

Theoretical Study of Chlorophyll *a* Hydrates Formation in Aqueous Organic Solvents

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A theoretical analysis of chlorophyll *a* (Chl*a*) hydration processes in aqueous organic solvents has been carried out by means of quantum chemistry calculations. A detailed knowledge of the thermodynamics of these processes is fundamental in order to better understand the organization of chlorophyll molecules *in vivo*, specifically the structure of chlorophyll pairs in photosystems I and II. In the present work, we assumed a Chl*a* model in which the phytyl chain is replaced by a methyl group. Calculations were performed at the B3LYP/6-31G(d) level corrected for basis set superposition errors and dispersion interaction energy. This computational scheme was previously shown to provide data close to MP2/6-311++(2d,2p) results. Solvents effects were taken into account using either continuum (for nonpolar solvents) or discrete-continuum (for polar coordinating solvents) methods. In the latter case, we first examined the structure of Chl*a* in rigorously dry solutions. Two types of solvents were characterized according to Mg-atom coordination: In type I solvents (acetone, acetonitrile, DMSO), Mg exhibits five-coordination, whereas in type II solvents (THF, pyridine), Mg exhibits six-coordination. Hydration processes are quite dependent on solvent nature. In nonpolar or low-polarity solvents such as cyclohexane or chloroform, hydration is always exothermic and exergonic, despite a large entropy term that strongly opposes hydration. In polar solvents of type II, hydration is quite unfavorable, and essentially no hydrates are expected in these media, except perhaps at very large water concentrations (although, in such a case, the medium cannot be simply described as an organic solvent). In polar solvents of type I, the situation is intermediate, and dihydration is favorable in some cases (acetone, acetonitrile) and unfavorable in others (DMSO). It is interesting to note that first hydration processes in coordinating solvents (of either type I or type II), where a water molecule must displace a solvent molecule coordinated to Mg, exhibit values of $\Delta H > 0$ and $\Delta S > 0$, in sharp contrast to first hydration processes in nonpolar media. The present results represent the first theoretical attempt to rationalize the large amount of experimental data on hydration and aggregation of Chl*a* in aqueous organic media that have been accumulated over the past four decades. The data stress, in particular, the key role of Chl*a* dihydrates, a point that has been the object of intense debate in the literature. Clearly, dihydrates are found to be more stable than monohydrates owing to a particular structure in which cooperative interactions occur between the water molecules and Chl*a*. The calculations also explain the irregular behavior observed for Chl*a* in aqueous THF or pyridine: In these media, Chl*a* remains basically unhydrated because the Chl*a*–solvent adducts are stabilized by strong dispersion interactions.

1. Introduction

The mechanism of photosynthesis has been the focus of many experimental and theoretical works. Understanding how living systems operate to convert solar energy into chemical energy is important not only from the point of view of fundamental research but also for the development of new technologies.¹ A key issue is the relationship between the structure and the activity of chlorophyll molecules in photosystems I and II (PSI and PSII). Major advances in this direction have been made thanks to the determination of crystallographic structures,^{2–7} but nonetheless, many unanswered questions remain. For instance, available crystal structures of PSI and PSII have shown that their reaction centers involve chlorophyll *a* (Chl*a*) pairs (P700 and P680, respectively), which presumably are responsible for the high efficiency of energy conversion. The stability of these

specific arrangements (and, more generally, of Chl*a* organization in photosystems) is certainly connected to Chl*a*–Chl*a*, Chl*a*–water, and Chl*a*–protein interactions, but the relative strengths of these interactions are not well-known. Likewise, the reason why five-coordination of the Chl*a* Mg atom is almost exclusively observed in biological environments whereas six-coordinated complexes are observed in certain solvents^{8–11} is unclear. Note that histidine is a typical axial ligand in proteins, although Mg can also bind other residues (Gln, Asp, Glu, and Tyr), as well as water molecules.^{2,3,6}

In fact, the role of water molecules in the self-assembly of chlorophyll molecules has attracted attention for a long time. Former models of Chl*a* pairs, in which Chl*a* molecules are cross-linked by water molecules, were proposed by Katz and Norris,¹² Fong,¹³ Chow et al.,¹⁴ and Shipman et al.¹⁵ The so-called Strouse model^{14,16} was based on the first detailed crystallographic structure reported for a chlorophyll derivative, the ethyl chlorophyllide *a* dihydrate, and was obtained from an acetone–water mixture. Since these pioneering works, the role of water in Chl*a*

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aggregation has been the subject of many publications. Most of these works have regarded the aggregation mechanisms of Chla (or other chlorophylls) in aqueous organic solvents as a function of water concentration.^{8,17–40} The nature of the solvent has been shown to be decisive. A seminal study using IR spectroscopy was carried out by Ballschmider and Katz¹⁹ in the 1960s. The authors considered the case of nonpolar solvents in the absence or presence of water and discussed in some detail the formation of Chla dimers and oligomers, as well as that of different hydrates. The formation of both 2Chla–H₂O and Chla–H₂O species in CCl₄ and benzene (and also in aliphatic hydrocarbons for low Chla concentrations) was postulated. In aliphatic or cycloaliphatic hydrocarbons with increasing Chla concentration, formation of micelles of colloidal dimensions corresponding to the motif Chla–H₂O was suggested. This interpretation was reconsidered a few years later by Fong and Koester,²¹ who suggested that the formation of Chla hydrates in nonpolar media probably corresponds to the adduct (Chla–2H₂O)_n. Comparison between nonpolar and polar solvents was made by Fujiwara and Tasumi⁸ on the basis of IR and Raman data. These authors classified the solvents into two groups depending on the coordination number of the Mg atom. Five-coordination was observed in solvents such as *n*-hexane, CCl₄, diethylether, and acetone, whereas six-coordination was observed in tetrahydrofuran (THF) and pyridine. In the case of the nonpolar solvents, the five-coordination could be due to Chla–Chla interactions or to coordination of Mg with trace water molecules. Agostiano et al.³² investigated aqueous solutions of Chla in acetone, dimethylformamide, and acetonitrile; Chla–2H₂O monomers were observed up to high water mole fractions, after which dimerization and oligomerization occurred. The same group^{30,31,33,35} used optical and other techniques to investigate nanoscale self-assembly of Chla in the water-rich region. In such conditions, hydrophobic interactions play an essential role, and the observed data suggested the formation of spherical-shaped aggregates of Chla, with the phytol chains lying in the inner part and the macrocyclic heads pointing toward the bulk water solvent. Perhaps the most systematic study on Chla self-assembly in aqueous organic (coordinating) solvents was reported by Vladkova³⁸ using absorption, fluorescence spectroscopy, and fluorescence lifetime measurements. The nature of the solvent was shown to modify both the molar fraction of water required to induce the Chla monomer → aggregate transition and the type of aggregate formed. The author concluded that the hydrogen-bonding ability and the dipole–dipole interactions of the solvent–water mixtures are the main factors promoting Chla self-assembly. In some solvents (methanol, ethanol, acetone, acetonitrile), water can replace the coordinated solvent molecules, and for a sufficiently high molar fraction of water, polymeric aggregates (Chla–2H₂O)_n do form. In THF and pyridine, however, this is not possible, and the absorption spectra after complete transition exhibit the typical features of colloidal aggregates of Chla.

All of these experimental studies have provided valuable information on Chla aggregation mechanisms in organic solvents, but a thorough understanding of the role of water is still lacking. There are several reasons for that. First, the interpretation of experimental data is not always unambiguous owing to the complexity and the multiplicity of species that can be present under some specific conditions. In addition, the precise structures of Chla hydrates are unknown; mono- and dihydrates have often been proposed depending on experiments, but there is no clear-cut determination of their relative stability and how the medium influences it. Often, low solubilities (water in nonpolar media

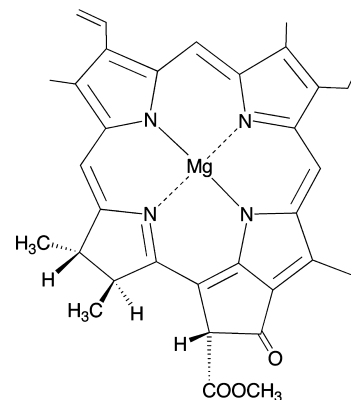


Figure 1. Chlorophyll *a* model employed in this work (Chla-73).

or Chla in water, for instance) represent a drawback as well. Finally, the marked affinity of Chla for water combined with the difficulty of preparing rigorously dry Chla solutions introduces errors that are difficult to evaluate. As a consequence, the thermodynamics of hydration and aggregation processes in aqueous organic solvents is poorly known.

In principle, the estimation of thermodynamic quantities that are not easily accessible (or are inaccessible) to experiment can be achieved computationally. However, the main difficulties are encountered in dealing with Chla hydrates. On one hand, quantum chemical calculations can become too expensive because of the large size of Chla molecules, even for its simplest models, because the porphyrin macrocycle must always be explicitly considered. On the other hand, molecular mechanics techniques are not well adapted to describe the energetics associated with changes in the Mg-atom coordination sphere (from four to six). These two reasons probably explain why there has not been any systematic investigation on Chla hydration phenomena to date. Indeed, only a few theoretical studies considering Chla hydrates have been reported.^{41–46} Recently, we carried out the first theoretical analyses of Chla–water complexes formation using either density functional theory (DFT)^{45,46} or MP2⁴⁶ approaches. These works focused on complex formation in the gas phase, although a rough estimation of the effects of dielectric solvent in nonpolar media was reported in ref 45.

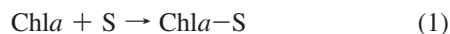
With the aim of gaining a deeper insight into the thermodynamics of Chla hydration in aqueous organic solvents, we have carried out a detailed theoretical study of monomer hydrates using both continuum and discrete-continuum solvation models. The formation of Chla aggregates will be considered in a forthcoming work. This article is organized as follows: First, we describe the models and the methods employed. Afterward, we report the calculations for the mono- and dihydration processes in nonpolar and in coordinating solvents. The latter case requires a prior examination of the structures of Chla–solvent adducts in the absence of water (five vs six coordination of the Mg atom). Finally, we discuss the results in relation to available experimental data and clarify a number of controversial issues.

2. Method of Calculation

In this study, we use the Chla-73 model (involving 73 atoms) proposed in our previous work.⁴⁵ All chemical groups in Chla are included except for the phytol ester side chain, which is replaced by a methyl group (Figure 1).

Quantum chemical calculations were carried out with the Gaussian 03 program⁴⁷ using density functional theory (DFT)

at the B3LYP/6-31G(d) level. This approach represents an excellent compromise between accuracy and computational cost, as it has been shown⁴⁶ to provide results comparable to MP2/6-311++(2d,2p) calculations provided that (1) the energies are corrected for basis set superposition errors (BSSEs) and (2) energies are corrected to account for intermolecular dispersion contributions, which are lacking in standard density functionals. Here, BSSEs were corrected using the counterpoise method.⁴⁸ Dispersion energy was estimated as proposed before,⁴⁶ that is, using the DFT + D method.^{49,50} Electrostatic bulk solvent effects were evaluated using the polarizable continuum model⁵¹ and standard cavities in Gaussian 03. Nonelectrostatic solvation effects were neglected. Numerical estimations for cavitation, dispersion, and repulsion energy contributions were reported before for Chla–water complexes in liquid water.⁴⁵ It was shown that the nonelectrostatic solvation energy is roughly additive, and therefore, it plays a minor role in the estimation of complex formation energies. The geometries of all systems were fully optimized in the gas phase. The structures are reported as Supporting Information (Tables 1S and 2S), and for the sake of conciseness, they are not discussed in detail here. Calculations in the continuum were carried out with the gas-phase geometries because preliminary tests showed that further geometry relaxation does not modify the results significantly.^{45,46} Frequencies of vibration were computed in the gas phase to verify that the structures do correspond to energy minima (real frequencies) and also to evaluate contributions to Gibbs free energies. We used the ideal gas approximation and assumed $T = 298.15$ K and 1 atm (gas phase) or 1 M concentration (solution). The computation of association free energies in solution was done according to typical procedures⁵² based on thermodynamic cycles. Accordingly, solvation free energies include a correction to account for change in reference state (1.89 kcal/mol at 298.15 K). In addition, when the complex involves solvent molecules, one has to account for solvent concentration in order to obtain quantities comparable to experiment. For instance, the appropriate ΔG for the process



is

$$\Delta G = \Delta G' - RT \ln [\text{S}] \quad (2)$$

where [S] stands for the concentration of solvent and $\Delta G'$ is the free energy computed as $\Delta G' = G(\text{Chla-S}) - G(\text{Chla}) - G(\text{S})$. Using solvent densities at 298.15 K, one estimates $RT \ln [\text{S}]$ as 1.51 kcal·mol⁻¹ (pyridine), 1.49 kcal·mol⁻¹ (THF), 1.55 kcal·mol⁻¹ (acetone), 1.76 kcal·mol⁻¹ (acetonitrile), and 1.57 kcal·mol⁻¹ [dimethyl sulfoxide (DMSO)].

3. Results

We present in the following subsections the relative energies of mono- and dihydrates of Chla in different media. For clarity, we summarize in Figure 2 the different types of complexes that will be discussed. Calculations have been carried out for some other possible structures exhibiting lower interest; brief comments on the corresponding results will be made in some cases.

A. Hydration of Chla in Nonpolar or Low-Polarity Solvents. To start, we focus on hydration processes in nonpolar or low-polarity solvents that, in addition, are assumed to be noncoordinating. We shall consider cyclohexane as a prototype, but we have also included calculations in chloroform to evaluate the effect of increasing the solvent dielectric constant. In principle, chloroform can exhibit specific interactions with Chla, but such interactions are not expected to play an important role and are neglected here.

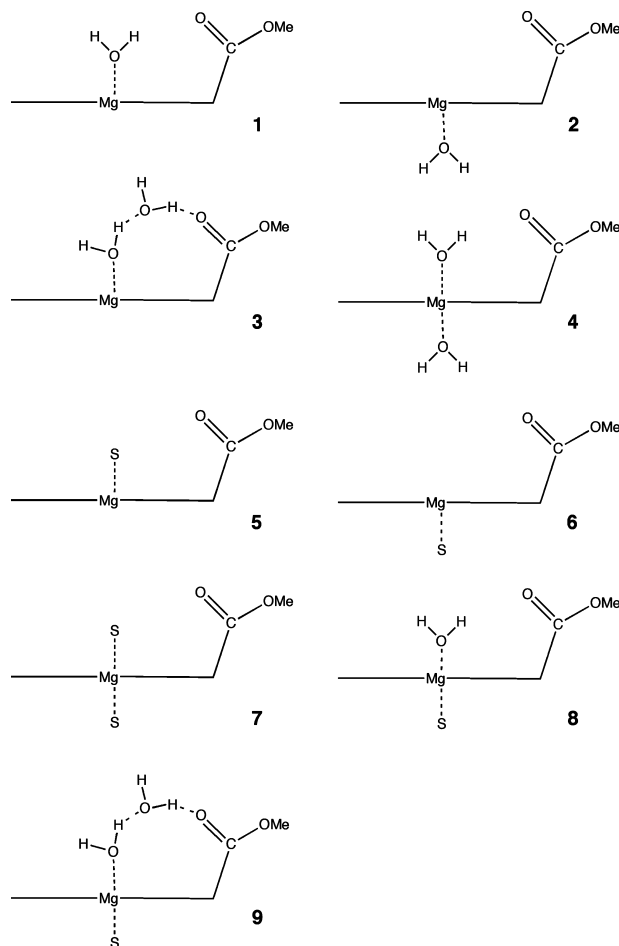


Figure 2. Schematic representation of the studied Chla complexes. S represents a solvent molecule.

The mono- and dihydrates of Chla in these media can be represented by structures 1–4 in Figure 2. The energetics for gas-phase hydration processes are summarized in Table 1. The present calculations are comparable to those reported in ref 45, although that work neglected BSSE and dispersion corrections (as shown in Table 1, these contributions have the same order of magnitude but opposite signs). They confirm the following trends: (1) all hydration processes are strongly exothermic; (2) they are exergonic, despite a quite unfavorable entropic term; (3) the first hydration is more favorable when the water molecule lies in the syn-periplanar position with respect to the ester group (structure 1); (4) the second hydration is much more favorable for the five-coordinated structure 3 than for the diaxial structure 4 because of cooperativity between the Mg–water interaction and water–water and water–carbonyl hydrogen bonds; and (5) the dihydrate is considerably more stable than the monohydrate. These results are consistent with experimental findings. On one hand, the predicted five-coordinated structure for the Chla dihydrate complex is in agreement with crystallographic data on dihydrates of ethyl chlorophyllide *a*, methyl chlorophyllide *a*, and ethyl chlorophyllide *b*.^{14,53–55} On the other hand, the preference for Mg hydration in the ester syn-periplanar position corresponds to the general trend observed for Chla complexes.^{56,57} Note that, in the rest of this article, with the aim of conciseness, we shall limit the discussion to coordination processes that lead to hydrates of this type.

Bulk solvation effects on the first hydration, $\text{Chla} + \text{H}_2\text{O} \rightarrow 1$, and the second hydration, $1 + \text{H}_2\text{O} \rightarrow 3$, are summarized in Table 2. In this table, and in the subsequent tables, $\delta\Delta G_{\text{sol,el}}$

TABLE 1: Energetics (kcal·mol⁻¹) for the First and Second Hydration Processes of Chla in the Gas Phase^{a,b}

	ΔE^{uncorr}	ΔE	ΔE_{disp}	ΔZPE	$\delta \Delta H(T)$	ΔH	$-T\Delta S$	ΔG_g
Chla + H ₂ O → 1	-18.0	-12.3	-6.5	1.6	-0.2	-17.4	8.5	-8.9
Chla + H ₂ O → 2	-17.6	-12.0	-6.4	1.8	-0.4	-17.0	9.0	-8.0
1 + H ₂ O → 3	-22.8	-17.0	-8.5	3.3	-1.3	-23.5	11.8	-11.7
1 + H ₂ O → 4	-12.9	-6.7	-7.0	1.9	-0.5	-12.3	10.0	-2.2

^a Calculations at the B3LYP/6-31G(d) + D level. ^b Meanings of symbols are as follows: ΔE^{uncorr} and ΔE represent the BSSE-uncorrected and BSSE-corrected electronic energy differences, respectively; ΔE_{disp} is the dispersion correction to the B3LYP energy; ΔZPE contains the contribution due to zero-point energy; $\delta \Delta H(T)$ stands for thermal corrections to the enthalpy change at 0 K; ΔH , $-T\Delta S$, and ΔG_g correspond to the enthalpy, entropy, and free energy changes, respectively, at 1 atm and $T = 298.15$ K.

TABLE 2: Energetics (kcal·mol⁻¹) for the First and Second Hydration Processes of Chla in Hydrophobic Solvents^{a-c}

	Chla + H ₂ O → 1			1 + H ₂ O → 3			total
	ΔG_g	$\delta \Delta G_{\text{sol,el}}$	ΔG_s	ΔG_g	$\delta \Delta G_{\text{sol,el}}$	ΔG_s	ΔG_s
cyclohexane	-8.9	3.5	-7.3	-11.5	3.5	-9.9	-17.2
chloroform	-8.9	6.5	-4.3	-11.5	6.9	-6.5	-10.8

^a Calculations at the B3LYP/6-31G(d) + D level for processes 1 and 3. ^b Total free energies correspond to the sum of first and second hydration processes. ^c ΔG_g and ΔG_s correspond, respectively, to the free energy changes in the gas phase and solution at $T = 298.15$ K and 1 atm (gas phase) or 1 M (solution). $\delta \Delta G_{\text{sol,el}}$ stands for the difference in electrostatic solvation energies. Note that ΔG_s does not necessarily correspond to the sum of ΔG_g and $\delta \Delta G_{\text{sol,el}}$ because it also includes the concentration correction, as explained in the Method of Calculation section.

represents the electrostatic effect of the dielectric continuum on the free energy for the process, computed with the PCM method and defined as

$$\delta \Delta G_{\text{sol,el}} = \sum_{i=\text{products}} \Delta G_i^{\text{sol,el}} - \sum_{j=\text{reactants}} \Delta G_j^{\text{sol,el}} \quad (3)$$

where $\Delta G_i^{\text{sol,el}}$ is the electrostatic solvation free energy term.

From Table 2, it can be seen that all $\delta \Delta G_{\text{sol,el}}$ quantities are positive. In other words, increasing the dielectric constant of the medium opposes hydration. Nonetheless, the formation of **1** and **3** remains quite exergonic in cyclohexane and chloroform. As in the gas phase, the second hydration is particularly favorable because of cooperative interactions. The total free energy for the formation of Chla dihydrate, $\text{Chla} + 2\text{H}_2\text{O} \rightarrow \mathbf{3}$, is remarkably large in both media. Therefore, our calculations predict that addition of water to solutions of Chla in nonpolar or low-polarity solvents will spontaneously lead to the formation of Chla hydrates, with the dihydrates being much more stable. Actual concentrations of monohydrates and dihydrates of the Chla monomer will depend on the initial Chla and water concentrations, but also on other equilibria (Chla dimerization, oligomerization) not considered here.

B. Chla in Coordinating Solvents: Five- vs Six-Coordination. We consider now hydration in coordinating solvents, typical representatives of which are THF, pyridine, acetone, acetonitrile, and DMSO. The first two (THF and pyridine) are experimentally known^{8-11,58,59} to form diaxial complexes with chlorophyll and magnesium porphyrins, the Mg atom being six-coordinated. These solvents are referred to here as type II solvents. The other three (acetone, acetonitrile, and DMSO) form monoaxial complexes with chlorophylls and lead to five-coordination of the Mg atom. They are referred to here as type I solvents. To discuss the formation of Chla hydrates in these media, the structures and stabilities of Chla-S complexes and the origin of differences between type I and type II solvents need to be examined.

Free energy calculations have been done for the first and second ligation processes: $\text{Chla} + \text{S} \rightarrow \mathbf{5}$ (or **6**) and $\mathbf{5} + \text{S} \rightarrow$

7. The results are summarized in Table 3 for the species in the gas phase. We do not include the calculations for complex **6** because complex **5** was systematically found to be more stable (by 0.4 kcal·mol⁻¹ for acetone, 3.2 kcal·mol⁻¹ for acetonitrile, 2.3 kcal·mol⁻¹ for DMSO, 1.1 kcal·mol⁻¹ for THF, and 1.7 kcal·mol⁻¹ for pyridine). First ligation is in all cases very exothermic and exergonic. Second ligation is exothermic too, but owing to entropic contributions, it is endergonic for type I solvents and slightly exergonic for type II solvents. A comparison of ΔH and ΔG values with those for Chla coordination to water in Table 1 shows that water behaves like the less nucleophilic solvents (acetone, acetonitrile) in the first ligation processes and like the most nucleophilic ones (THF, pyridine) in the second axial ligation. Two other interesting remarks can be made. First, the enthalpy for second coordination stems mostly from the dispersion contribution, as was already found for pyridine ligation to magnesium porphyrin.⁴⁶ Second, the position of the Mg atom with respect to the mean N-pyrrol plane in the 1:1 Chla-S complexes (see Table 2S in the Supporting Information) is correlated with the interaction energy and is as large as 0.42 Å in the case of DMSO.

Bulk solvent effects on these free energies are reported in Table 4 (we use the dielectric constant of the pure solvent). Bulk solvation disfavors Chla-S complex formation, as all $\delta \Delta G_{\text{sol,el}}$ values are positive. However, although the absolute values of the free energies change with respect to those obtained in the gas phase, the signs are not modified. In other words, we predict that Chla should be six-coordinated in THF and pyridine but five-coordinated in acetone, acetonitrile, and DMSO. These coordination modes are in excellent agreement with available experimental spectroscopic evidence.⁸⁻¹¹ According to our calculations, five- or six-coordination is principally determined by the magnitude of the dispersion energy, which is significantly larger in type II solvents.

C. Hydration in Coordinating Solvents of Type I. Upon addition of water to the solution, solvent and water molecules enter into competition for coordination to Chla. In principle, the reaction of the initial five-coordinated Chla-S complex with one water molecule (first hydration process) could lead to the formation of the diaxial complex **8**, but exploratory calculations showed that these complexes are quite unstable for type I solvents. Hence, we limit the discussion here to the S-water exchange process: $\mathbf{5} + \text{H}_2\text{O} \rightarrow \mathbf{1} + \text{S}$. Gas-phase results are gathered in Table 5, which shows that the process is endothermic, especially for S = DMSO, and exhibits a favorable entropic term. Indeed, the entropy diminution due to water coordination is overcompensated by the release of a solvent molecule. Accordingly, the first hydration in type I solvents contrasts to that in nonpolar media, which, as discussed above (Table 1), is a highly exothermic, entropically disfavored process. All in all, the first hydration of Chla was found to be slightly exergonic in acetone and acetonitrile and significantly endergonic in DMSO.

TABLE 3: Energetics (kcal·mol⁻¹) for the First and Second Axial Bindings to Chla in the Gas Phase^{a,b}

	ΔE_{uncorr}	ΔE	ΔE_{disp}	ΔZPE	$\delta \Delta H(T)$	ΔH	$-T\Delta S$	ΔG_g
Chla + S → 5								
THF	-17.2	-11.9	-12.3	0.8	0.5	-22.9	11.0	-11.9
pyridine	-18.6	-13.8	-13.4	0.8	0.6	-25.8	11.1	-14.7
acetone	-15.0	-9.8	-9.5	0.6	0.5	-18.2	10.6	-7.6
acetonitrile	-14.7	-10.3	-10.1	0.7	0.3	-19.3	10.7	-8.6
DMSO	-23.8	-16.4	-11.4	0.9	0.4	-26.5	12.0	-14.5
5 + S → 7								
THF	-6.7	-1.5	-12.3	0.7	0.7	-12.4	11.4	-1.0
pyridine	-6.2	-1.8	-12.3	0.5	0.7	-12.9	11.2	-1.7
acetone	-7.7	-2.3	-9.9	0.9	0.3	-11.0	11.6	0.6
acetonitrile	-3.1	0.2	-6.0	0.3	0.7	-4.8	7.7	2.9
DMSO	-7.2	-0.5	-9.1	0.4	0.6	-8.6	11.1	2.5

^a Calculations at the B3LYP/6-31G(d) + D level. ^b For the meanings of symbols, see footnote ^b to Table 1.

TABLE 4: Energetics (kcal·mol⁻¹) for the First and Second Axial Bindings to Chla in Solution^{a,b}

	ΔG_g	$\delta \Delta G_{\text{sol,el}}$	ΔG_s
Chla + S → 5			
THF	-11.9	7.2	-8.1
pyridine	-14.7	9.0	-8.9
acetone	-7.6	8.5	-2.6
acetonitrile	-8.6	11.2	-1.1
DMSO	-14.5	11.8	-6.1
5 + S → 7			
THF	-1.0	4.0	-0.3
pyridine	-1.7	5.1	-0.3
acetone	0.6	6.5	3.7
acetonitrile	2.9	3.9	3.1
DMSO	2.5	8.2	7.3

^a Calculations at the B3LYP/6-31G(d) + D level. ^b For the meanings of symbols, see footnote ^c to Table 2.

TABLE 5: Energetics (kcal·mol⁻¹) for S–Water Ligand Exchange in the Gas Phase (5 + H₂O → 1 + S)^{a,b}

	ΔE_{uncorr}	ΔE	ΔE_{disp}	ΔZPE	$\delta \Delta H(T)$	ΔH	$-T\Delta S$	ΔG_g
acetone	-3.0	-2.5	3.0	1.0	-0.7	0.8	-2.1	-1.3
acetonitrile	-3.3	-2.0	3.6	0.9	-0.5	2.0	-2.3	-0.3
DMSO	5.8	4.1	4.9	0.7	-0.6	9.1	-3.5	5.6

^a Calculations at the B3LYP/6-31G(d) + D level. ^b For the meanings of symbols, see footnote ^b to Table 1.

TABLE 6: Energetics (kcal·mol⁻¹) for the First and Second Hydration Processes of Chla in Coordinating Solvents of Type I^{a-c}

	5 + H₂O → 1 + S			1 + H₂O → 3			total
	ΔG_g	$\delta \Delta G_{\text{sol,el}}$	ΔG_s	ΔG_g	$\delta \Delta G_{\text{sol,el}}$	ΔG_s	
acetone	-1.3	0.1	0.3	-11.7	9.6	-4.0	-3.7
acetonitrile	-0.3	-2.3	-0.8	-11.7	10.1	-3.5	-4.3
DMSO	5.6	-2.7	4.5	-11.7	9.9	-3.7	0.8

^a Calculations at the B3LYP/6-31G(d) + D level. ^b Total free energies correspond to the sum of first and second hydration processes (5 + 2H₂O → 3 + S). ^c For the meanings of symbols, see footnote ^c to Table 2.

The second hydration is simply described by the process 1 + H₂O → 3. This is the same process as the one described in noncoordinating solvents. The corresponding gas-phase results are included in Table 1 and will not be further discussed here.

Bulk solvation effects on free energies for the first and second hydrations are collected in Table 6. The role of the solvent contribution, $\delta \Delta G_{\text{sol,el}}$, can be summarized as follows: It is negligible for the first hydration in acetone, slightly favorable

for the first hydrations in acetonitrile and DMSO (by roughly 2–3 kcal·mol⁻¹), and strongly unfavorable for the second hydration in all cases (as was also found in nonpolar or low-polarity solvents). Overall, the computed free energies suggest that spontaneous formation of dihydrates is expected in acetone and acetonitrile whereas hydration in DMSO (either mono- or dihydration) seems very unlikely.

D. Hydration in Coordinating Solvents of Type II. We consider now the case of type II solvents in which Chla exhibits diaxial coordination with solvent molecules. The situation is a bit more complicated than before because of the larger number of hydrates that can be envisaged. For the first hydration, two types of processes can be considered depending on the number of solvent molecules released: 7 + H₂O → 8 + S and 7 + H₂O → 1 + 2S. In the former, one could also envisage a structure 8' derived from 8 by exchanging the positions of the water and S ligands. Calculations show that 8' is less stable than 8, and therefore, only the latter is considered herein. The second hydration can lead to either five-coordinated or six-coordinated Chla with a water dimer bonded to Mg and to the ester CO group in both cases, according to the reactions 8 + H₂O → 9 or 8 + H₂O → 3 + S.

The hydration energetics for complexes in the gas phase are presented in Table 7 and show that, whereas the first hydration is endothermic, the second hydration is exothermic. As expected, entropy contributions do strongly favor the 7 + H₂O → 1 + 2S processes, because two solvent molecules are released, but in terms of free energy, these processes remain quite unfavorable and can be safely ignored. The results in solution are summarized in Table 8. Clearly, the dielectric solvent effects oppose hydration, and according to our calculations, neither monohydrates nor dihydrates of Chla are thermodynamically stable in type II solvents. In other words, water will not be able to displace the solvent molecules in the Mg coordination shell.

4. Discussion

Hydration and aggregation of Chla in aqueous organic solvents have experimentally been shown to be strongly interrelated processes. Indeed, although a precise description of aggregation processes would require further studies, knowledge of the hydration free energies allows one to obtain some insight into the experimental observations and could be useful for the design of new experiments.

According to the present calculations, in nonpolar solvents, Chla will readily form hydrated complexes in the presence of water, even in the form of traces. The monohydrate/dihydrate equilibrium will be strongly shifted toward the formation of

TABLE 7: Energetics (kcal·mol⁻¹) for the First and Second Hydration Processes of Six-Coordinated Complexes (7) in the Gas Phase (Chla-2S, S = Pyridine, THF)^{a,b}

	ΔE_{uncorr}	ΔE	ΔE_{disp}	ΔZPE	$\delta \Delta H(T)$	ΔH	$-T\Delta S$	ΔG_{g}
Pyridine								
$7 + \text{H}_2\text{O} \rightarrow 8 + \text{S}$	-2.4	-0.7	6.0	1.1	-0.9	5.4	-2.0	3.4
$7 + \text{H}_2\text{O} \rightarrow 1 + 2\text{S}$	6.7	8.1	19.2	0.3	-1.3	26.2	-13.8	20.4
$8 + \text{H}_2\text{O} \rightarrow 9$	-19.8	-14.1	-6.9	3.1	-1.1	-19.0	11.0	-8.0
$8 + \text{H}_2\text{O} \rightarrow 3 + \text{S}$	-13.7	-12.3	4.8	2.5	-1.7	-6.7	0.0	-6.7
THF								
$7 + \text{H}_2\text{O} \rightarrow 8 + \text{S}$	-3.4	-2.3	5.6	1.0	-0.9	3.4	-1.8	1.6
$7 + \text{H}_2\text{O} \rightarrow 1 + 2\text{S}$	5.9	6.4	18.0	0.1	-1.3	23.3	-13.9	9.5
$8 + \text{H}_2\text{O} \rightarrow 9$	-18.9	-13.1	-7.3	3.1	-1.1	-18.4	11.0	-7.4
$8 + \text{H}_2\text{O} \rightarrow 3 + \text{S}$	-13.5	-13.0	3.9	2.4	-1.6	-8.3	-0.3	-8.6

^a Calculations at the B3LYP/6-31G(d) + D level. ^b For the meanings of symbols, see footnote ^b to Table 1.

TABLE 8: Energetics (kcal·mol⁻¹) for the First and Second Hydration Processes of Chla in Type II Solvents^{a-d}

	$7 + \text{H}_2\text{O} \rightarrow 8 + \text{S}$			$8 + \text{H}_2\text{O} \rightarrow 9$ $8 + \text{H}_2\text{O} \rightarrow 3 + \text{S}$			total
	ΔG_{g}	$\delta \Delta G_{\text{sol,el}}$	ΔG_{s}	ΔG_{g}	$\delta \Delta G_{\text{sol,el}}$	ΔG_{s}	
pyridine	3.4	0.3	5.2	-8.0	7.7	-2.2	2.9
				-6.7	2.8	-2.4	2.8
THF	1.6	1.1	4.2	-7.4	6.9	-2.3	1.8
				-8.6	3.2	-3.9	0.3

^a Calculations at the B3LYP/6-31G(d) + D level. ^b Only one process is considered for the first hydration ($7 + \text{H}_2\text{O} \rightarrow 8 + \text{S}$), whereas two different processes are considered for the second hydration ($8 + \text{H}_2\text{O} \rightarrow 9$ and $8 + \text{H}_2\text{O} \rightarrow 3 + \text{S}$). ^c Total free energies represent the sum of first and second hydration processes. ^d For the meanings of symbols, see footnote ^c to Table 2.

the dihydrate, so that, roughly speaking, monohydrates can be expected only for relative concentrations of water below the 1:2 stoichiometric ratio. These results agree with the experimental findings that, in nonrigorously dried nonpolar solvents, Chla can easily form a five-coordinated complex,^{8,17,19,21} as well as with the fact that, in the presence of small amounts of water, there is formation of mono- and dihydrated Chla species, as well as their corresponding dimers.^{21,23,24} Not surprisingly, upon further addition of water to a solution inducing Chla aggregation, the equilibrium mixture is greatly shifted in favor of the dihydrated polymeric Chla form (Chla-2H₂O)_n.^{21,22,24,60} Note that the structure of this aggregate has been the subject of extensive debate in the literature. Both a cylindrical micelle and a planar sheetlike structure have been proposed.^{28,37,60,61} Evidence for the presence of neutral structural water molecules forming a hydrogen-bonded network in an arrangement similar to that of crystals of the ethyl chlorophyllide¹⁴ has been reported.^{36,37,60,61} Other experimental studies have suggested the possibility that polymeric (Chla-2H₂O) aggregates are stabilized in hexameric units.²⁵

In sharp contrast to nonpolar media, in coordinating solvents of type II such as THF or pyridine, Chla is essentially in the form of the unhydrated diaxial solvate. In principle, a significant concentration of Chla hydrates could not be reached, except perhaps at large water concentrations. However, in that case, our results in Table 8 would have to be revised. Indeed, as the molar fraction of water rises, the dielectric constant of the medium is expected to increase, implying a change in the $\delta \Delta G_{\text{sol,el}}$ contributions as well. This latter quantity has a positive sign for solvents of type II, and therefore, an increase in solvent dielectric constant would lead to slightly larger values, that is, to still less favorable hydration processes. Hence, hydration in these solvents appears to be particularly unfavorable, and not

surprisingly, experimental measurements by Vladkova³⁸ were consistent with the formation of colloidal aggregates of Chla and not with the formation of (Chla-2H₂O)_n polymeric aggregates.

In principle, the effects of solvents of type I can be considered as intermediate between the effects of nonpolar solvents (where dihydrates are formed readily) and those of solvents of type II (where basically no hydrates are expected). However, a closer examination of the computed thermodynamic properties in Table 6 shows that hydration can exhibit subtle characteristics in this type of medium. The bulk solvent effect for the first hydration, estimated by the $\delta \Delta G_{\text{sol,el}}$ value, is relatively small and negative (except for acetone, which displays a very small positive value). On the contrary, the solvent effect on the second hydration is always positive and large. Overall (cf. total ΔG_{s} values in Table 6), spontaneous dihydrate formation is expected to occur (acetone, acetonitrile) or not (DMSO) depending on the nucleophilic character of the solvent. An increase of the water concentration will obviously displace the equilibria toward hydrate formation, but owing to the associated increase in dielectric constant, bulk solvation will favor monohydrates at the expense of dihydrates. Several experimental observations^{29,31,32,34,35,40} support this theoretical analysis. For instance, studies using triangular-sweep adsorption voltammetry and absorption and fluorescence spectroscopies reported by Agostiano et al.^{29,31,32} for Chla in binary mixtures of water with acetone and acetonitrile showed that Chla-2H₂O dihydrate is the main species up to 40 vol % water in acetone. Beyond this value, Chla undergoes dimerization and then oligomerization. They also noted that lower water contents are required for aggregation in acetonitrile as compared to acetone. On the other hand, studies of IR and Raman spectra of Chla in DMSO/water mixtures at different water/DMSO ratios and different pigment concentrations^{26,27} suggested the formation of Chla-DMSO adducts, without appreciable Chla-Chla or Chla-water interactions.²⁶ The results indicated that the aggregates formed in 30–80% (v/v) water/DMSO solutions incorporate DMSO but not water.²⁶

5. Conclusions

The stability of Chla hydrates in aqueous organic solvents has been the focus of many experimental studies in the literature using a variety of techniques. However, because of the complexity of the systems, the low solubility of Chla in some media, and the high affinity for Chla by water, the interpretation of the observed quantities is not straightforward. We have presented here the first systematic theoretical study of Chla hydration in organic solvents, which has allowed us to rationalize experimental observations. The strong dependence of hydrates stability on the nature of the solvent has been explained

by making a detailed analysis of enthalpic and entropic contributions. It is important to recall here that dispersion energy has been found to play an important role in the stability of Chla–water and Chla–solvent adducts. Thus, standard DFT methods are not expected to lead to accurate results unless corrected for such a contribution, as was done here.

Our results provide numerical estimations for hydration processes involving the Chla monomer, and they, in turn, constitute a basis toward the understanding of aggregation phenomena. Further investigation of this topic will require explicit treatments of dimerization and oligomerization processes, which represents a difficult computational task. The implementation of molecular dynamics simulations is obviously an exciting perspective in this respect but efficient molecular mechanics (MM) force fields for Chla are unfortunately lacking. The present investigation does provide a convenient set of data to develop and test new MM methods. Such a goal seems to be extremely important because it would open the door to the computer modeling of photosystems.

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Supporting Information Available: Optimized geometries in the gas phase and total energies of the described complexes. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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