FULL PAPER

Theoretical Study of the Dimerization of Chlorophyll (a) and Its Hydrates: Implication for Chlorophyll (a) Aggregation

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Dimeric structures chlorophyll (a) (Chla) and their mono- and dihydrated have been suggested to play an important role in the mechanism of photoreaction center chlorophyll special pairs PSI and PSII. Despite their functional importance, the molecular basis structures for interacting two Chla molecules and the structural stabilization role of H_2O in the formation of hydrated Chla dimer complexes is poorly understood. In this article, the different coordination modes between two interacting Chla molecules and the configurational (orientation and distance) features between the dimer and bound H_2O molecules are characterized by means of super molecule approach the density functional theory DFT. An estimation of the thermodynamic quantities is made for Chla dimerization and hydration processes. The results indicate that structure including ester linkages *via* H_2O hydrogen bonding is the most favorable conformation for the dihydrated chlorophyll (a) dimer at B3LYP/6-31G*-DCP level of calculation. The dispersion interaction is shown to be of great significance for the Chla dimer stabilization. In aqueous nonpolar solvent, the thermodynamics show that Chla has a slightly stronger driving force for full hydration than for dimerization and that hydration of the dimers is rather weakly exergonic. The tetrahydrated dimers having a similar arrangement to that in crystals of ethyl chlorophyllide (a) dihydrate are found to be more stable than the Chla dihydrated dimer. The data underscore the key role of H-bonding in the stability of Chla-H_2O adducts and, in particular, the great importance of the Chla monomeric dihydrated species in the hydration and dimerization of Chla in aqueous media. Clearly, the Chla dihydrates (Chla-2 H_2O) are found more stable than the monohydrates (Chla-H_2O) and the Chla dimers (Chla_2), owing to a particular structure in which cooperative interactions occur between the H_2O molecules and Chla. Calculations also indicate that the most thermodynamically preferred pathway for the formation of Chla dimer hydrates can be represented by two steps: the first corresponds to the formation of Chla monomeric dihydrates and the second is the dimerization of the dihydrates on to tetrahydrated Chla dimers. These results allow to obtain a new possible pathway for Chla dimer formation processes and could provide new insights to the aggregation of chlorophyll (a) in solution.

Introduction. – Photosynthesis is well-known to be the one of the most important energy-conversion mechanisms carried out for living systems. The initial processes of photosynthesis take place in a photosynthetic unit membrane which is described as a segment of lipid bilayer in which protein-pigment complexes are bound. Two complexes within the photosynthetic unit are considered the key reaction centers, photosystems I and II (PSI and PSII) [1]. Pigments such as chlorophyll a (Chla), located within PSI and PSII, play a major role in the capture of light energy and the subsequent charge transfer that results in CO_2 reduction [2]. The different roles played by such pigment are performed in differently organized forms and environments as evidenced from many studies *in vivo* [3][4]. Dimeric structures of chlorophyll (Chl) are frequently observed in various apparatus engaged at the initial stage of photosynthesis including light harvesting antennas and electron-transporting reaction centers. Typically, two Chla molecules are shown to be exiton-coupled *in vivo* to form the dimeric primar donor or specialized pairs named P700 and P680 as revealed by X-ray determinations [5][6]. Two monomeric chlorophyll molecules are placed in edge-edge position relative to the specialized pairs and interact strongly with the functional groups of the protein moeity in which they are imbedded, principally with carotenoids, imidazole groups, amide and peptides side chains, and in particular with H_2O as *Lewis* base to form stable Chla pigment-protein light harvesting complexes [5 – 7]. A detailed knowledge on the chemistry of the formation and stabilization of Chla dimer complexes and aggregates is thus of key importance for understanding the complex chemistry of chlorophylls *in vivo*.

Fundamental advances in the understanding of Chla association processes have come from solvation studies carried out in organic solvents of variable polarity. The formation of chlorophyll (a) dimers in concentrated solution of saturated hydrocarbons was first reported by *Lavorel* [8] and by *Weber* and *Teale* [9], based on the comparison of absorption spectra with action spectra for fluorescence of the solution. Further studies based on the analysis of absoption spectra of Chla in different solvents by *Sauer et al.* [10] and *Tomita* [11], demonstrate the existence of monomer-dimer equilibrium. From these studies and from nuclear magnetic resonance, infrared spectrophotometry, and molecular weight measurements by *Katz* and co-workers [12 – 17] and by *Anderson* and

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Calvin [18], important characteristics of the structure of chlorophyll dimers in non polar solvents have been established. Their studies conclude that Chla dimers results chiefly from interaction of the central magnesium of one chlorophyll molecule with a CO functional group of the second. The keto CO group of ring *V* appears to be strongly involved and acts as a donor to the Mg atom of another to generate chlorophyll dimers and higher oligomers in apolar solvents. *Fong* and *Koester* [19][20], however, proposed a symmetrical structure for the Chla dimer in which the carbomethoxy (–O–C=O) functions are used as electron donors to Mg. The presence of two donor functions in Chla considerably complicated matters and whether there are two populations of Chla dimer structures remains a doubtful question. This type of dimerization process is highly dependent on the extent to which extraneous nucleophiles such as H_2O molecules compete for coordination with the Mg. The role of H_2O in the formation of Chla dimer complexes has frequently been invoked to account for complex features of the absorbtion spectra of plant chloroplast and bacterial chromotaphores and, therefore, there has been a longstanding interest in the aggregation of hydrated Chla. First models of Chla special pairs (PSI and PSII), describing the formation of Chla dimers held together *via* H_2O bridges, were proposed by *Katz* and *Norris* [21], *Fong* [22], *Chow et al.* [23], and *Shipman et al.* [24]. In all these models, interactions between Chla and H_2O molecules play an important role with the two Chla molecules being cross-linked by one or two bridging H_2O molecules. The crystal data reported by *Chow et al.* [23] was the first detailed crystallographic structure reported for a chlorophyll derivative, the ethyl chlorophyllide (a) dihydrate obtained from an acetone/H_2O mixture. In this structure, a H_2O dimer is datively bonded to the central Mg atom and forms a H-bond with the keto CO group of the the methyl ester at $C(13^2)$. Since these pioneer works, subsequent investigations have regarded the aggregation mechanisms of Chla in aqueous mixtures as a function of H_2O concentration [19][25 – 38]. *Ballschmiter* and *Katz* in 1960s [25], considered the case of non polar solvents in the presence of H_2O and discussed in some detail the formation of Chla dimers and oligomers, as well as that of different Chla hydrates. They showed that, in aqueous CCl_4 and benzene solutions (and also in aliphatic hydrocarbons for low Chla concentration), H_2O acts as a nucleophile to form monohydrated dimeric and monomeric Chla species. In aliphatic or cycloaliphatic hydrocarbons with increasing Chla concentration, formation of micells of colloidal dimensions corresponding to the motif Chla-H_2O was suggested. In such structure, the H_2O coordinated to the Mg of one chlorophyll molecule and is H-bonded to the ketone and carbomethoxy CO functions to another chlorophyll molecule. This interpretation was reconsidered a few years later by *Fong* and *Koester* [19] who suggested that the formation of Chla hydrates in non polar media probably correspond to the dihydrated $(Chla\text{-}2\,H_2O)_n$ polymeric adduct. *Agiostiano et al.* [34] reported data for Chla hydration and aggregation in binary mixtures of H_2O with acetone, DMF, and MeCN indicating that dihydrated Chla monomers (Chla-2 H_2O) and their corresponding dimers $(Chla\text{-}2\,H_2O)_2$ were formed for sufficiently to high H_2O mol fractions. The same group [32][33][35] [38] investigated the aggregation behaviour of Chla in different aqueous organic solvents, paying special attention to the pigment response in the H_2O rich region. *Vladkova* [39] reported a detailed study on Chla self-assembly in polar solvent-H_2O mixtures. The author showed that in some solvents (MeOH, EtOH, acetone, MeCN), H_2O can replace the coordinated solvent molecule, and for a sufficiently high molar fraction of H_2O, polymeric aggregates $(Chla\text{-}2\,H_2O)_n$ do form. Further investigations have studied the aggregation state of the Chla in *Langmuir–Blodgett* (LB) films using several techniques [40 – 42]. In particular, *Chapados et al.* [40], using electronic and infrared spectroscopies, suggested a multilayer arrangement implying two molecules of H_2O per molecule of Chla. On the contrary to the interpretation of *Chapados* [40], the observations of *Bourque et al.* [42] using scanning tunnelling microscopy are consistent with a model composed only of dimers of Chla. However, *Boussaad* [41] considered that H_2O molecule is a key element in the formation of such dimers.

All of these experimental studies have provided valuable informations on Chla aggregation in organic solvents as well as in mono- and multilayer films of Chla. From all these studies, different forms of Chla-H_2O self associations were suggested ($Chla_2$-H_2O, $(Chla\text{-}H_2O)_2$, $(Chla\text{-}2\,H_2O)_2$), but their structural details, their relative stability, and how the medium influences it, are not accurately known.

Theoretically, there were only few studies on the Chla hydrates, as well as on Chla dimer complexes [43 – 51]. Recently, we carried out the first detailed theoretical study of Chla-H_2O monomer complexes using either DFT theory [46 – 48] or MP_2 [47] approaches, as well as both continuum and discrete-continuum solvation models. Our results provided evidence of the importance of dispersion interactions on the stability of Chla-L and Chla-L_2 (where L was a H_2O or another coordinating molecule such as acetone, THF, DMSO…) monomeric complexes. We showed that the fifth and the sixth coordination of the the Mg in Chla is essentially governed by dispersion interactions. *Nilsson Lill* [49] optimized the structure of a non-covalently linked Chla dimer (coordinated with two histidine molecules) taken from crystal structure using the same approach applied in our previous theoretical study and taking into account the dispersion energy. The author estimated the magnitude of the dimerization of Chla and showed that the dispersion interactions were of great significance for the stabilisation of Chla dimer complex. *Karki* and *Roccatano* [51] have applied molecular dynamical simulations to study the structural and dynamic properties of Chla in three different solvents: H_2O, MeOH, and benzene. The results of their simulations indicated that only two possible different structures of Chla dimers could be observed in benzene. The related geometries of the observed dimers included essential interactions between the ester carbonyl group of one molecule and the Mg of another molecule in contrast to several experimental results in benzene and other similar apolar solvents. In the simulation in H_2O, the authors observed a stable dimer and a pentamer respectively with one and two H_2O molecules sandwiched between the Chla molecules. Though, other dimer and multimeric conformations and or/configurations could also be expected to form in apolar solvents as well as H_2O rich region in solvent/H_2O media as indicated in several studies. They could not be observed in the work of *Karki et al.* [51], and this may be because of the limited size of the system and the length of the simulations they did. In spite of these few recent theoretical studies on the dimers of Chla, there was no definitive model of the association of H_2O with Chla in the hydrated dimers and aggregates. Likewise, fundamental questions closely related to the aggregation of Chla in solution arise. What is the most stable structure of the Chla dimer hydrates? What is the precise role of H_2O molecules in the stabilisation of Chla dimer hydrates? What is the most favorable pathway for the aggregation process?

In the present study, we try to give an answer to these questions using a detailed theoretical study of Chla dimer hydrates at the density functional level of theory. We present data on the different coordination modes of bound H_2O molecules and their effects in the stability of these complexes and the different possible mechanisms (processes) of formation of the dimer complexes in the gas phase and in solution using the continuum salvation model. The results are analyzed and discussed in the light of our previous theoretical studies on the structures of hydrated monomers and in relation to the available experimental data.

Computational Methods. – In this study, calculations were based on a Chla model containing 73 atoms (Chla-73) reported in *Fig. 1* and which was proposed in our previous work [46 – 48]. This model remains the structure of Chla except for the phytyl ester side chain which was replaced by a Me group to reduce the number of atoms for computation. Removal of the phytol chain was considered as a reasonable approximation from several previous studies on chlorophyll a, which shows negligible changes in the absorbtion and redox properties of the chlorophyll molecules in the absence of phytol chain. Quantum chemical calculations were carried out with the Gaussian 03 program [52] using density functional theory (DFT) at the B3LYP/6-31G(d) level. This approach represents an excellent compromise between accuracy and computational cost [47], as it has been shown 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 functional theory. BSSEs were corrected using the counterpoise method [53]. Dispersion energy was estimated as proposed before [47][48], that is using the DFT + D method [54][55].

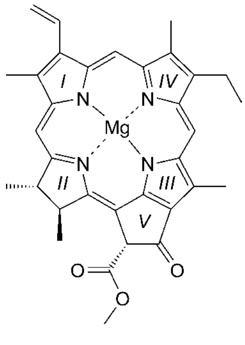

Fig. 1. *Chlorophyll (a) model employed in this work (Chla-73)*

Electrostatic bulk solvent effects were evaluated using the polarisable continuum model [56] and standard cavities in Gaussian 03. Non-electrostatic salvation effects were neglected. Numerical estimations for cavitation, dispersion, and the repulsion energy contributions were reported in a previous work [46]. For Chla-H_2O complexes in liquid H_2O, 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 the different configurations of Chla dimers and their mono- and dihydrated complexes were fully optimized in the gas phase. The structures are reported as Supporting Information and they are discussed in the subsequent section. 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 [48]. Frequencies of vibration were computed in the gas phase for all the monomers of Chla complex systems and their hydrates. The computation of vibration frequencies for dimer complexes was performed only for the Chla dihydrated dimer complex as a test of calculation. The results confirm that the geometry calculated is a minimum (all normal modes are positive) and show that there are a large number of low-frequency modes, which formally should not be treated as harmonic vibrations, indicating that the entropy terms can not correctly be estimated for the system considered. We used the ideal gas approximation and assumed $T = 298.15$ K and 1 atm (gas phase) or 1M concentration (soln.). The computation of association free energies in solution was done according to typical procedures [57] based on thermodynamic cycles. Accordingly, solvation free energies include a correction to account for change in reference state (1.89 kcal mol^{-1} at 298.15 K).

Results and Discussion. – *DFT Optimized Structures. Monomer (Chla) and Dimer of Chlorophyll (a) (Chla)$_2$.* On the basis of the NMR results combined with molecular modelling studies of physically linked chlorin–chlorin dimers [19][58 – 62], three different configurations **A**, **B**, and **C** have been optimized as most convincing models for the Chla dimer structure ($Chla_2$). The **A**, **B**, and **C** configurations result from specific interactions between nucleophilic sites $C(13^1)$ and $C(13^2)$, and the electrophilic

Mg ion. The structure resulting from non covalent π–π interactions between monomers of Chla is expected to be less stable; this was confirmed in exploratory calculations. Optimized structures in gas phase are shown in *Fig. 2*. *Table 1* summarizes ground-state geometrical parameters of chlorophyll (a) monomer (Chla) and those of the three different configurations of chlorophyll (a) dimer ($Chla_2$) in the gas phase.

The main results of the geometry optimization of the Chla model structure of the chlorophyll (a) monomer in vacuum was that the Mg ion is located at the centre and in the plane of the chlorin ring in good accord with experimental X-ray results for chlorophylls and bacteriochlorophylls in proteins [63 – 65]. A closer look at the bond distances of Chla reveals that the Mg–N distance towards ring (II) (the Mg–N(II) distance) is longer than the Mg–N distances towards the other three rings. A similar trend is observed in the experimental X-ray structure of ethyl chlorophyllide (a) dihydrate [23]. Similar results have been obtained from a density functional study of Chla molecule and HF studies of Mg-porphyrins, Mg-chlorins, and Mg-bacteriochlorins [66 – 68].

Due to the dimer formation, the C(13^1) or C(13^2) CO group from one of the monomers (Chla unit1) coordinates to the Mg atom in the second monomer (unit2) making this cationic atom fivefold coordinated in the three optimized structures **A**, **B**, and **C** of $Chla_2$ (*Fig. 2*).

Coordination of the Mg with both nucleophilic CO groups does not change the order of Mg–N bond distances: Mg–N(II) > Mg–N(IV) > Mg–N(I) > Mg–N(III); however, the Mg–N bond asymmetry becomes more manifest since their strength is different. This enhanced asymmetry induces an apparent bending due to the displacement of the Mg atom out of the Chla ring plane towards the axial C=O ligand: 0.41 – 0.43 Å in both chromophores of the configuration of type **A** and 0.37 – 0.4 Å in each one chromophore of the structures of types **B** and **C**. In addition to Mg coordination, the structure alterations detected between the different calculated configurations resulted from the specific orientation of Chla units with respect to one another. In the minimum energy structure of **A**, the Chla molecules are displaced face to face with C(13^2) ester carbonyl groups coordinated with the Mg inducing a nearly C_2 symmetric structure. The distances out of plane

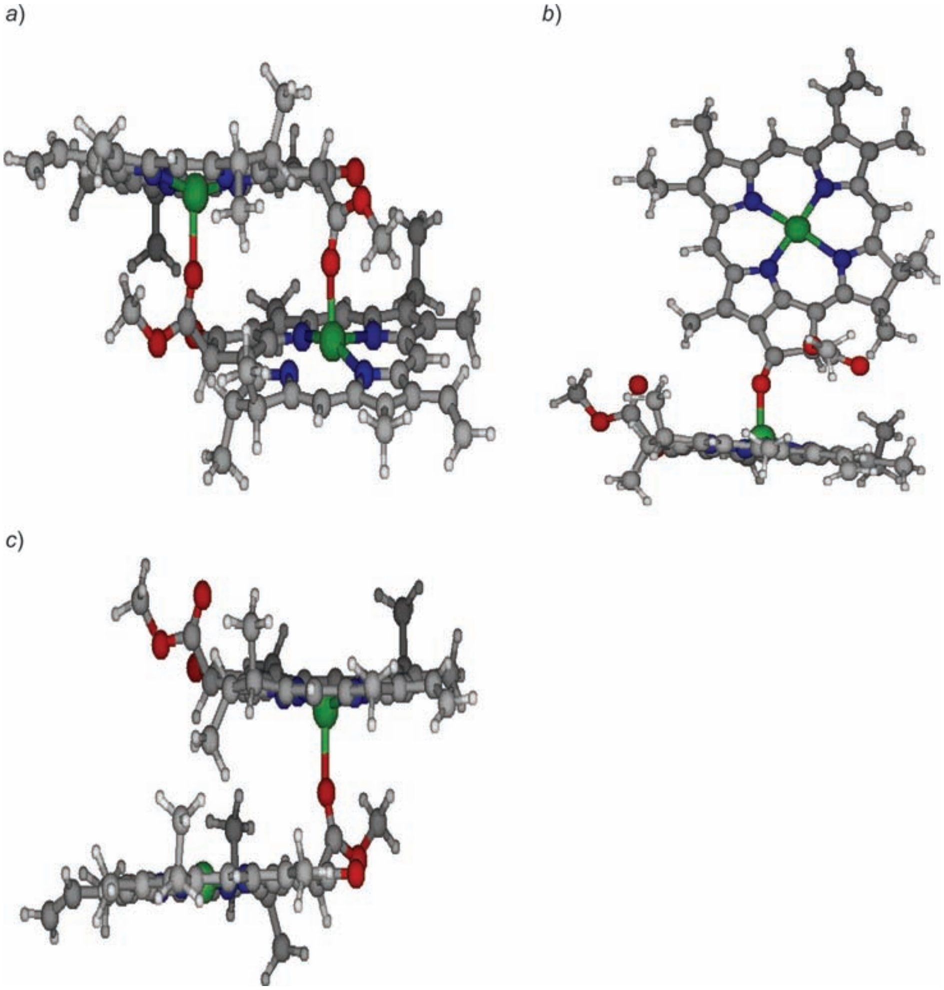

Fig. 2. *Configurations of Chla dimer ($Chla_2$) optimized at B3LYP/6-31G* level: a) configuration of type* **A**, *b) configuration of type* **B**, *and c) configuration of type* **C**

Table 1. *Structural Parameters* [Å] *Characterizing the Optimized Minimum Structures of Chlorophyll (a) monomer (Chla) and the Three Configurations of Types* **A**, **B**, *and* **C** *of Chla Dimer (Chla$_2$) Optimized at B3LYP/6-31G* Level in the Gas Phase*

Bond distances	Chla	Chla$_2$					
		A		**B**		**C**	
		Unit1	Unit2	Unit1	Unit2	Unit1	Unit2
Mg–N(I)	2.031	2.078	2.078	2.030	2.067	2.067	2.030
Mg–N(II)	2.149	2.205	2.205	2.147	2.192	2.201	2.151
Mg–N(III)	2.017	2.054	2.055	2.019	2.053	2.055	2.021
Mg–N(IV)	2.074	2.112	2.112	2.074	2.115	2.123	2.074
> –N > [a])		2.112	2.112	2.068	2.107	2.112	2.069
Mg···O	–	2.101	2.099	2.075		2.129	
Mg···N(I)N(II)N(III)	0.004	0.412	0.409	0.013	0.397	0.402	0.012
Mg···N(I)N(II)N(IV)	0.004	0.411	0.409	0.014	0.398	0.401	0.012
Mg···N(II)N(III)N(IV)	0.005	0.428	0.424	0.012	0.370	0.418	0.029
Mg···N(I)N(II)N(IV)	0.004	0.428	0.424	0.011	0.372	0.417	0.028
>···NNN > [a])	0.004	0.419	0.416	0.012	0.384	0.410	0.020

[a]) < Mg···NNN > Mg is the average distance from the planes of the N–N–N chlorine cycle. It is this distance that informs about the deviation from the planes of the macrocycles. In contrast, < Mg–N > is the average distance with respect to the bond lengths of Mg–N bond in chlorin cycles.

(d.o.o.p.) Mg···N(II)N(III)N(IV) and Mg···N(I)N(II)N(IV) in each Chla unit of the configuration of type **A** are found slightly larger than Mg···N(I)N(II)N(III) and Mg···N(I)N(III)N(IV), resulting in a somewhat bent structure in the side chains of the chlorophyll dimer structure (*Fig. 2,a*). Both are slightly larger in unit1 compared to those detected in unit2. This is can be due to the steric interactions between the two macrocycles, originated from the orientation of the MeO (–O–CO) groups which were found very close to the macrocycles.

The optimized asymmetric structure of type **C**, in contrast, no longer has the possibility of both C(13^2) carbomethoxy groups coordinating to the Mg, as in one molecule, this group points away from the adjacent molecule (*Fig. 2,c*). The structure allows both one carbomethoxy and one or both of the C(13^1) keto groups to participate in the bonding and in this sense is very different from the configuration of type **A**. The two macrocycles are found nearly parallel with some overlap at the rings (III) and (II). The Mg atom remains in the plane of the macrocycle of Unit1 but it is found significantly distorted from its plane in Unit2 (< Mg–NNN > = 0.4 – 0.42 Å). These distortions from planarity are found smaller than those detected in the structure of type **A**. This is due to the steric effects originated by the proximity of only one MeO group from the Chla ring of unit1, in contrast to the structure of type **A** which involve the proximity of both two MeO groups from the chlorin macrocycles as indicated above. The larger electrostatic repulsion in **A** decreases the Mg···O bond distance by distorting the Mg atom from its plane. However, the optimized minimum structure of type **B** involves the direct coordination of the C(13^1) keto group to the Mg atom of another molecule in a non planar skew dimer structure. The two Chla macrocycle planes have an orthogonal or nearly so orientation (*Fig. 2,b*) so as to optimize the directionality of the keto C=O···Mg coordination and minimize overlap repulsions. In this arrangement, the Mg is placed 0.39 – 0.37 Å out of the N(I)–N(II)–N(III)–N(IV) planes in the direction of the intermolecular coordination bond and the length of the Mg···O coordination is calculated to be equal to 2.075 Å. Moreover, the computed Mg–N, d.o.o.p., and Mg···O bond distances are found in quite agreement with those corresponding to axial ligated Chla computed at B3LYP level in our previous works [46 – 48] and compare well with the corresponding values calculated by *Linnanto* and coworkers [68] and by *Heimdal* [43]. They are found in good accord with the values observed in the structure of ethyl chlorophyllide (a) dihydrate determined by X-ray diffraction [23].

Hydrated Dimers of Chlorohyll (a). To study the effect of H_2O ligation on the structure properties of dimers of Chla, we built several molecular models of H_2O ligation of Chla dimers resulting from different types of interactions. We focus on hydrates in which at least one H_2O molecule directly interacts with the Mg atom. Structures in which H_2O molecules are H-bonded to Chla O-atoms, but not to the Mg atom are expected to be less stable. For the monohydrated Chla dimer complex (Cha$_2$-H_2O), several conformations of the monohydrate have been envisaged, but we only report here the most stable one. However, three molecular models **D**, **E**, and **F** for the dihydrated Chla dimer structure (Cha$_2$-2 H_2O), have been studied in this work resulting from three different types of interactions.

Two sets of ground-state conformations **G** and **H** have been studied for the Chla tetrahydrated dimers (Cha$_2$-4 H_2O). Both structures have been suggested on the basis of the X-ray crystal structure of the polymeric ethyl chlorophyllide (a) dihydrate [23]. In *Figs. 3* and *4*, the optimized structures of the hydrates, in the gas phase at the B3LYP/6-31G.* levels, are reported.

Analysis of the structural parameters characterizing the optimized geometries of the hydrated dimers, reported in *Tables 2* and *3*, reveal that the coordination of Mg with H_2O molecules in the mono-, dihydrated, and tetrahydrated dimers does not change the order of Mg–N bond distances:

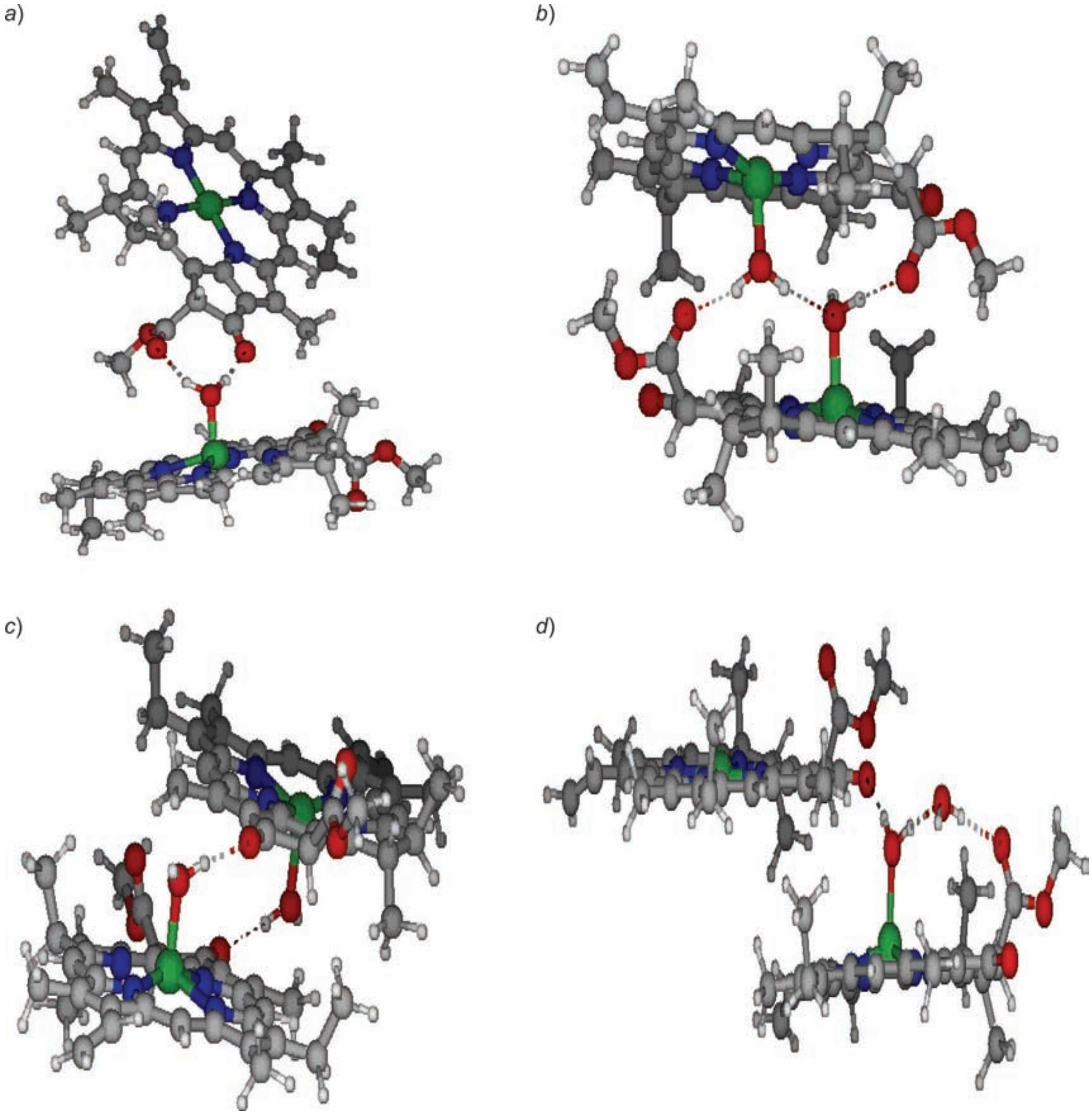

Fig. 3. *a) Structure of monohydrated Chla dimer* ($Chla_2$-H_2O) *optimized at B3LYP/6-31G* level. b), c) and d) Configurations of types* **D**, **E**, *and* **F** *of dihydrated Chla dimer* ($Chla_2$-2 H_2O) *respectively, optimized at B3LYP/6-31G* level.*

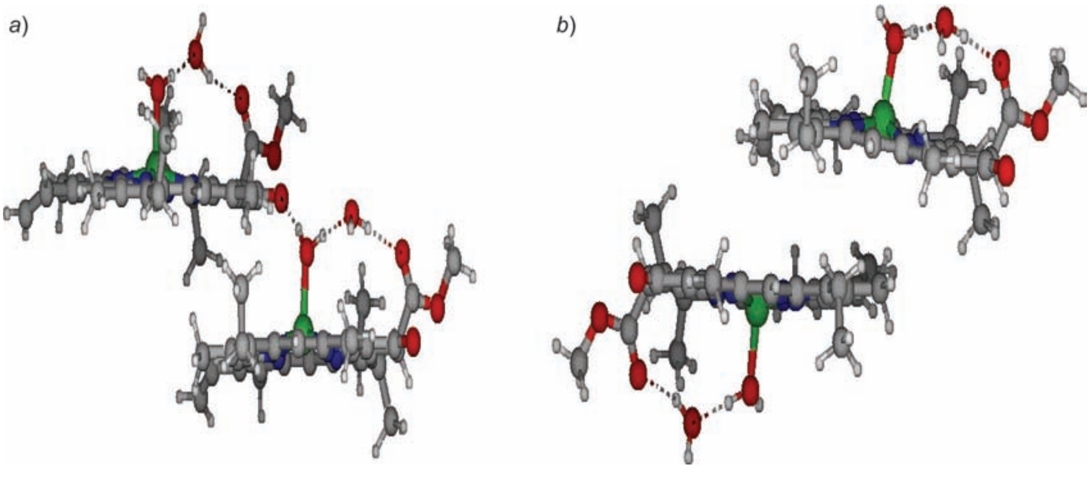

Fig. 4. *Ground state configurations of tetrahydrated Chla dimer* ($Chla_2$-4 H_2O) *optimized at B3LYP/6-31G* level: a) Configuration of type* **G**, *b) Configuration of type* **H**

Table 2. *Structural Parameters* [Å] *Characterizing the Optimized Minimum Structures of the Different Configurations of Types* **D**, **E**, *and* **F** *of Hydrated Chlorophyll (a) Dimers, Optimized at B3LYP/6-31G* Level in the Gas Phase*

Bond distances	$Chla_2–H_2O$		$Chla_2–H_2O_2$					
			D		**E**		**F**	
	Unit1	Unit2	Unit1	Unit2	Unit1	Unit2	Unit1	Unit2
Mg–N(I)	2.030	2.069	2.058	2.070	2.070	2.068	2.031	2.075
Mg–N(II)	2.149	2.194	2.182	2.202	2.196	2.197	2.148	2.206
Mg–N(III)	2.019	2.059	2.047	2.062	2.052	2.049	2.018	2.061
Mg–N(IV)	2.072	2.121	2.106	2.117	2.115	2.116	2.075	2.124
> –N >	2.068	2.111	2.098	2.112	2.108	2.108	2.068	2.117
Mg···O	2.083		2.139	2.086	2.101	2.090	2.053	
Mg···N(I)N(II)N(III)	0.009	0.433	0.334	0.437	0.382	0.381	0.002	0.440
Mg···N(I)N(III)N(IV)	0.009	0.434	0.334	0.438	0.382	0.381	0.002	0.440
Mg···N(II)N(III)N(IV)	0.010	0.380	0.327	0.406	0.406	0.393	0.006	0.433
Mg···N(I)N(II)N(IV)	0.009	0.380	0.327	0.408	0.405	0.392	0.006	0.433
< Mg···NNN > [a])	0.009	0.407	0.329	0.422	0.394	0.387	0.004	0.437

[a]) For the meaning of symbols, see footnote [a]) to *Table 1*.

Table 3. *Structural Parameters* [Å] *Characterizing the Optimized Minimum Structures of the Two Ground State Configurations of Types* **G** *and* **H** *of Tetrahydrated Chlorophyll (a) Dimers ($Chla_2$-4 H_2O), Optimized at B3LYP/6-31G* Level in the Gas Phase*

Bond distances	$Chla_2–4\ H_2O$			
	G		**H**	
	Unit1	Unit2	Unit1	Unit2
Mg–N(I)	2.067	2.074	2.063	2.067
Mg–N(II)	2.194	2.207	2.191	2.196
Mg–N(III)	2.049	2.061	2.059	2.046
Mg–N(IV)	2.106	2.124	2.114	2.100
< Mg–N >				
Mg···O	2.091	2.052	2.096	2.097
Mg···N(I)N(II)N(III)	0.370	0.440	0.410	0.347
Mg···N(I)N(III)N(IV)	0.370	0.440	0.411	0.347
Mg···N(II)N(III)N(IV)	0.369	0.432	0.358	0.346
Mg···N(I)N(II)N(IV)	0.369	0.433	0.361	0.346
< Mg···NNN > [a])	0.370	0.436	0.385	0.346

[a]) For the meaning of symbols, see footnote [a]) to *Table 1*.

Mg–N(II) > Mg–N(IV) > Mg–N(I) > Mg–N(III); however, differences of about 0.001 – 0.015 Å in the Mg–N bond distances are detected between the dimers and their hydrates.

The optimized minimum structure of the mono-hydrated dimer of Chla with a H_2O molecule coordinating both the keto and ester CO groups is found very similar to the configurations of type **B** and **C** of the Chla dimer indicating that mono-ligation of the dimer with H_2O does not significantly alter the structure of the Chla-free H_2O dimer complex. Our results show that the H_2O ligation causes the Mg ion of Chla to move slightly away from the center of the chlorin ring so that all Mg–N bond distances (except Mg–N(I) belonging to the coordinated chromophore noted as unit2) increase by 0.002 – 0.009 Å. Calculation reveals that both the $C(13^2)$ ester and the $C(13^1)$ keto CO groups and the chlorin plane are neither parallel nor perpendicular, being in an intermediate direction. The optimized minimum structure of the dihydrated dimer of type **D** involving bi-ligation of the carbomethoxy CO groups at position $C(13^{2\bullet})$ with H_2O molecules (one molecule for each CO group; *Fig. 4,b*), is calculated to be slightly asymmetric in comparison with the non-biligated dimer structure of type **A**. The calculated distances (d.o.o.p.) indicate a Mg ion more shifted from the plane of one chromophore than from that of the other; the maximum difference between the d.o.o.p. is of 0.103 Å. However, the optimized minimum structure of the configuration of type **E**, in which the two H_2O molecules provide the structural linkage by coordination to the Mg of one chromophore and H-bonding to the keto CO group in the other (*Fig. 4,c*), is found more symmetric than the configuration of type **D** as indicated by the values of bond distances reported in *Table 2*; the large discreapances between the Mg–N and d.o.o.p. distances of the two macrocycles of two chromophores (sub-units) is found equal to 0.003 Å and 0.013 Å, respectively. In this type of configuration, the two chlorin planes are approximately parallel, making rings (III) and (V) as the region of maximum overlap of the ring systems. The interplanar separation is found 3.78 Å compared to 5.6 Å in the configuration of type **D** where the planes are much further separated. These values are found in quite good agreement with those corresponding to the models of Chla special pairs modelled by *Pellin et al.* [69]. In contrast to the configurations of types **D** and **E**, the minimum structure corresponding to the dihydrated dimer configuration of type **F** is found to be the more asymmetric one with Mg atom significantly shifted from the plane of the macrocycle of unit1. The bonding interactions within $(Chla)_2$-2 H_2O are assumed to be equivalent to a symmetrical addition of a Chla molecule to a Chla-2 H_2O system. These interactions are accordingly seen to be equivalent to the subunit interactions in the ethyl chlorophyllide (a) dihydrate polymeric aggregates [23].

In this structure, the optimized Mg···O distance is 2.053 Å, the average Mg–N distance is 2.093 Å, the Mg distance to N(I)N(III)N(IV) plane is 0.44 Å. The corresponding X-ray values [23] are 2.035, 2.086, and 0.385 Å, respectively, in fair agreement with our results. Both configurations of types **D** and **F** generate a cooperative H-bond pattern comparable to that observed in the crystallographic structure of ethyl chlorophyllide (a) dihydrate [23] in which the two H_2O O-atoms are separated by 2.8 Å. The optimized values in **D** and **F** are found equal to 2.9 and 2.7 Å, respectively, in fairly agreement with those observed values in ethyl chlorophyllide (a) dihydrate [23].

For the tetrahydrated Chlorophyll (a) dimers, the results of calculations reveal the non-planarity of the chlorin ring in both chromophores (unit1 and unit2) of the first ground-state dimer structure. This is documented by the values of the distances out of plane between Mg and the four N-atoms N(I), N(II), N(III) and N(IV): 0.37 – 0.36 Å and 0.432 – 0.44 Å, respectively, in unit1 and unit2 (*Table 3*). Comparison between the first ground-state configuration of type **G** of the tetrahydrated dimer and the optimized structure of the dihydrated dimer of type **F** indicate that the nonplanarity detected in both Chla units of structure of type **G** is due to outer two strong H-bonds in the H_2O dimer molecules between Mg ion and $C(13^2)$ CO group which are absent in the configuration of type **F** of the dihydrated dimer. The optimized **G** structure has three H-bonds between the two Chla units, but one is a stronger $C(13^1){=}O\cdots H{-}O$ interaction, as opposed to the weaker $C(13^1){=}O\cdots H{-}O$ bond found in the structure **F** dihydrated dimer structure. The ground-state configuration of type **G** is found more stable than the mimimum optimized structure of the configuration of type **H** as well as than all the different configurations of the dihydrated dimers (see the available supplementary material) at the B3LYP/6-31G* level. This confirms the remarkable cooperative character of the interactions in the configuration of type **G** of the tetrahydrated dimers.

Comparison of the calculated electronic energies of the dihydrates (available in the supplementary material) indicate that the configuration of type **D** is more stable than that of type **E** and **F** at B3LYP/6-31G* level. The ester C=O group at $C(13^2)$ and the cooperatively interactions between Mg···O and H-bond play a key role on this remarkable stabilization. The difference in minimum energy between the ground-state structures of types **D** and **E** is only 3.9 kcal mol^{-1} at this level of calculation and thus a rapid equilibrium between the two configurations can be predicted in solution. Therefore, mixtures of predominantly configurations of types **D** and **E** may be predicted in solution.

Dimerization of Chlorophyll (a) and Hydrated Chlorophyll (a) Monomers. The chlorophyll (a) dimer-monomer equilibria can be described by *Eqns. 1 – 8*:

$$2\ \text{Chla} \leftrightarrow \text{Chla}_2 \tag{1}$$

$$2\ \text{Chla-2}\ H_2O \leftrightarrow \text{Chla}_2 + 4\ H_2O \tag{2}$$

$$2\ \text{Chla-}H_2O \leftrightarrow \text{Chla}_2 + 2\ H_2O \tag{3}$$

$$2\ \text{Chla-2}\ H_2O \leftrightarrow \text{Chla}_2\text{-}H_2O + 3\ H_2O \tag{4}$$

$$2\ \text{Chla-}H_2O \leftrightarrow \text{Chla}_2\text{-}H_2O + H_2O \tag{5}$$

$$2\ \text{Chla-2}\ H_2O \leftrightarrow \text{Chla}_2\text{-2}\ H_2O + 2\,H_2O \tag{6}$$

$$2\ \text{Chla-2}\ H_2O \leftrightarrow \text{Chla}_2\text{-4}\ H_2O \tag{7}$$

$$2\ \text{Chla-}H_2O \leftrightarrow \text{Chla}_2\text{-2}\ H_2O \tag{8}$$

In the following subsections, we present the energetics for dimer formation of anhydrous and hydrated Chla dimers, in the gas phase and in non-polar media. To study the formation of anhydrous Chla dimers, the three configurations of types **A**, **B**, and **C** of Chla_2 have been considered. In aqueous non-polar media, calculations have been carried out for the most stable conformation of the hydrated Chla monomers: monohydrate (Chla-H_2O) and the dihydrate (Chla-2 H_2O) (with Chla 5-coordinated). In the most stable structure of the monohydrate using the Chla-73 model, the H_2O molecule coordinated to the Mg atom is synperiplanar with respect to the ester group. The dihydrate structure exhibits Mg–H_2O dimer interactions, the outer H_2O molecule interacts also with the CO ester group. This generates a cooperative H-bond pattern comparable to that observed in the crystallographic structure of the ethyl chlorophyllide (a) dihydrate [23]. For the hydrated dimers, calculations were done for the different configurations proposed, but we only report the results for the most stable configuration of type **D** for dihydrated dimers (Chla_2-2 H_2O) and the ground-state of type **G** for the tetrahydrated species (Chla_2-4 H_2O).

Interaction Energy, Entropy, and Free Energy Changes for the Chla Dimerization Processes. In Dry Non-polar Solvents. We focused on the dimerization processes in non polar solvents which were assumed to be noncoordinating. We considered the cyclohexane as a prototype. In *Table 4*, the energetics for the dimerization process 1 in gas phase and in cyclohexane solution are summarized.

The results of our calculations indicate that the dimerization of Chla at the present geometries are favorable in the gas phase and in solution, in good accord

Table 4. *Energetics* [kcal · mol^{-1}] *for the Dimerization Processes of Chla in the Gas Phase* a) b) *and in Cyclohexane Solution*

Eqn.		ΔE^{uncorr}	ΔE	ΔE_{disp}	ΔE^{el}	$\delta\Delta G_{\text{sol,elec}}$	ΔG_{g}	ΔG_{s}
1a	2 Chla → $(\text{Chla})_2$ (**A**)	– 28.2	– 15.0	– 30.2	– 20.6	7.5	– 36.1	– 30.5
1b	2 Chla → $(\text{Chla})_2$ (**B**)	– 18.7	– 11.0	– 15.8	– 14.2	4.5	– 17.7	– 15.1
1c	2 Chla → $(\text{Chla})_2$ (**C**)	– 14.3	– 7.3	– 18.7	– 9.4	4.9	– 16.9	– 13.9

a) Calculations at the B3LYP/6-31G(d) + D level. b) Meanings of symbols are follows: ΔE^{uncorr} and ΔE represent the BSSE-uncorrected and BSSE-corrected electronic energy differences, resp.; ΔE_{disp} is the dispersion correction to the B3LYP energy. ΔE^{el} represents the electrostatic effect of the continuum on the electronic energy of the process, computed with PCM method. ΔG_{g} and ΔG_{s} correspond, resp., to the free energy changes in the gas phase (including the dispersion correction and BSSE error) and in solution at $T = 298.15$ K and 1 atm. ΔG_{g} is calculated using $\Delta E + \Delta E_{\text{disp}} +$ 9.1 kcal · mol^{-1}. $\delta\Delta G_{\text{sol,elec}}$ stands for the difference in electrostatic solvation energies defined as

$$\delta\Delta G_{\text{sol,elec}} = \sum_{\text{i=products}} \Delta G_{\text{i}}^{\text{sol,elec}} - \sum_{\text{j=reactants}} \Delta G_{\text{j}}^{\text{sol,elec}}$$

($G_{\text{sol,elec}} = E^{\text{el}} - E_{\text{g}}$ with E_{g} = the electronic energy calculated at B3LYP/6-31G*). Note that ΔG_{s} does not necessarily correspond to the sum of ΔG_{g} and $\delta\Delta G_{\text{sol,elec}}$, because it also includes the concentration correction, as explained in the method of calculation section.

with some of experimental results [12 – 18]. The value of the dimerization energy (ΔE) at the most stable configuration of type **A** is found equal to – 28.2 kcal mol^{-1} at the B3LYP/6-31G* level, in quite good agreement with the value – 22 kcal mol^{-1} calculated by *Wang* and *Hu* [70] using MP2 method and the modified basis set 6-31G*(0.25). Inclusion of the dispersion correction and BSSE errors shifts the calculated dimerization energy (ΔE) from – 28.2 kcal mol^{-1} to – 45.2 kcal mol^{-1}. It is important to note here the important effect of the dispersion interaction. The latter has been found to play an important role in the stability of Chla-H_2O and Chla-solvent adducts [46 – 48]. Standard DFT methods are not expected to lead to accurate results unless corrected for such a contribution, as was done here.

According to our preceeding studies on monomeric mono-ligated (Chla-L) and bi-ligated Chla-L_2 complexes at this B3LYP/6-31G* level of calculation [46 – 48], correction terms between relative enthalpies and relative potential energies (sum of ΔZPE and $\delta\Delta$H) were calculated in the range from 1.4 kcal mol^{-1} (for the first hydration of Chla) to 2 kcal mol^{-1} (for the second hydration). These values of thermal corrections were found when small compared with the corresponding free energy of complexation (varying from – 8.9 kcal mol^{-1} (for the first hydration) to – 11.5 kcal mol^{-1}(for the second hydration) in the gas phase [48]. To test this assumption in the case of the Chla dimer, the magnitude of the correction terms for the dimerization process was calculated in this study and is found equal to 2 kcal for dimer formation of dihydrated Chla dimer complex (Chla$_2$-2 H_2O; at the most stable configuration of type **D**). These values of thermal corrections are found small comparing with the calculated free dimerization energy of about – 22 kcal mol^{-1}, and, therefore, these terms could be neglected in the present study. In addition, the entropy term for the dimer formation has been determined to be – 30.6 ± 2 cal K^{-1} mol^{-1} from the experimental results on the dimerization of Chla in methylcyclohexane solution [71]. At room temperature, the value of – 30.6 cal K^{-1} mol^{-1} for the entropy change corresponds to – 9.1 kcal mol^{-1} in methylcyclohexane solution. The free dimerization energy could thus be evaluated from the difference between the relative potential energy and $T\Delta S$. The corresponding values were found – 36.1, – 17.7 kcal mol^{-1}, and – 16.9 kcal mol^{-1}, respectively, for the dimerization of Chla at the configurations of types **A**, **B**, and **C** (*Table 4*).

In cyclohexane solution, the results of calculations (*Table 4*) indicated that, solvation opposes dimer formation, as all processes become less negative (less exothermic if we neglect the thermal correction terms). As a consequence, all the three dimerization processes were exergonic indicating that these processes are quite favorable in apolar cyclohexane solution and room temperature. The free dimerization energy at the configuration of type **A** was estimated to be significantly lower (by 15.4 – 16.7 kcal mol^{-1}) than those corresponding at the configurations of types **B** and **C**, showing that the carbomethoxy-C(13^2) ester linkages as the preferred configuration for the Chla dimer. This was found in accord with the classical model of *Fong* [19][20] and with the analysis of fluorescence spectrum of Chla dimer in apolar solution by *De Wilton et al.* [72]. It was interesting to note the similarity in stability between the configurations of types **B** and **C** at B3LYP-DCP level, in spite of the different interactions and orientations of macrocycles in the related structures. This result confirmed the experimental observations from the infrared absoption and nuclear magnetic resonance studies in dry nonpolar solution indicating that both nucleophiles, keto C=O at C(13^1) and C(13^2) ester carbomethoxy carbonyl can be involved in the interaction between Chla molecules and models involving such interactions are both quite possible for Chla dimer structure. The *Process 1a* is more exergonic than either *1b* or *1c* and thus, an equilibrium between the three dimer species with predominantly, configuration of type **A** of the dimer can be predicted in non-polar solution.

In Aqueous Nonpolar Media. In the study on the hydration of monomeric Chla [47], we showed that addition of H_2O to solutions of Chla in nonpolar solvents will spontaneously lead to the formation of both monohydrated (Chla-H_2O) and dihydrated (Chla-2 H_2O), monomeric Chla species (Chla-2 H_2O), with the latter being much more stable. Therefore, a chemical reaction in which four H_2O molecules are added to Chla to form two molecules of the dihydrated Chla monomer according to the following equation: $2\ \text{Chla} + 4\ H_2O \leftrightarrow 2\ (\text{Chla-}2\ H_2O)$, was considered as one of the most common (even the most favorable) hydration processes in aqueous nonpolar solvents. The other possible equilibria existing in solution such as monohydration ($2\ \text{Chla} + 4\ H_2O \leftrightarrow 2\ (\text{Chla-}H_2O) + 2\ H_2O$) and the dimerization process of Chla and its hydrates (*Processes 2 – 8*) are also considered here. All these postulated equilibria have, experimentally, been shown to be strongly interrelated processes and a precise description of the dimerization processes requires a knowledge and studies on the hydration free energies.

In *Table 5*, we did not include calculation for the *Processes 4* and *5*, because exploratory calculations showed that the formation of monohydrated Chla dimer is highly disfavored at B3LYP/6-31G*. Thus, we have limited the discussion here to the formation of dihydrated and tetrahydrated dimer species.

Gas-phase results showed that the *Process 2* exhibits a highly positive dimerization energy ($\Delta E = 43.3$ kcal mol^{-1}) at the DCP/B3LYP level and a favorable entropic term ($-T\Delta S = -31.5$ kcal mol^{-1}). It was found endergonic in the gas phase at the DCP/B3LYP level. The bulk solvent effect, estimated by $\delta\Delta G_{\text{sol,elec}}$, was found significantly negative, favoring the formation of the Chla dimer in cyclohexane. Despite the negative value of $\delta\Delta G_{\text{sol,elec}}$, the dimerization process remained endergonic in cyclohexane. These results predicted that Chla dimers cannot be formed from dimerization of dihydrated Chla monomers. This was found in quite good agreement with the experimental works on the state of aggregation of Chla in apolar solution which indicate that anhydrous Chla dimers (Chla_2) dissociate in aqueous solutions [27]. The dimerization energy for the *Process 3* was found negative ($\Delta E = -7.6$ kcal mol^{-1}) at DCP/B3LYP level. Owing to the release of two H_2O molecules, *Process 3* was calculated to be less favorable entropically ($-T\Delta S = -7.9$ kcal mol^{-1}) than *Process 2*. It was found strongly exergonic in gas phase at DCP/B3LYP level. In cyclohexane, *Process 3* remained highly exergonic in spite of a rather positive value of $\delta\Delta G_{\text{sol,elec}}$, indicating that dimers (Chla_2) could be formed from dimerization of the monomeric monohydrates in sharp contrast with the results obtained for *Process 2*. The dimerization of the dihydrates with two H_2O molecules are released (*Process 6*) was found to be entropically favored ($T\Delta S$ *ca.* -7.9 kcal mol^{-1}) and slightly exergonic in gas phase. The bulk solvent effect was relatively small and negative, favoring the formation of the dihydrated dimer in solution. The free dimerization energy was found rather slightly positive indicating a weakly endergonic process in cyclohexane. The results of calculations for *Processes 7* and *8* indicated that dimerization of mono- ($\text{Chla-}H_2O$) and dihydrated Chla ($\text{Chla-}2\ H_2O$) monomers were both quite favorable in the gas phase at DCP/B3LYP level. Both processes were exergonic, despite a quite unfavorable entropic terms due to H_2O coordination. The dimerization of mono-hydrated Chla monomers appeared to be more favorable than that of the dihydrated Chla monomers. The $\delta\Delta G_{\text{sol,elec}}$ quantities are both positive in cyclohexane. Nonetheless, the formation of dihydrate and tetrahydrate remained quite strongly exergonic in cyclohexane.

To further discuss the formation of Chla hydrates in gas phase and in solution, free energy calculation of all hydrates and their dimers species relative to a common standard ($2\ \text{Chla} + 4\ H_2O$) have been done. The results are summarized in *Table 6*. It can be seen from these results, that all hydration processes are strongly exergonic. Chla has a slight preference for full hydration than for dimeri-

Table 5. *Energetics* [kcal · mol^{-1}] *for the Dimerization Processes of Mono- and Dihydrated Chla Monomers in the Gas Phase*[a])[b]) *and in Cyclohexane Solution*

Eqn.		ΔE^{uncorr}	ΔE	ΔE_{disp}	ΔE^{el}	$\delta\Delta G_{\text{sol,elec.}}$	ΔG_{g}	ΔG_{s}
2	$2\ (\text{Chla-}2H_2O) \rightarrow (\text{Chla})_2 + 4H_2O$	53.4	43.6	– 0.3	46.9	– 6.6	5.1	4.2
3	$2\ (\text{Chla-}H_2O) \rightarrow (\text{Chla})_2 + 2H_2O$	7.8	9.6	– 17.2	8.3	0.5	– 18.3	– 15.9
6	$2\ (\text{Chla-}2H_2O) \rightarrow (\text{Chla}_2\text{-}2H_2O) + 2H_2O$	14.1	13.4	– 5.7	12.6	– 1.5	– 0.2	0.2
7	$2\ (\text{Chla-}2H_2O) \rightarrow (\text{Chla})_2\text{-}4H_2O$	– 10.5	– 5.2	– 11.8	– 8.4	2.1	– 4.6	– 4.4
8	$2\ (\text{Chla-}H_2O) \rightarrow (\text{Chla}_2\text{-}2H_2O)$	– 31.5	– 20.6	– 22.7	– 25.9	5.6	– 23.6	– 19.9

[a]) Calculations at the B3LYP/6-31G(d) + D level. [b]) For the meaning of symbols, see footnote [b]) to *Table 4*.

Table 6. *Energetics* [kcal · mol^{-1}] *for the Hydration Processes of Chla Monomers in the Gas Phase*[a])[b]) *and in Cyclohexane Solution*

Eqn.		ΔE^{uncorr}	ΔE	ΔE_{disp}	ΔE^{el}	$\delta\Delta G_{\text{sol,elec.}}$	ΔG_{g}	ΔG_{s}
1a-2	$2\ \text{Chla} + 4H_2O \rightarrow 2\ (\text{Chla-}2H_2O)$	– 81.6	– 58.6	– 30	– 67.5	14	– 41.2	– 34.4
1a-3	$2\ \text{Chla} + 4H_2O \rightarrow 2\ (\text{Chla-}H_2O) + 2H_2O$	– 36	– 24.6	– 13	– 28.9	7	– 17.8	– 14.6
1a	$2\ \text{Chla} + 4H_2O \rightarrow \text{Chla}_2 + 4H_2O$	– 28.2	– 15.0	– 30.2	– 20.6	7.5	– 36.1	– 30.5
1a-3 + 8	$2\ \text{Chla} + 4H_2O \rightarrow \text{Chla}_2\text{-}2H_2O + 2H_2O$	– 67.5	– 45.2	– 35.7	– 54.8	12.6	– 41.4	– 34.5
1a-2 + 7	$2\ \text{Chla} + 4\ H_2O) \rightarrow (\text{Chla}_2\text{-}4H_2O)$	– 92.1	– 63.8	– 41.7	– 75.9	16.2	– 45.8	– 38.8

[a]) Calculations at the B3LYP/6-31G(d) + D level. [b]) For the meaning of symbols, see footnote [b]) to *Table 4*.

zation. As predicted above, the formation of the dihydrated monomer with a five coordinated Mg structure was the most favorable hydration process. The hydration of the dimer was weakly exergonic compared with the hydration of Chla monomeric species. The predicted structure of the tetrahydrated dimer complex was more stable than the dihydrated dimer because of cooperatively between the Mg–H_2O interaction and H_2O–H_2O and H_2O–CO H-bonds. According to these results, in non polar solvent, Chla will readily form monomeric and dimeric hydrated complexes in the presence of H_2O, even in the form of traces. The dihydrate/tetrahydrate equilibrium will be slightly shifted toward the formation of the tetrahydrated dimers. These results agreed with the experimental findings that, in the presence of small amounts of H_2O, there is formation of mono and dihydrated Chla species, as well as their corresponding dimers [19][28]. They corroborate the experiments facts that upon further addition of H_2O to a solution inducing Cha aggregation, the equilibrium mixture is greatly shifted in favour of the dihydrated polymeric Chla form (Chla-2 H_2O)$_n$ [19][27][72]. It has to be noted that the structure of this aggregate has been the subject of extensive debate in the literature [31][37][73]. Evidence for the presence of neutral structural H_2O molecules forming a H-bonded network in an arrangement similar to that of crystals of ethyl chlorophyllide (a) dihydrate [23] has been reported [36][37][73].

On the other hand, comparison with the available experimental results obtained by *Hoshino et al.* [71] reveals differences in the stoechiometry between H_2O and Chla molecules in the structure of the hydrated Chla dimer as well as in the pathways for the dimer formation. From their analysis of the absorption spectra at ambient and low temperatures, *Hoshino et al.* [71] indicated that monohydrated monomeric Chla is formed at the first stage and this one is dimerized at low temperature to form the dihydrated dimeric structure ($Chla_2$-2 H_2O). However, our results predict that the formation of dihydrated monomeric Chla (Chla-2 H_2O) is more favorable at the first stage and thereafter, this dihydrate can dimerize to form the tetrahydrated Chla dimer ($Chla_2$-4 H_2O).

It is to be noted that there was no direct structure determination of the hydrated dimer complex, and the assignment of the dimer structure was done qualitatively based on studies reported by *Wasielewski et al.* [74] and *Boxer et al.* [75] and by comparison with the results reported for the formation of dimeric pheophytins [76]. Indeed, the assignment of the ($Chla_2$-2 H_2O) structure was based on their observations made at low temperature below 240 K. It is known that in non-polar solvents, the absorption spectrum of Chla varies substantially depending on the procedures of preparing the solution, in particular at low temperature, and the amount of H_2O causes substantial changes in the absorption spectrum of Chla in hydrocarbon solvents. No further experimental studies at ambient temperature were reported in the literature, and our results may offer a new possible pathway for hydrated Chla dimer formation processes. They can be considered helpful to resolve the doubtful questions about the stoechiometry between bound H_2O and Chla molecules in the hydrated Chla dimer which was a controversial one in literature. Our results provide new insights to the aggregation of chlorophyll (a) in solution.

Conclusions. – We have presented here the first theoretical study of the Chla dimer hydration process in solution, in order to rationalize experimental observations. The structure of the chlorophyll dimer and their hydrates has been optimized for the first time using dispersion-corrected density functional theory (B3LYP-DCP). The magnitude of the interaction energy within the dimer is determined. It is important to recall here the important effect of the dispersion energy on the stabilization of Chla dimers. Our results obtained provide numerical estimations of the thermodynamic quantities for hydration processes involving the Chla monomer and Chla dimers which are not easily accessible (or inaccessible) to experiment. The estimated values of the hydration free energies allow to obtain knowledge about the most thermodynamically favorable pathway forming Chla dimer hydrates and to elucidate the structural stabilization role of H_2O molecules in the formation of such complexes. According to the present calculations, in dry apolar solvent, dimer formation of anhydrous $Chla_2$ having a symmetric configuration of type **A** is the most favorable dimerisation process at B3LYP-DCP level. Calculations reveal the similarity in stability between the two nonsymmetrical configurations of types **B** and **C** of the Chla dimer at this B3LYP-DCP level, in spite of the different interactions and orientations of chlorin macrocycles in the related structures. This result confirms the experimental observations from IR absorption and NMR studies in dry apolar solution indicating that both nucleophiles, keto C=O at C(13^1) and the ester carbomethoxy CO at C(13^2) can be involved in the interaction between Chla molecules and models involving such interactions are quite possible for the Chla dimer. Otherwise, this also can provide an explanation why there were many controversies and a debate along many years in assigning a fixed model for the Chla dimer, since the two possible Chla dimer configurations were calculated to be very close in energy. Therefore, equilibrium between the different possible configurations, with predominantly dimers at the configuration of type **A**, can be predicted in dry apolar solution and ambient temperature. Addition of H_2O to solutions of Chla in apolar solvents will spontaneously lead to the formation of monomeric and dihydrated and tetrahydrated dimer complexes. The dihydrate/tetrahydrate dimer equilibrium will be slightly shifted toward the formation of the tetrahydrated dimers. Both the dimerization and hydration are cooperative and proceed according to two steps of equilibrium: in the first step, our calculations predict that the formation of the dihydrated monomers is the most favorable process. In a second step, the dihydrated Chla (Chla-2 H_2O) can dimerize on to the tetrahydrated Chla dimer complex ($Chla_2$-4 H_2O). These theoretical results may offer a new possible pathway for hydrated Chla dimer formation process. They can provide new insights to the aggregation of chlorophyll (a) in solution and could be considered useful for the design of new experiments.

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Supplementary Data. Optimized geometries in the gas phase and total energies of the described Chla complexes can be found in the supporting information. This is available free of charge *via* the internet at http://www.wileyonlinelibrary.com.

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