using the lactonic carbonyl stretching as a marker band to investigate the different photophysical role of each Per in the protein complex, this study represents an important step to understand the experimental spectra and to identify the Per(s) molecule(s) bearing the triplet in PCPs.

Keywords: Peridinin; carotenoids; QM/MM calculations; triplet state; hydrogen bonds; solvent effects on vibrational bands

1. Introduction

Peridinin (Per) is a carbonyl-containing carotenoid found in the light-harvesting complexes of dinoflagellates. It is present both in the membrane-bound Chl *a* –c2 Per binding proteins and in the water-soluble Peridinin-Chlorophyll *a* -Proteins (PCPs) [1]. Several PCP complexes have been identified and for some of them the crystal structure is known: each Chlorophyll *a* (Chl *a*) is surrounded by 3 or 4 Per molecules, located in non-equivalent positions [2]. The presence of Per enables light harvesting in the spectral region where Chl a poorly absorbs [3]. The excitation is then transferred to Chl *a* with high efficiency (~90%) via ultrafast energy transfer. Per plays also an important photoprotective role, because it can quickly and efficiently (100%) quench the ^{3}Chl *a* state which could be a potential generator of singlet oxygen [4].

Per has a peculiar molecular structure (see Fig. 1) characterized by a unusual C_{37} skeleton (instead of the usual C_{40} skeleton found in most carotenoids). A butenolide ring and an allene moiety are conjugated with the π system of the polyenic backbone. An ester group is located on one β ring with a tertiary alcoholic group; an epoxy group with a secondary alcohol is located on the opposite β ring.

Due to their relevance in light harvesting and photoprotection in dinoflagellates, and to their use for technological [4, 5] and biomedical [6] applications, in the last two decades PCPs have been the object on an increasing number of spectroscopic studies (see [3,4] for recent reviews). In particular, the role of Per in PCP photophysics (notably in the photoprotective mechanism) has been recently studied by time-resolved infrared (IR) spectroscopies ([7-12], reviewed in [4, 13]). This has prompted a number of experimental and theoretical investigations on Per vibrational properties [8-10; 14-18], in particular on the influence of the surrounding environment on the lactonic C=O stretching band [8-11;15,16,18]. The use of *ab initio* molecular dynamics, also within the Quantum Mechanics / Molecular Mechanics (QM/MM) framework, is recently emerging as a powerful technique for the accurate study of structural and spectroscopical properties of biomolecules in different solvents [19,20]. IR spectra and finite-temperature effective normal modes can be extracted directly from the computed dynamics, providing a precious support to the interpretation of experimental data [21]. In the case of Per in its ground state (hereafter called ^{1}Per), a joint QM/MM and Resonance Raman study in different solvents [15] has made it possible to investigate in details the effect of the dielectric constant of the environment, and of the presence of hydrogen bond (H-bond) donors on the C=O stretching band. This greatly helped the interpretation of IR difference spectra of PCPs (most of which reflects ^{3}Per formation), by leading to tentatively assign several vibrational bands to ^{1}Per [15]. In addition, the QM/MM approach shed light on the role of the dynamics played by solute-solvent interactions [15].

Following a similar approach, in this paper, we have used Density Functional Theory (DFT) and QM/MM calculations to study how the environment can influence the vibrational properties of Per in its triplet state (^{3}Per). We have chosen, as in the previous study [15], a Per model system (PMS), as defined in Fig. 1, and three prototypical solvents: cyclohexane (CHX), apolar; acetonitrile (ACN)[1], polar, aprotic; methanol (MeOH), polar, protic. This approach may result very useful in the interpretation of IR difference spectra [13] reflecting triplet formation in one or more Per in PCPs, especially by making it possible to tentatively assign bands to the lactonic C=O of the different ^{3}Pers. It is worth mentioning that the assignment of band belonging to Pigment triplet states is a major problem in the spectroscopic investigation, by IR difference spectroscopy, of triplet state formation in photosynthetic proteins ([22-27]; see also [13] for a recent review on light harvesting systems). Furthermore, in most cases the pigment bearing the triplet state is characterized by a C=O moiety conjugated to the π system (as it happens for Per), whose stretching band has been used as a probe to study the nature of the triplet state [23-25] and/or the specific pigment-protein interactions [22, 25].

FIGURE 1 HERE

2. Materials and Methods

All DFT calculations were performed in spin-Unrestricted Kohn and Sham (UKS) scheme in local spin density approximation using the Perdew-Burke-Ernzerhof (PBE) functional [28] in the triplet electronic state.

Model Systems. As mentioned in the introduction, the calculations were performed on the Model System PMS shown in Fig. 1 in order reduce the computational cost, similarly to our previous work [15]. This PMS preserves the chemical groups which characterize the photophysical behaviour of Per, and its main vibrational bands appearing in IR difference spectra of PCPs or in Resonance Raman spectra of Per ([15-18] and refs. therein): the carbonyl group on the lactone moiety, the allene group and the polyene chain. The missing two sides (β-ring with ester group on the right side and β -ring with epoxy group on the left side of Per, see Fig. 1) are not significantly affecting the vibrational properties of the polyenic chain or of the lactonic C=O, as previously shown [15].

Gas phase and implicit solvent calculations. Gas phase PMS calculations were performed with the Car-Parrinello molecular dynamics (CPMD; http://www.cpmd.org) program using Troullier-Martins [29] normconserving pseudo-potentials. Kohn-Sham orbitals were expanded using plane waves up to an energy cutoff of 70 Ry. Implicit solvent calculations were obtained using ORCA package [30] with the conductor-like screening model (COSMO) [31] feature for the description of the solvents and a triple zeta valence plus polarization (TZVP) Ahlrichs basis set. For gas phase calculations harmonic frequencies are calculated by normal mode analysis at the optimized geometry.

QM/MM calculations. QM/MM simulations were carried out treating PMS at DFT level and all the solvent molecules at the level of classical force-fields. Details of the QM/MM scheme are described in

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[1] In order to be consistent with our previous work on ^{1}Per [15], we have chosen to use deuterated ACN (ACN-d_3) in our simulations.

Ref. [15]. Energy conserving QM/MM Born-Oppenheimer molecular dynamics is performed following the Hamiltonian scheme described in Ref. [32, 33]. To describe the classical part of the system we used the Amber force field [34, 35], whereas for the QM region was treated using the PBE functional and the same setup as that for plane-wave gas phase calculations.

The three QM/MM systems were built adding to optimized structure of ^{3}PMS several solvent molecules within periodic boundary conditions. In particular the system includes 440 CHX molecules for QM/MM in CHX, 1750 ACN molecules for QM/MM in ACN, and 3742 MeOH molecules for QM/MM in MeOH. Each system was pre-equilibrated using classical force field at constant room pressure and temperature. Finally, constant energy QM/MM production runs were propagated for about 10 ps for CHX and ACN and 17 ps for MeOH solutions.

Vibrational density of state (VDOS) is obtained along dynamics through the Fourier Transform of the velocity autocorrelation function. With the aim to decompose the VDOS into a sum of modes vibrating at the same frequency we have used the effective normal mode approach described in ref. [36], already used to analyse the data on ^{1}PMS dynamics [15]. In such scheme, the VDOS is decomposed in movements which are linear combinations of internal coordinates being highly localised in frequency. These modes are called “effective normal modes” and they are the analogous of the normal modes at finite-temperature.

3. Results and Discussion

3.1. Molecular Orbitals of PMS in Singlet and Triplet state

The triplet spin state promotes one of the two electrons of the singlet HOMO (highest occupied molecular orbital) to the triplet HOMO (see Fig. 2). The triplet HOMO-1 is indeed similar to the singlet HOMO and does not apparently produce large changes in the electronic structure. The triplet HOMO has surely a significant effect on the electron distribution. This orbital affects the central part of the polyenic chain, shifting by one unit the bond length alternation order of the C-C polyenic chain. As a result, there is a general weakening of double bonds between the carbon atoms and a strengthening of the single bonds, as it will be described in the next section. This effect is also clear from the spin density (Fig. 2D) which is basically reflecting the difference between the localization of the triplet HOMO and the singlet HOMO. The picture shows that the triplet is delocalized on several nuclei, and in particular in the center of the molecule, in which mainly two bonds are involved (the bond 7 and bond 9; reference is made to the numbering scheme in Fig. 1).

FIGURE 2 HERE

3.2.Geometrical description of ^{3}PMS in the three solvents

The bond length alternation, namely the average difference between the lengths of single bonds and that of double bonds, is an important geometrical parameter for polyenes, modulating its electronic properties in the ground and excited states. Different DFT functionals provide also rather different geometrical parameters, if compared with high-level correlated methods like Quantum Monte Carlo, as recently shown for the case of Per [37, 38].

In Table 1 the calculated bond lengths for polyene chain of ^{3}PMS are listed both for DFT calculations and along dynamics. The results from both techniques show that the electronic reorganization passing from the singlet state to the triplet greatly affects the geometrical properties of the polyene skeleton group of PMS.

TABLE 1 HERE

In Fig. 3 the average length of the C-C bonds for ^{3}PMS in different solvents is shown, and compared to the same parameters for ^{1}PMS. The first evidence is the poor influence of the solvent, as already noticed for the singlet state [15]. Secondly, the regular pattern showing alternation of single and double bonds found in ^{1}PMS, is lacking in ^{3}PMS. In fact, in the triplet state the polyenic chain is divided into two parts, each one composed by a conjugated state extending over four bonds (bonds 3-4-5-6 and 10-11-12-13, see Fig. 3).

FIGURE 3 HERE

In order to investigate the flexibility of ^{3}PMS, in Fig. 4 the average of the dihedrals angles for ^{3}PMS in the three solvents are plotted. Here differences can be observed in the three solvents. The saw shape in the angle values going from a C atom to the following one, which ensures, in polyenes in their ground state, an almost planar configuration (see for instance the dihedral angles of ^{1}PMS in the supplementary information of ref. [15]) is here somehow altered. In fact for ^{3}PMS there is always an "irregular point" that interrupts the regular saw shape in the polyene chain (in Fig. 4 bond n. 9 in CHX; bond n. 8 in ACN; bond n. 6 – close to the lactonic ring – for MeOH).

FIGURE 4 HERE

3.3.Solute-solvent interactions

As we did previously for ^{1}PMS [15], the dynamics of specific solute-solvent interactions can be studied in details. A very interesting situation is found for ^{3}PMS in MeOH. In this case, looking at the radial pair distribution function g(r) between the carbonyl oxygen and the MeOH molecules, it is possible to assess the number of H-bonds involving the C=O moiety.

We used as definition for H-bonding an O-H distance shorter than 2.3 Å, and an angle O (acceptor)–H–O(donor) less than 30°. In a previous paper, it has already been shown that the lactonic C=O of ^{1}PMS can accept up to two hydrogen bonds from solvent molecules [15]. For the lactonic C=O of ^{3}PMS the H-bond basicity (i.e. the tendency to accept H-bonds) of the carbonyl oxygen is even more pronounced, as in some cases it can accept up to 3 H-bonds from MeOH molecules (see the lowest trace of Fig. 5a, especially between 10 and 15 ps). Fig. 5a shows also the formation/disruption of H-bonds involving the C=O as an acceptor and each of four MeOH molecules (named A,B,C,D) as donors during the dynamics. Compared to previous results for ^{1}PMS [15], it appears that the triplet state can increase the tendency of the lactonic C=O to accept H-bonds. This may have some consequences after the formation of ^{3}Per in protein environments (e.g. inside PCPs). Fig. 5b shows the corresponding number of hydrogen bonds between the MeOH molecules A, B, C (and D) involved in the C=O----H-O H-bond interactions. (N.B. The MeOH A, B, C molecules act as hydrogen bond donors towards C=O, but as acceptors – using the two lone pairs of the oxygen atom – when interacting with other MeOH molecules).

FIGURE 5 (A & B) HERE

3.4.Vibrational analysis

Polyenic vibrations. Fig. 6 shows the VDOS of ^{3}PMS extracted from QM/MM molecular dynamics in the three solvents, superposed to the VDOS obtained from ^{1}PMS QM molecular dynamics [15]. Each panel of the figure shows the results associated to a different solvent, in the range 1400 to 2000 cm^{-1}. The stretching frequencies of the high-frequency polyenic modes in this spectral region (1400-1600 cm^{-1}) is also obtained by the VDOS effective normal mode decomposition and reported in Table 2. These seven frequencies correspond to the combination of the frequencies of the seven double bonds of the polyenic chain of the molecule.

The interpretation of the polyenic modes is not easy because of the conjugation delocalizes π electrons over several bonds along the carbon chain. Effective normal mode analysis is able to decompose every peak into a combination of two or more C=C stretching. In Table 2 the corresponding frequencies are also compared with those obtained from normal mode analysis in gas phase. Differently from what observed in our previous the QM/MM dynamics of ^{1}PMS, the solvent has an influence on the position of several vibrational modes of ^{3}PMS (this is particularly evident for the modes Pol-1, Pol-2, Pol-3, and Pol-6, see Table 2). Interestingly, very different solvent effects on ^{3}PMS are predicted by DFT and QM/MM: for instance, in the case of Pol-3, QM/MM calculations foresee a strong downshift when passing from CHX to ACN (25 cm^{-1}) whereas the calculated downshift using DFT (with implicit solvent) is very small (3 cm^{-1}). An opposite effect (when comparing the same two solvents) is found for Pol-6: QM/MM calculations foresee a 17 cm^{-1} upshift when passing from CHX to ACN, whereas in DFT calculations there is a small downshift (4 cm^{-1}). The reasons for these differences cannot be easily rationalised, but they are most probably related to the differences between the C=C vibrational modes, as already noticed by DFT analysis of the whole Per in the triplet state [9]. We underline however that there is no clear indication in the published experimental IR data on ^{3}Per for a solvent – or environmental – effect on the position of C=C bands.

FIGURE 6 HERE

TABLE 2 HERE

Lactonic C=O stretching and role of solvation dynamics. The most important part of the analysis concerns the lactonic C=O mode. As experimentally observed [7,8,10] and suggested by DFT calculations [8,9] this vibrational frequency strongly downshifts upon triplet formation. As previously reported for ^{1}PMS [15], a quite pronounced solvent effect on the frequency of the lactonic C=O stretching band is also observed in the present calculations on ^{3}PMS.

The most interesting situation is found in the case of PMS in MeOH. For ^{1}PMS, the formation of H-bonds involving the lactonic C=O as an acceptor is a key interaction in determining the position of the C=O stretching band [8, 9, 15]. Furthermore, the investigation of ^{1}Per solution in MeOH (both by Resonance Raman spectroscopy and QM/MM simulations) has pinpointed that the solvation dynamics plays a key role in determining not only the position, but also the shape of the C=O stretching band [15]. For this reason, in the analysis of the ^{3}PMS dynamics we have paid particular attention to the processes of formation/disruption of the H-bonds and to their effect on the position (and shape) of the C=O stretching band in the VDOS analysis.

To this aim, as already mentioned, we have identified 4 MeOH molecules forming, at least once, a H-bond with the O atom of the lactonic C=O of ^{3}PMS during the whole dynamics. These molecules have been named A, B, C, D. The time evolution in the formation and disruption of the H-bond between each of these molecules and the C=O is shown in Fig 5b. The D molecule has a marginal role, as it forms an H-bond only during some steps within a single 0.5 ps interval. Conversely, the A molecule has a predominant role, because it forms a stable H-bond since the beginning of the simulation, with an effective disruption of the bond (the molecule goes away from the neighbourhood of the C=O) only towards the end of the simulation (15.2 ps). Two significant interruptions of the H-bond can also be observed around 13.4 and 13.8 ps. The B molecule seems to be weakly H-bonded during the first 5 ps, but afterwards for most of the time the H-bond interaction is present (in some intervals, e.g. between 7 and 8.7 ps, the H-bond is very stable). The C molecule does not form any H-bond for the first half of the dynamics; then it establishes an almost stable H-bond with the C=O.

It should however be taken into account that a MeOH molecule can also accept H-bonds since the oxygen has two lone pairs, and this makes possible the formation of H-bond networks around the C=O of ^{3}PMS, involving both solvent----O=C and solvent-solvent hydrogen bond interactions. We can expect that the formation of solvent-solvent H-bonds between the MeOH molecules A,B,C,D may have a (slight) effect on the strength of the solvent-C=O interactions, and therefore also on the shape and position of the C=O stretching band. In Fig. 5b the number of H-bonds involving (as acceptor) the oxygen atom of the MeOH molecules A, B, C, D is shown. The oxygen of the D molecule never "receives" an H-bond. Conversely, the A, B, C molecules are deeply involved in mutual solvent-solvent interactions, each one acting at least once as donors towards the other two.

To further investigate the role of H-bonding on C=O vibrations, we have divided the dynamics of ^{3}PMS in MeOH into 5 parts, each characterized by a peculiar H-bond network around the C=O (see Fig. 7).

FIGURE 7 HERE

We have then calculated the VDOS for the 5 sections of the dynamics, as well as for the three subsection of section n. 4 (see three drawings on the bottom of Fig. 7). The results are shown in Fig. 8 and in Table 3.

It is now possible, by looking simultaneously at Fig. 7 and Fig. 8 and at Table 3, to establish some clear links between peculiar MeOH-----O=C interaction configurations and band position and shape. Part 1 (0 - 5.2 ps) is characterized by a stable H-bond (with MeOH-A) with a second – unstable – H bond (with MeOH-B). This behaviour is reflected by the presence of two peaks at 1690 and 1676 cm^{-1}, corresponding to the two situations (one H-bond and two H-bonds, respectively). An interesting point is the quite low intensity of the peaks. Part 2 (5.2 – 7.8 ps) shows a situation with two stable H-bonds involving the C=O (with a very short period characterized by 3 H-bonds, due to the sudden, short and unstable formation of the hydrogen bond between the C=O and MeOH D). This is consistent with the observed peak at 1670 cm^{-1} (due to the two H-bond situation) with high intensity. A weaker band is observed at 1625 cm^{-1}, most probably reflecting the transient formation of 3 H-bonds (due to the additional formation of a short H-bond between MeOH-D and the C=O, taking place between 6.2 and 6.6 ps). Part 3 (7.8 – 10.2 ps) shows a predominance of a 3 H-bond situation for the C=O, even though quite often only 2 H-bonds are observed (due to brief separations of MeOH-B or MeOH-C). This could explain the presence of a peak at 1625 cm^{-1} (3 H-bond situation) and a broader one at 1660 cm^{-1}

(the broadness and the position of this band are probably related to the instability of one of the two H-bonds and possibly also due to the peculiar network of H-bonds involving also the solvent-solvent H-bond between MeOH-A and MeOH-C). As observed in part 1, the intensity is quite low; this may be due to the presence of several H-bonds network conformations alternating, and as a consequence the intensity of the vibrational mode of the C=O is distributed on several frequencies. Part 4 (10.2 – 12.2 ps) is characterized by 3 H-bonds (all of them quite stables, as they are present for the majority of the time interval) and this may account for the presence of a single, quite intense and narrow peak at 1632 cm^{-1}. It is interesting to analyse in detail the three subsections of the time interval: the first one, Part 4a (10.2 – 12.2 ps) is characterized by a "weakened" 3-H bond character (and in fact in the corresponding calculated VDOS spectrum, beside a 1635 cm^{-1} shoulder, a 1650 cm^{-1} peak appears representative of a 2 H-bond situation. In the other two subsections (Part 4b, 12.2 – 13.4 ps; and Part 4c, 13.4 – 15.2 ps) the 3 H bond situation is more defined, and this is reflected by the presence of sharp peaks at 1640 cm^{-1} (second subsection) and at 1630 cm^{-1} (third subsection).

Part 5 (15.2-16.6 ps) is characterized by two stable H-bonds, which explains the presence of an intense peak at 1655 cm^{-1}. However, two weak shoulders can be observed at 1632 and 1672 cm^{-1}.

It should be underlined that the interpretation described above relies on a very simple assumption (H-bond yes/no, with cut-off distance at 2.3 Å and the requirement of the O----H----O angle to be smaller than 30°). We did not mention the strength of the interaction or other factors such as the value of the O----H----O angle. Nevertheless, an approximate "scale" can be established: the calculated C=O frequency for the lactonic C=O involved in one H-bond is around 1690 cm^{-1}; for the two H-bond scenario, the frequency is around 1670 cm^{-1}; for three H-bonds, the frequency is around 1630 cm^{-1}. Between 2 and 3 H bonds, however, several intermediate situations can be found, resulting in the presence of other peaks, in shifts of the peak position and in broadening of the corresponding bands. In Fig. 6 the VDOS for the lactonic C=O of ^{3}PMS in MeOH (whole dynamics) is shown.

Allene. The allene asymmetric stretching of PMS has a frequency red-shift from singlet to triplet state that indicates a slight weakening of the C=C bonds. The shift seems to be unaffected by the environment. We refrained to examine more in details this vibrational mode, as the lack of the β ring adjacent to the allene moiety is supposed to induce a quite strong difference between the properties of the allene asymmetric stretching band of ^{3}PMS and ^{3}Per.

FIGURE 8 (A & B) HERE

TABLE 3 HERE

3.5 Comparison with experimental data

Solvents. In a previous work [15], we have used a joint experimental/computational approach in order to determine in details how the microenvironment surrounding Per in its ground state can affect the vibrational properties of Per. In the present case, the formation of the (short-lived) ^{3}Per needs the use of a sensitizer, as in the photophysics of isolated Per the triplet state is formed in negligible yield. So far, to our knowledge no IR spectra of ^{3}Per have been reported in the three solvents studied here, most probably because of technical difficulties (insufficient solubility of Per and/or of the sensitizer, triplet lifetime, parallel photochemical reactions, etc). . The only reported ^{3}Per IR spectrum (actually, a ^{3}Per/Per FTIR difference spectrum obtained with the step-scan FTIR technique coupled to a chemometrical treatment of data using Chl-*a* as a sensitizer) was obtained in tetrahydrofuran (THF) by Bonetti and co-workers [10]. Looking at THF dielectric constant (ε = 7,58), this solvent can be considered as a "half-way situation" between the apolar cyclohexane (ε = 2,02) and the polar – but aprotic – acetonitrile (ε = 37,5). However, a direct and precise comparison between experimental and calculated absolute values for the carbonyl stretching frequency of the lactonic C=O of ^{3}Per is difficult, due to intrinsic limitations of the DFT and QM/MM analysis in the calculations of absolute vibrational frequencies. Instead, the frequency shift Δ (C=O) when passing from ^{1}Per to ^{3}Per is more robust. From Table 4 it can be seen that the calculated Δ (C=O) using DFT on PMS is roughly the same in the three considered solvents (and in the gas-phase), showing that the dielectric constant of the environment does not influence it. It should be noticed that there is an almost perfect agreement with the experimental value for the experimental Δ (C=O) for Per in THF.

The Δ(C=O) values calculated from QM/MM (where the solute-solvent interaction dynamics is taken into account) are conversely lower (21-22 cm^{-1}) in CHX and ACN, whereas a precise calculation is not possible – and not meaningful – for MeOH, due to the important role played by the formation/disruption (on the sub-ps scale; we recall that the lifetime of ^{3}Per is on the microsecond scale) of MeOH----O=C H-bonds. It should be also recalled that QM/MM data show an increased tendency of the lactonic C=O of ^{3}Per, compared to ^{1}Per, in accepting hydrogen bonds from MeOH solvent molecules.

Furthermore, it should also mentioned that, for ^{1}Per in aprotic solvents, a Fermi resonance interaction between the C=O stretching and an underlying vibration (most likely a C-H bending) takes place [16]. This phenomenon is not taken into account by our QM/MM simulations. However, the shape of the lactonic C=O stretching band attributed to ^{3}Per in THF [10] seems to suggest that a Fermi resonance effect could also take place in ^{3}Per.

Implications for PCP Proteins. It is also useful to compare the results concerning ^{3}Per vibrations reported in different PCPs using step-scan FTIR difference spectroscopy [7-10; 12]. Table 4 compares the shifts induced in the main vibrations of Per – compared to the resting singlet state - upon triplet formation. It should be noticed that in most cases band assignment for ^{3}Per in PCPs is still to be considered as tentative; furthermore, the number and identity of the involved Per and the values of their lactonic C=O stretching bands (both in the ground and triplet state) are still debated (see [4, 13]). It is interesting to note however, from the values in Table 4, that the reported shift of the C=O frequency upon triplet formation seems to depend on the studied PCP. This may be due to different conformations or different interactions with the environment of the involved Per, and/or to other processes such as triplet delocalization on more than one pigment, and/or possibly to the increased H-bond acceptor basicity of the carbonyl of ^{3}Per suggested by our QM/MM calculations for ^{3}PMS in MeOH. A final remark on the C=O of ^{3}Per must be done. According to the present QM/MM calculations on ^{3}PMS, and to their comparison to previous QM/MM results on ^{1}PMS, a strong downshift (> 60 cm^{-1}) of the lactonic C=O stretching mode can in principle take place upon the ^{1}Per → ^{3}Per transition for a Per molecule with its lactonic C=O moiety lying in a protic environment (because of the increased tendency of C=O to accept H-bonds in the triplet state, and because of the strong sensitivity of the C=O stretching bands to the presence of H-bonds involving the carbonyl oxygen). It is therefore in principle possible that, in step-scan FTIR difference spectra on PCP, some positive bands due to the C=O stretching of one (or more) ^{3}Per has not been identified (or proposed for possible identification) because they lay in an "unusual" (and too downshifted, compared to the position of the corresponding lactonic C=O stretching of ^{1}Per) spectral region (in numerical terms, below 1700 cm^{-1}). This hypothesis is quite unlikely – in most cases the identification of twin ^{1}Per C=O (negative) and ^{3}Per C=O (positive) stretching bands in step-scan FTIR difference spectra is intuitive, looking at the existing literature – but cannot be completely ruled out.

In a previous paper [9] some IR bands due to the polyenic chain of ^{3}Per were identified in the step-scan FTIR difference spectrum obtained on A-PCP (PCP from *Amphidinium carterae*) at low temperature, using DFT calculations (with appropriate correction factor) on whole Per. On the other hand, the present QM/MM calculations on ^{3}PMS (see Table 1) strongly suggest that some of the vibrational bands of ^{3}Per due to the polyenic chain should be sensitive to the surrounding environment. If this holds true, in principle the exact position of such ^{3}Per bands in PCP proteins can be used to distinguish among spectral contribution arising from different Pers, each lying in a peculiar proteic environment. More detailed step-scan FTIR experiments (with increased signal-to-noise ratio, compared to the published works [7-10; 12]) are needed in order to clarify this point.

Finally, more broadly speaking, the present work suggests that QM/MM simulations of ^{3}Per (and ^{1}Per) in their binding sites in PCPs could be particularly useful to identify and assign vibrational bands of ^{3}Per (or ^{1}Per) in the IR difference spectra reflecting the process of triplet formation in PCPs.

TABLE 4 HERE

4. Conclusions

In summary, our DFT and QM/MM studies of ^{3}PMS in the three prototypical solvents (CHX, ACN, MeOH) show that:

1) The ^{1}PMS → ^{3}PMS transition strongly modifies the electronic configuration of the molecule and, as a consequence, deeply affects its vibrational spectrum. The same effect takes place also in Per, as confirmed by experimental IR difference spectra [7-10; 12];
2) In ^{3}PMS, differently from ^{1}PMS, the localization of the effective normal modes of the polyenic chain is more difficult but some of them show higher sensitivity to the environment. This makes them good candidates as marker bands in the identification, in PCPs, of Per molecules where the triplet is localized;
3) The lactonic C=O stretching band of ^{3}PMS, as observed previously for ^{1}PMS [15], is strongly sensitive to the polarity of the environment and to the formation of H-bonds involving the carbonyl;
4) The lactonic C=O of ^{3}PMS has an increased capacity of accepting hydrogen bonds compared to ^{1}PMS. This may have some unknown consequences in the photophysics of ^{3}Per in PCPs, and in the spectral position of the C=O band of ^{3}Per in PCPs;
5) As observed for ^{1}PMS [15], QM/MM dynamics can rationalize, in term of specific C=O---- solvent H-bonds, the shape and position of the lactonic C=O stretching band.

This work also represents a new approach to investigate how the vibrational properties of pigments in their triplet state are affected by the surrounding environment, and therefore is a new tool to help the assignment of pigment triplet bands in IR difference spectra. Beside PCPs, IR difference spectroscopy has been applied to investigate triplet formation in many other photosynthetic proteins [22-27], and in most cases (like for Per) the most interesting band was an environment-sensitive C=O stretching vibrational mode. From a methodological point of view, this work demonstrates the relevance of theoretical approaches to investigate the properties of triplet states (including their sensitivity to environmental effects) for a better interpretation of spectroscopic data (see for instance [43]). QM/MM studies of pigment triplet states in prototypical solvents may result very useful in order to better understand all the factors governing the relationship between band position, and environment properties (both structural and dynamics).

More broadly speaking, QM/MM calculations on prototypical environments, along with QM/MM calculations on protein binding sites [39] and with innovative chemometrical strategies for spectral data treatment ([40-42] and references therein) represents new software tools for the assignment of bands in IR difference spectra on photosynthetic proteins and on other biological systems.

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Captions of the figures

Fig. 1. Structural formula of Peridinin (Per) and of Peridinin model system (PMS) used for calculations. The bond order shown in the figure follows the one of the singlet state.

Fig. 2. PMS orbitals. In all the panels the red is associated to positive values and blue to negative one. From top to bottom:

(A) Singlet HOMO from RKS-DFT calculation (doubly occupied orbital).

(B) Triplet HOMO-1 of alpha electron from UKS-DFT calculation.

(C) Triplet HOMO of alpha electron from UKS-DFT calculation.

(D)Triplet spin density.

Fig. 3. Average Length of the bonds C-C in the polyenic chain in triplet ^{3}PMS (upper trace) and singlet ^{1}PMS ground state (lower trace). Vertical lines are standard deviations.

Fig. 4. Average Dihedral angles of polyenic chain in ^{3}PMS. The dihedral angle corresponds to the bond where the torsion acts. See text for further details

Fig. 5 (A) H-bond formation/disruption between the C=O of ^{3}PMS (acceptor) and the four closest MeOH molecules (donors) counts between ^{3}PMS and four closest MeOH molecules along the QM/MM dynamics. In the upper four traces, the formation of H-bond between each of four MeOH solvent molecules (acting as donors and named A,B,C,D) and the C=O of ^{3}PMS (acting as acceptor) is shown. In the lower trace, the overall number of H-bonds involving the C=O of ^{3}PMS is shown. (B) H-bond formation/disruption between the MeOH molecules A, B, C, D of Fig. 5(A) (acting as acceptor through the two lone pairs of their oxygen atoms) and the OH groups of other MeOH molecules. A more detailed pictorial description can be found in Fig. 7.

Fig. 6. VDOS spectra of ^{1}PMS and ^{3}PMS in different solvents. From top to bottom: CHX, ACN, MeOH.

Fig. 7. Pictorial representation of representative interactions involving the lactonic C=O taking place during the dynamics of ^{3}PMS in MeOH. A continuous line indicates a stable (or almost stable) H-bond; a dashed line indicates an instable H-bond (broken and re-formed several times on a very short time-scale (<<1ps).

Fig. 8. VDOS from ^{3}PMS trajectory divided in the 5 parts described in Fig. 7 (upper five graphs). In the bottom graphs, the three sub-parts of the fourth time interval (as indicated in Fig. 7) are shown. Peaks at 1600-1610 cm^{-1} are given by the polyenic chain.