The structure of chlorophyll a–water complexes: insights from quantum chemistry calculations†

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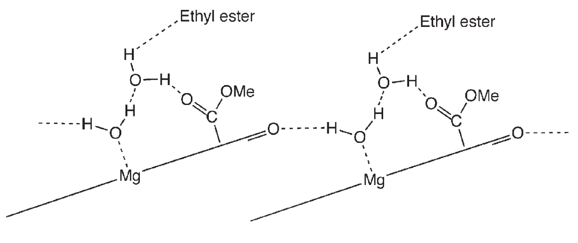
Computations show that chlorophyll a is able to coordinate a maximum of two water molecules in hydrophobic media that form a bridge between the Mg atom and the methyl ester carbonyl group.

Photosynthesis involves two reaction centres (PSII and PSI) that associate special pairs of chlorophyll a (Chl.a), P680 and P700, respectively. Though crystallographic data for several PSI[1] and PSII[2] systems are available, definitive conclusions on structure and reaction mechanisms deserve further investigation. Four models have traditionally been employed for Chl.a pairs based on works by Katz and Norris,[3] Fong,[4] Chow *et al.*[5] and Shipman *et al.*[6] In all these models, interactions between Chl.a and water molecules play an important role[7] with the two Chl.a molecules being cross-linked by one or two bridging water molecules. The crystallographic data reported by Chow *et al.*[5] concerned a dihydrate of the ethyl chlorophyllide a, obtained from an acetone–water mixture, and represented the first detailed structure of a chlorophyll derivative. The Mg atom was shown to be coordinated to a single water molecule. The second water molecule is hydrogen bonded to the first water molecule, to the methyl ester carbonyl oxygen atom of one chlorophyllide molecule and to the carbonyl oxygen atom of the ethyl ester of the other chlorophyllide molecule (Scheme 1). Subsequent investigations have either supported these models[8] or proposed other arrangements for Chl.a pairs.[9]

Fundamental advances in the understanding of Chl.a association processes have also come from solvation studies carried out in aqueous mixtures with solvents of variable polarity. In a pioneer work, Ballschmiter and Katz[10] showed that in aqueous CCl_4, benzene and aliphatic hydrocarbons media, Chl.a–H_2O and 2Chl.a–H_2O species are formed at low Chl.a concentration. In aliphatic hydrocarbons with increasing Chl.a concentrations, micelles are obtained. The authors suggested that Chl.a–$2H_2O$ complexes could also be formed for larger water concentrations. Fujiwara and Tasumi[11] reported Chl.a–solvent complexes with Mg exhibiting 5- or 6-coordination, depending on media. More recently, Agostiano *et al.*[12] published data for Chl.a hydration and aggregation in binary mixtures of water with acetone, dimethylformamide and acetonitrile showing that Chl.a–$2H_2O$ monomers or dimers are formed for sufficiently high water mole fractions. The same group[13] investigated the aggregation behaviour of Chl.a in different aqueous organic solvents paying special attention to the pigment response in the water rich region. Vladkova[14] has reported a detailed study on Chl.a self-assembly in polar solvent–water mixtures. This author has shown that in some solvents (methanol, ethanol, acetone, acetonitrile), water can replace the coordinated solvent molecules leading to the formation of polymeric aggregates (Chl.a–$2H_2O$)$_n$. In other media (THF, pyridine), this is not possible and Chl.a is 6-coordinated with two solvent molecules.



Scheme 1 Hydrogen-bond pattern in the X-ray structure of the ethyl chlorophyllide a dihydrate.[5]

Theoretical studies on Chl.a structure have also been carried out (for a review see ref 15). For instance, in a very recent paper, Ryde and co-workers[16] discussed the role of axial ligands on the structure, reduction potentials and absorption spectra of model chlorophylls. They also examined the preference of 5- *vs.* 6-coordination of the Mg atom in the case of histidine showing that 6-coordination is especially unfavourable in polar media. Calculations for chlorophyll–water complexes have deserved some attention[16–18] but no systematic investigation on hydration phenomena has been reported yet in spite of the biological implications connected to it. Fundamental questions closely related to the structure of reaction centres *in vivo* and to the aggregation of Chl.a molecules in solution are: how many water molecules can be coordinated to Chl.a?, what is the most stable structure of the hydrates and the coordination number of Mg?, what is the role of interactions with the medium?, and what is the precise role of the ester lateral chain in the stabilisation of hydrates?.

In the present study, quantum chemical calculations have been carried out with the Gaussian 03 program[19] at the density functional level of theory (B3LYP/6-31G*), which has proved successful in describing related systems.[18,20–23] Calculations were based on two Chl.a models (Scheme 2). In model Chl.a-46, all groups attached to the main macrocycle, except the vinyl one, are replaced by hydrogen atoms. In model Chl.a-73, all groups are

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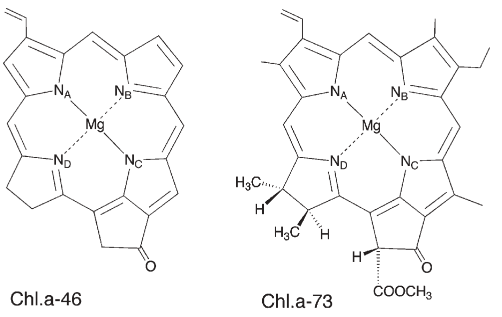

Scheme 2 Chlorophyll a models considered in this work.

included except the phytyl ester side chain, which is replaced by a methyl group. Comparison of results obtained with these two models will allow analysing the role of the methyl ester group that, according to the crystallographic structure in Scheme 1, is expected to contribute to Chl.a hydrates stabilization. We focus on hydrates in which at least one water molecule directly interacts with the Mg atom. Structures in which water molecules are hydrogen-bonded to Chl.a oxygen atoms but not to the Mg atom are expected to be less stable; this was confirmed in exploratory calculations. Several conformations of the hydrates have been envisaged but we only report here the most stable one for each coordination type: monohydrate (1w), dihydrate with 5-coordination (2w) and dihydrate with 6-coordination (2w′). Optimized structures in gas phase are shown in Fig. 1 (cartesian coordinates and some interatomic distances are available as supplementary material†).

Before considering the stability of the hydrates, a few general comments can be made on their structure. First, in the most stable structure of the 1w monohydrate in the Chl.a-73 model, the water molecule coordinated to the Mg atom is *syn*-periplanar with respect to the ester group. Second, both 2w structures exhibit Mg–water dimer interactions but in Chl.a-73, the outer water molecule interacts also with the carbonyl ester group. This generates a cooperative hydrogen-bond pattern comparable to that observed in the crystallographic structure of the ethyl chlorophyllide a dihydrate,[5] as noted above. Moreover, computed interatomic distances are similar to experimental ones. Thus, the predicted Mg···O distance is 2.087 Å, the average Mg–N distance is 2.107 Å and the Mg distance to the $N_AN_BN_C$ plane is 0.406 Å. The corresponding X-ray values[5] are 2.035 Å, 2.086 Å and 0.385 Å, respectively, not far from our results. It is interesting to note also that the calculations predict a large change of these parameters in going from the monohydrate 1w to the dihydrate 2w (values for 1w in Chl.a-73 are 2.152 Å, 2.081 Å and 0.275 Å, respectively), which confirms the remarkable cooperative character of the interactions in the dihydrate. Finally, a remark can be made on the relative orientation of the water molecules in the most stable conformations of the diaxial 2w′ complexes. Chemical intuition would suggest an anti-parallel orientation, favoured by dipole moment interactions. Structures in Fig. 1 show this is not the case (especially in Ch.a-46). The reason seems to be related to various negative charges carried by N atoms as a consequence of the asymmetry of the porphyrin ring (Mulliken charges are −0.741, −0.699, −0.747 and −0.639 for N_A, N_B, N_C and N_D, respectively in Chl.a-46).

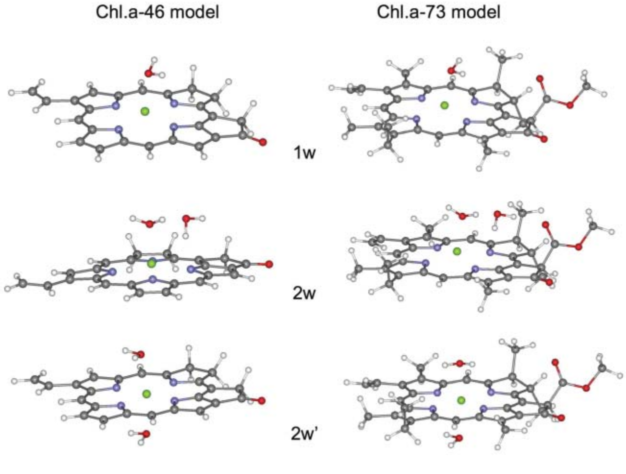

Fig. 1 Optimized structures of hydrated Chl.a models.

Table 1 summarizes the computed thermodynamic data in gas phase and cyclohexane at $T = 298$ K for the following hydration processes:

$$H_2O + \text{Chl.a} \rightarrow \text{Chl.a-1w}\ \mathbf{1}$$

$$H_2O + \text{Chl.a-1w} \rightarrow \text{Chl.a-2w (5-coordinated)}\ \mathbf{2a}$$

$$H_2O + \text{Chl.a-1w} \rightarrow \text{Chl.a-2w}' \text{ (6-coordinated, diaxial)}\ \mathbf{2b}$$

Gas-phase calculations are reported for both Chl.a-46 and Chl.a-73. Computations in cyclohexane are reported for Chl.a-73 only using optimized geometries in gas phase and the polarisable continuum approach[24] ($\varepsilon = 2.023$).

Let us first comment the energetics for Chl.a-46 in gas phase. The three hydration processes (**1**, **2a**, **2b**) are exothermic and exergonic, in spite of a significant entropy decrease. ΔH and ΔG are significantly larger (in absolute value) for first water coordination (process **1**). Comparison of **2a** and **2b** shows that the former is a little more favourable. In other words, the 5-coordinated dihydrate (2w) is more stable than the 6-coordinated one (2w′), the energy differences being $H^{2w'} - H^{2w} \sim 3$ kcal mol^{-1} and $G^{2w'} - G^{2w} \sim 1$ kcal mol^{-1}. Since there are no Chl.a ligands able to interact with water molecules in Chl.a-46, energy differences must be ascribed to intrinsic properties of the Mg atom in the porphyrin environment, namely to its weak capacity to

Table 1 Energetics (kcal mol^{-1}) for studied hydration processes in gas phase and in cyclohexane for Chl.a-46 and Chl.a-73 models at $T = 298$ K

Process	ΔE	ΔH	$-T\Delta S$	ΔG
Chl.a-46 model, gas phase				
1	−18.1	−16.0	7.1	−9.0
2a	−16.6	−14.7	10.5	−4.2
2b	−13.0	−11.7	8.6	−3.1
Chl.a-73 model, gas phase				
1	−18.0	−16.6	8.5	−8.1
2a	−22.8	−20.8	11.9	−9.0
2b	−12.9	−11.4	10.1	−1.4
Chl.a-73 model, cyclohexane				
1	−13.1	−11.7	8.5	−3.2
2a	−19.1	−17.1	11.9	−5.3
2b	−9.6	−8.2	10.1	1.8

form 6-coordinated complexes, as also found with histidine ligands.[16] However, a free energy difference around 1 kcal mol^{-1} cannot explain why diaxial hydrates of Chl.a are not observed in mixture aqueous solution whereas diaxial coordination with some nucleophilic solvents do exist. Other factors have therefore to be invoked.

Comparison of gas phase results for Chl.a-46 and Chl.a-73 models provides the explanation. Thus, though energetics of processes **1** and **2b** depend moderately on which Chl.a model is employed, process **2a** is substantially favoured in Chl.a-73. As a consequence, it is both more exothermic and more exergonic than either **1** or **2b**. The larger stability of 2w with respect to 2w′ is significantly enhanced in Chl.a-73, so that $H^{2w'} - H^{2w} \sim 9$ kcal mol^{-1} and $G^{2w'} - G^{2w} \sim 8$ kcal mol^{-1} now. As mentioned, the ester C=O group and the cooperativity between Mg···O and hydrogen-bond interactions play a key role on this remarkable stabilization.

The effect of an apolar solvent is illustrated by calculations in cyclohexane (Table 1). Clearly, solvation opposes complex formation, as all processes become less exothermic. The main consequence is that process **2b** is predicted to be endergonic in cyclohexane and accordingly, diaxial dihydrates 2w′ should hardly be present in apolar media, in conformity with experimental observations. Analysis of the calculations (supplementary material†) shows that solvent effects are mainly related to the electrostatic component of the solvation energy. The hydrate destabilisation effect of the solvent should be enhanced with the dielectric contact so that one can predict that in sufficiently polar media, no hydrates will be formed. Indeed, calculations for **1** in THF ($\varepsilon = 7.5$) and acetone ($\varepsilon = 20.7$) predict slightly endergonic processes ($\Delta G = 0.5$ and 1.5 kcal mol^{-1}, respectively). Obviously, comparison with experimental data will require taking into account possible solvent coordination with Chl.a. This will be examined in detail elsewhere.

Considering the previous results, one might wonder whether coordination to a third water molecule would be a feasible process or not. We have carried out calculations for several Chl.a-73 trihydrated complexes (B3LYP/6-31G*//B3LYP/3-21G level in this case). As before, 5- or 6-coordination for the Mg atom has been considered. Optimized structures exhibit a stabilisation enthalpy with respect to 2w + H_2O of 7–10 kcal mol^{-1} in gas phase and 4–5 kcal mol^{-1} in cyclohexane, well below the computed enthalpy for second water coordination. Free energies for the third coordination are positive in cyclohexane suggesting that complexes of Chl.a with more than two water molecules should not be formed in hydrophobic solvents.

Due to limitations of the computational level and to basis set superposition errors, our hydration energies cannot be considered as quantitative. Nevertheless, error compensation is expected to be large when comparing different processes or when comparing gas phase and solution results. Thus, main conclusions of this study may be summarized as follows: (1) the 2w dihydrate is significantly more stable than the monohydrate, (2) the structure of the dihydrate corresponds to 5-coordination for the Mg atom, (3) formation of hydrates with a larger number of water molecules is very unlikely, (4) diaxial coordination of the Mg atom with water is plausible for isolated systems but becomes unlikely in solution, even in hydrophobic solvents, (5) dielectric solvent effects opposes complex formation. Calculations are consistent with reported experimental facts and emphasize the role of water interactions with the methyl ester group.

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