

Self-Association of Amphotericin B in Water. Theoretical Energy and Spectroscopy Studies

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This present work is devoted to the study of the behavior in water of important antifungal drug, the amphiphilic Amphotericin-B (AmB) molecule. Experimental spectroscopy data, namely, the modification of the AmB UV absorption spectrum in water (hypsochromic frequency shift) with the increasing AmB concentration, indicate the self-association of the molecule in water. In this work, a possible three-dimensional structure of the AmB self-associated species present in water has been determined by using in concert both spectroscopy and energy information and not by fitting the calculated UV absorption spectrum to the experimental one. The calculations have been performed upon AmB dimers and helicoidal oligomers involving 2–8 unit cells. The total energy of any helicoidal oligomer has been evaluated taking into account, besides the intermolecular interactions (computed using an accurate *intermolecular interaction potential*), (i) the “thermal” effects evaluated utilizing the well-known formulas of *statistical thermodynamics* and (ii) “solvent” effects calculated in the framework of an adequate *continuum* model. It has been shown that all energy results obtained for the different helicoidal oligomers may be extended to infinite helicoidal aggregates. In conclusion, (i) The study of the evolution with Nuc (the number of unit cells) of the total energy (involving all here-above cited terms) of both *single* and *double* helices has shown that the highest energy gain (*per* AmB molecule) is obtained with the *double* helix. (ii) Such a stabilization mainly proceeds from van der Waals forces together with entropy factors. (iii) This optimized *double* helix, which is characterized by an helicoidal angle, $\theta = 30^\circ$, and an intercell distance R as 5.7 Å, (thus a pitch of 62.7 Å), leads to an hypsochromic frequency shift ($\Delta\nu_+$) calculated as 3950 cm^{-1} , a value which is quite close to the experimental one, 4368 cm^{-1} .

Introduction

The goal of this work was a theoretical determination of the structure of aggregates formed by the self-association in water of amphotericin-B (AmB), (Figure 1) an amphiphilic molecule characterized by (i) a rigid heptaene unit and a polyol chain fused together into a quasi planar macrolactone ring and (ii) a flexible polar head containing a carboxylate group and a mycosamine ring. AmB is a polyene antibiotic used in deep-seated mycotic infections.^{1–6} The assumption of presence of AmB self-associated complexes in water^{7–12} mainly proceeds from experimental observation in water of a change of the UV absorption spectrum (first $\Pi \rightarrow \Pi^*$ transition) when the AmB concentration increases:^{7–9} (i) at low AmB concentration (below 10^{-6} M), the absorption spectrum is similar to the one observed in natural conjugated polyenes (heptaenes); it presents a vibrational structure and the main band (0–0 transition) is located at 24450 cm^{-1} (409 nm), (ii) while above 10^{-6} M, a totally new spectrum (without any vibrational structure) is observed, the absorption maximum detected at 28818 cm^{-1} (347

nm) is blue shifted, $\Delta\nu_+ \approx 4368 \text{ cm}^{-1}$ ($\Delta\lambda = 62 \text{ nm}$). In the framework of the *excitonic* theory,¹³ such an hypsochromic shift ($\Delta\nu_+$) should proceed from some parallel (HH) or antiparallel (HT) *stacked* dimers (Figure 2 a and b), *In-plane* dimers (Figure 3)) or some aggregates (*single* or *double* helicoidal ones). On the basis of the value of $\Delta\nu_+$ (calculated within *excitonic* model), depending on (i) the relative orientation of the different chromophores involved in the molecular complex and (ii) the nearest-neighbor intermolecular distances, one could determine, in theory, the most important geometrical parameters of some aggregate models by fitting the theoretical UV absorption spectrum (calculated within the excitonic theory for instance) to the experimental one. In practice, such a strategy is not so simple because in most of cases (i) one has to consider more than one or two geometrical parameters and (ii) several sets of geometrical parameters could match with experiment. In fact, we think that the different AmB self-associated structures proposed to explain the experimental data, should be borne out by computer modeling, thus both energy and spectroscopy theoretical calculation techniques have to be used in concert to settle an accurate structure of the AmB self-associated species. Due to the complexity of the AmB molecule, the completion of such a curriculum is not so easy, both spectroscopy and

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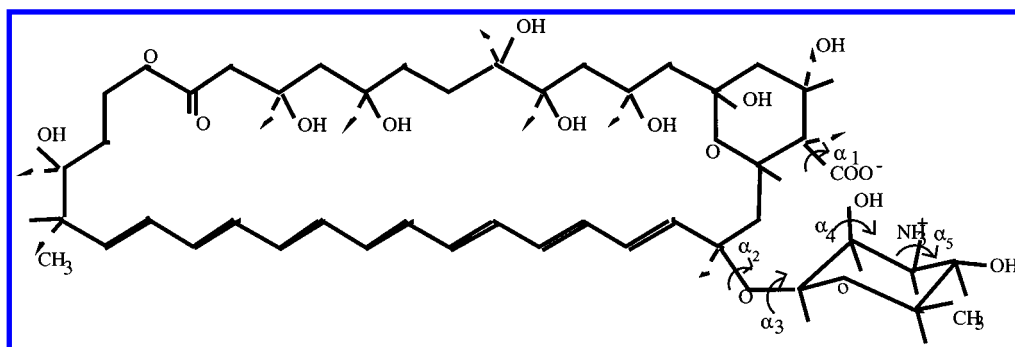


Figure 1. Structure of Amphotericin B molecule (AmB). Polar head Degrees of freedom are indicated.

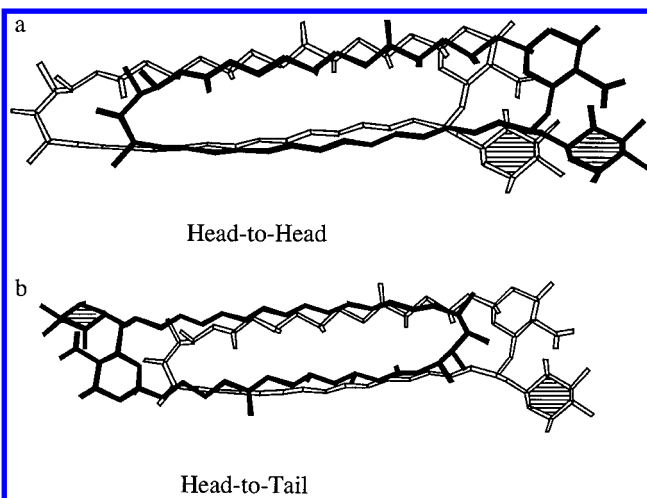


Figure 2. AmB stacked dimers (HH and HT, a and b, respectively) optimized in water.

energy calculations have to be simplified, but one must be cautious in adopting any simplification in order to obtain results as reliable as possible. As an example, some years ago, following the simplified way used to evaluate the excitonic matrix elements, it has been proposed several AmB aggregate structures, as different as stacked dimer, channel-like complex or double helix.^{7,9,11} Our work has thus involved two parts, the *first* one being devoted to energy optimizations of some AmB self-association (dimers, *single* and *double* helicoidal oligomers) and accounting for temperature and solvent effects, while the *second* one is related to a spectroscopy study, more precisely the calculated theoretical UV hypsochromic shift related to the optimal structure of each of the here-above cited aggregates. All our results and discussions are respectively collected in parts I and II of this paper.

I. Aggregates Total Energy

This part of our work gathers all results concerning the *energy* stabilization due to the AmB self-association in water at room temperature. Before any calculations one had to settle several computational details: the choice of the computational technics involving (i) an “intermolecular interaction potential”, (ii) the representation of the water solvent, and (iii) the taking into account of temperature. A survey of the computing techniques, which have been used in this work, is given in section I.1.2) For the choice of geometrical parameters to be optimized in water, such a choice depending on the AmB conformation in water, see section I.2. All the hereabove cited theoretical constraints being taken into consideration: *stacked* and *in-plane* dimers (See section I.3.1) and *single* or *double* helicoidal oligomers involving 2–6 unit cells (section I.3.2) have been

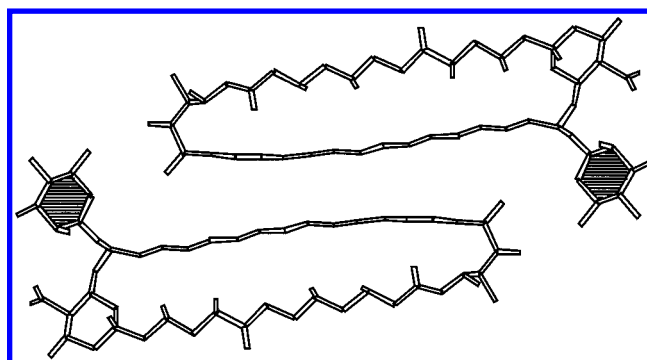
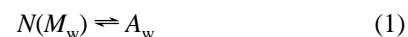


Figure 3. AmB in-plane dimer optimized in water.

optimized in water. The relative stability of these two helicoidal structures has been discussed in terms of intermolecular interactions, thermal, and solvent effects. At the end of this study, it has been shown that the results obtained for helicoidal oligomers may be enlarged to infinite helicoidal aggregates.

I.1. Computational Methods. Self-association of N AmB monomers (M) into a self-associated structure (A) at a temperature T and in water is related to the equilibrium reaction given by the eq 1 where the notation I_w is used: I denoting M or A and subscript w indicating that the equilibrium reaction is accomplished in water.



The determination of thermodynamical quantities of self-association at temperature T and in water calls out three concepts: (i) *association*, i.e., “intermolecular interactions”; (ii) *temperature* T , i.e., “thermal effects”; (iii) *water*, i.e., “solvent effects”. In the calculations of the thermodynamical quantities X (X standing for H , S , or G) corresponding to this equilibrium (at temperature T) in water, one has to take into consideration two terms, namely, (1) the one related to the association itself at molecular level $\Delta(\Delta X_{\text{ass}})$, which accounts for (i) the intermolecular interactions E_{int} , and (ii) the thermal differential motions occurring at a temperature T and (2) the one accounting for differential solvation effects $\Delta(\Delta X_{\text{solv}})$.

Thus one obtains

$$\Delta(\Delta X_{\text{tot}}) = \Delta(\Delta X_{\text{ass}}) + \Delta(\Delta X_{\text{solv}}) \quad (2)$$

I.1.1. Association Term. $\Delta(\Delta X_{\text{ass}})$ is evaluated by considering (i) the intramolecular electronic energy of each molecule as the energy zero and (ii) E_{int} , as the intermolecular interaction energy calculated at the bottom of the intermolecular potential well. Besides intermolecular interactions, it accounts for temperature effects proceeding from the thermal molecular motions, (translational (Tr), rotational (R), and vibrational (V)) associated with the thermodynamical quantities $\Delta X_{\text{mole}}(I)$.

$$\Delta X_{\text{mole}}(I) = \Delta X_{\text{Tr}}(I) + \Delta X_{\text{R}}(I) + \Delta X_{\text{V}}(I) \quad (3)$$

The reactional balance of the equilibrium given by eq 1 accounts for the variation ($\Delta(\Delta X_{\text{mole}})$) due to the association process.

$$\Delta(\Delta X_{\text{mole}}) = \Delta X_{\text{mole}}(A) - N\Delta X_{\text{mole}}(M) \quad (4)$$

In an association process, some translational and rotational degrees of freedom of monomers are turned into more constrained intermolecular vibrational degrees of freedom, thus association process leads to creation of order. As a whole, taking advantage of eq 4, one may write

$$\Delta(\Delta H_{\text{ass}}) = E_{\text{int}} + \Delta(\Delta H_{\text{mole}}) \quad (5)$$

$$\Delta(\Delta S_{\text{ass}}) = \Delta(\Delta S_{\text{mole}}) \quad (6)$$

Thus, following the Gibbs–Helmholtz equation, one obtains $\Delta(\Delta G_{\text{ass}})$.

This step of calculation requires the theoretical evaluation of E_{int} and $\Delta X_{\text{mole}}(I)$. (1) E_{int} has been calculated in the framework of molecular mechanics using sophisticated intermolecular interaction potential including five contributions, namely, electrostatic (E_{eel}), polarization (E_{pol}), dispersion (E_{disp}), first- and second-order perturbation Exchange contributions (respectively E_{rep} and $E_{\text{disp-exch}}$). Thus,

$$E_{\text{int}} = E_{\text{eel}} + E_{\text{pol}} + E_{\text{rep}} + E_{\text{disp}} + E_{\text{disp-exch}} \quad (7)$$

For more details concerning the computation of these different contributions, see refs 14–18. It has to be emphasized that the electrostatic and polarization contributions have been calculated using a multicentric (atoms and bond centers) multipolar (up to quadrupoles) charge distribution calculated from an *ab initio* wave function following the process developed by Vigné-Maeder et al.¹⁹ The total dispersion contribution includes (i) terms beyond R^{-6} , namely, R^{-8} and R^{-10} and (ii) the second-order exchange–dispersion. All the contributions included in this intermolecular potential have been fitted in such a way to satisfactorily reproduce the results of *ab Initio Symmetry Adapted Perturbation* calculations on some small complexes.^{17,18} Furthermore realistic results have been obtained (using this potential) in previous studies of interactions involving AmB.^{20–22} (2) $\Delta X_{\text{mole}}(I)$ has been calculated with the help of the usual formulas of *statistical thermodynamics* based upon the *partition function*. From a practical point of view, the calculation of vibrational frequencies (inherent in the evaluation of $\Delta X_{\text{V}}(I)$) can be simplified, by taking advantage of two facts: Intramolecular frequencies values have not to be computed, since (i) they are very high compared to the ones related to the intermolecular mode (thus not any coupling between these two modes) and (ii) they do not practically change upon molecular association. The force constants of the *intermolecular* vibration modes can be calculated from the curvature of the intermolecular interaction energy profiles evaluated as a function of the intermolecular displacement involved in the vibration. Thus, (1) in the dimer, besides the intermolecular stretching mode, some wagging and tilting vibration modes around the main intermolecular axis have been considered. (2) The *single* and *double* helicoidal AmB N-oligomers can be considered as *springs* having two main vibration modes: (i) stretching mode with regards to the mass center of the oligomer and (ii) torsion mode with regards to the main helicoidal axis. The calculated frequencies are ranging between 8 and 15 cm^{-1} for the different helicoidal AmB oligomers under study.

1.1.2. Solvation Terms. In the same way as discussed for thermal thermodynamical quantities, the balance of the equilibrium given by eq 1 involves the quantity $\Delta(\Delta X_{\text{solv}})$ calculated as the difference between the solvation energy of the aggregates and the N monomers, namely,

$$\Delta(\Delta X_{\text{solv}}) = \Delta X_{\text{solv}}(A) - N\Delta X_{\text{solv}}(M) \quad (8)$$

Each thermodynamical quantity ΔX_{solv} relative to the solvation of any species I is calculated within a *continuum* model which introduces statistically averaged information on the solvent effect by replacing the microscopic description of the solvent by a macroscopic *continuum* medium having suitable properties (dielectric constant, thermal expansion coefficient, solvent molar volume, etc.).^{23–26} We have used the methodology first proposed by Huron et al.^{23,24} within an adaptation of Langlet et al.²⁶ The essentials of this method are (i) choice of the exact shape of the cavity the volume of which is calculated as an union of van der Waals spheres of atoms constituting the solute molecule; each van der Waals radius is multiplied by a suitable parameter in order to take into account the so-called *free volume of solvation*; (ii) Representation of the solute charge distribution by the set of multicentric multipoles which has been used for the calculation of the intermolecular electrostatic contribution of the *intermolecular interaction* energy. Calculation of the thermodynamical quantities related to the solvation process involves (i) the *creation of a cavity in the solvent*, (ii) the *solute–solvent* interactions which explicitly involve electrostatic and dispersion–repulsion contributions, and (iii) solute polarization. Parameters used for the calculation of dispersion–repulsion contribution are the ones used for the calculation of the *intermolecular interaction* energy.

1.2. Geometrical Parameters. Bond lengths and bond angles has been fixed to the values obtained from X-ray study of the crystal structure of *N*-iodoacetyl AmB.²⁷

The Cartesian coordinates of AmB monomer have been calculated using the three main inertia axes as general coordinates axes OX_i , OY_i , and OZ_i , the origin point O_i is localized on the mass gravity center. The O_iZ_i and O_iY_i axes are situated in the mean plane containing the AmB macrolide ring skeleton with the O_iZ_i axes being directed along the long axis of the molecule i . The O_iX_i axis is perpendicular to the $O_iZ_iY_i$ plane. Then it has been generated (i) AmB *stacked* dimers obtained by superimposing the $O_iZ_iY_i$ ($i = 1,2$) planes, the second molecule being then rotated around the OX and OZ axis (respectively θ_{ox} and θ_{oz} angles) and translated along the OX , OY , OZ axis (respectively ΔX , ΔY , ΔZ translations). ΔX defines the intermolecular distance R , and ΔZ defines the relative position of the two AmB polar heads and heptaenic parts in a dimer. Energy optimizations have been performed as a function of the five geometrical parameters here-above-defined. (ii) AmB *in-plane* dimers obtained by a 180° rotation, θ_{ox} , of the second molecule followed by a translation, ΔY , defining the intermolecular distance D . Another geometrical parameter has to be considered in order to state the structure of this dimer, namely, the symmetrical translation ΔZ which defines the relative position between the two AmB heptaenic parts. (iii) In the case of *single* helices, the unit cell of which is an AmB monomer, the helicoidal axis OX passes through the AmB mass center. Two geometrical parameters are sufficient to optimize this aggregate, namely, the helicoidal angle (θ_{ox}) defined as the rotation around OX axis and the intercell distance ΔX denoted R . (iv) *Double* helices, the unit cell of which is an optimized *in-plane* dimer (as here-above-discussed, two geometrical parameters have to be optimized in D and ΔZ). The helicoidal

axis O'X' (parallel to OX axis) passes through this dimer mass center. In such an aggregate, besides the two here-above cited geometrical parameters related to the unit cell (D and ΔZ) one has to consider those which are inherent in the determination of any helicoidal structure, the helicoidal angle $\theta_{O'X'}$ and the intercell distance R . Thus four geometrical parameters are necessary to optimize such an aggregate.

I.3. Energy Results and Discussion. This section has to be referred to energy results obtained for some AmB dimers, *single* and *double* helicoidal oligomers. The results, thus obtained, will be extended to infinite *single* and *double* helicoidal aggregates. Before any calculation of associated structure, one had to settle all questions related to the AmB conformation in water. All details of this study being given elsewhere,^{20–22} we will only give here, the essential outcomes, namely, the macrolide ring has been kept rigid in water in the same way as in the crystal structure;²⁷ effectively, we may expect that such a property proceeding from the presence of the heptaenic chain should not depend of the surrounding medium. This hypothesis has been confirmed by theoretical molecular dynamics calculations^{20,28–30} and by experimental studies in solution.³¹ The conformation of the polar head depends on the surrounding medium because of the presence of the AmB amino sugar which is the most flexible fragment of the molecule due to allowed rotations denoted in Figure 1 by dihedral angles α_2 and α_3 . Theoretical calculations²² of AmB monomers in water have shown that the polar head is within a conformation denoted B which is intermediate between (i) a closed conformation (denoted C) stabilized by a network of intramolecular H-bonds which is optimal at an isolated state²² and (ii) the open one (denoted A) which has been experimentally determined in the crystal structure.²⁷

The conformation of the polar head is of an unquestionable interest in the structure of AmB associations since it interferes (i) on the value of the intermolecular distance between the different unit cells forming the AmB association, (ii) on the relative position of the polyenic chains (the AmB part responsible of UV absorption), (iii) the formation of possible intramolecular and intermolecular H-bond formation. At the light of preliminary results obtained when optimizing (in water) dimers and small helicoidal oligomers, it has appeared that, in all complexes studied in this work, the AmB polar head is within conformation B.

I.3.1. Dimers. A circumstantial study of different AmB dimers has been performed at an isolated molecular level²¹ and in water. Besides the *in-plane* dimer, we have considered two structures of AmB *stacked* dimers, *head-to-head* (HH), and *head-to-tail* (HT) differing by the relative position of the polar head of the two AmB molecules which can be (i) next to each other in HH structures or (ii) next to the tail of the other AmB in HT structures.

In this section, after an overview of the most important results concerning the different AmB dimers and their relative stability, we have analyzed the thermodynamics of dimer formation in water. Such an analysis has appeared to be very interesting for the understanding of the formation of bigger AmB aggregates and more generally association of amphiphilic molecules in water.

I.3.1.1. Overview of the Outstanding Results. (1) This work being devoted to self-associations in water, we do not give results obtained at a molecular level, they can be found in our previous paper.²¹ The geometrical optimized (in water) structures of HH and HT *stacked* and *in-plane* dimers are illustrated in Figure 2a and b and Figure 3, respectively. Our results have shown that the intermolecular distance R calculated in both HH

and HT dimers (R as 6.0 Å) is longer than the one usually determined for some biological aggregates (DNA, for instance). Furthermore, due to some translations along OZ axis, the polyenic chain of the two AmB molecules do not overlap. These two results proceed from the presence of a bulky polar head and of some methyl groups; thus rotational and translational motions of one AmB molecule relative to the second one are necessary in order to avoid repulsions. (2) Intermolecular forces (E_{int}) at a molecular isolated state stabilize HH *stacking* interactions (mostly by intermolecular H-bonds) over the pure van der Waals ones, HT *stacking* and (*in-plane*) interactions: for instance, E_{int} as -40.0 kcal/mol for the *stacked* HH dimer vs -31.0 kcal/mol for the *stacked* HT dimer (Figure 2a and 2b) and -14.0 (kcal/mol for the *in-plane* one (Figure 3)). (3) The values of the thermodynamical quantities related to thermal motions, namely, $\Delta(\Delta X_{\text{mole}})$ (eq 4) are quite equivalent for both *stacked* HH and HT dimers and for the *in-plane* one. As a consequence of these two last results, (2) and (3), both $\Delta(\Delta H_{\text{ass}})$, and $\Delta(\Delta G_{\text{ass}})$ favors the *stacked* HH dimer. (4) Water destabilizes the stacking interactions especially HH *stacked* dimers, a 60% E_{int} loss being obtained. In fact, in this case, E_{int} is not the prevailing part of the total optimized energy. As a consequence, the optimal geometrical structure of the *stacked* dimers (both HH and HT) in water is different from the one obtained at an isolated molecular state. (5) Water does not affect the van der Waals interactions between hydrophobic polyenic chains in an *in-plane* dimer, thus the geometrical structure remains unchanged. (6) Finally, as a balance, between intermolecular forces and solvent effects, the stability of *stacked* (HH and HT) and *in-plane* dimers is quite equivalent in water where both of them can be present at low AmB concentration. With an increase of AmB concentration, they can lead to bigger self-associated structures.

I.3.1.2. Thermodynamics of Association into In-Plane Dimer.

We will especially discuss results obtained for the association of two AmB molecules into an *in-plane* dimer for two reasons: its geometrical structure does not change when solvated in water and such an association is the unit cell of double helices. Nevertheless, all results which will be discussed here-after are qualitatively equivalent to the ones obtained with *stacked* dimers. The essential of our results given at $T = 300$ K may be summarized and discussed as follows: (1) *At a molecular isolated state*, we obtain: (i) a quite small value of the enthalpy proceeding from thermal motions ($\Delta(\Delta H_{\text{mole}})$ as 1.2 kcal/mol); consequently, the value of the enthalpy change $\Delta(\Delta H_{\text{ass}})$ (eq 5) is dominated by the *intermolecular interaction* energy and thus negative; (ii) A negative value of the entropy change (eq 6) ($\Delta(\Delta S_{\text{ass}})$ as -38.3 cal/mol/K); (iii) a negative small value of ($\Delta(\Delta G_{\text{ass}})$ as -1.3 kcal/mol.

It appears that notwithstanding an important stabilizing effect of the enthalpy change $\Delta(\Delta H_{\text{ass}})$ the value of the free enthalpy change $\Delta(\Delta G_{\text{ass}})$, is small due to a quite important negative value of the entropy change $\Delta(\Delta S_{\text{ass}})$, reflecting an increase of order during the association of two AmB monomers into an AmB dimer (this has been discussed in section I.1.1). (2) *In water*, we obtain (i) positive values of solvation enthalpy and entropy changes, $\Delta(\Delta H_{\text{solv}}) = +12.1$ kcal/mol and $\Delta(\Delta S_{\text{solv}}) = +50.3$ cal/mol/K, (ii) a negative value of the solvation free enthalpy change ($\Delta(\Delta G_{\text{solv}}) = -3.0$ kcal/mol) proceeding from an unfavorable solvation enthalpy change balanced by a favorable entropy one; such a result can be understood at the light of theory of hydrophobic forces.^{32–34} As is well-known, the exposition to water of hydrophobic part of a molecule (the polyenic chain in the case of AmB monomer) leads to an

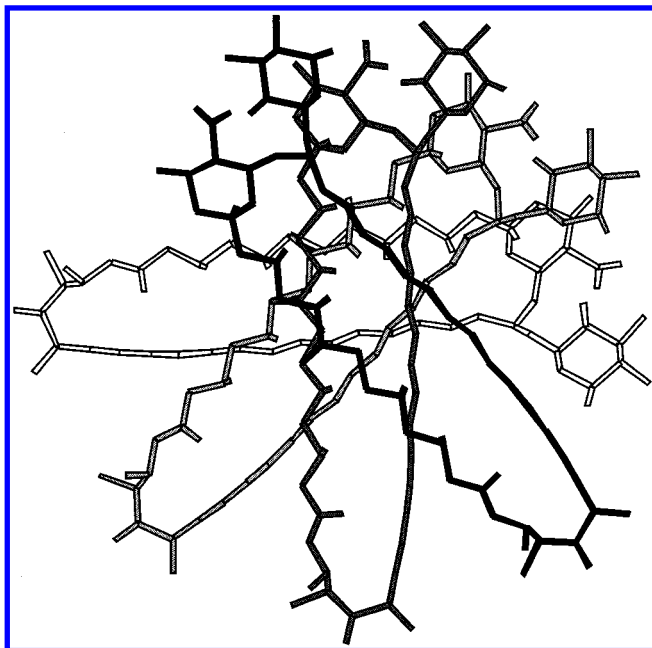


Figure 4. AmB single helicoidal oligomer (four unit cells) optimized in water.

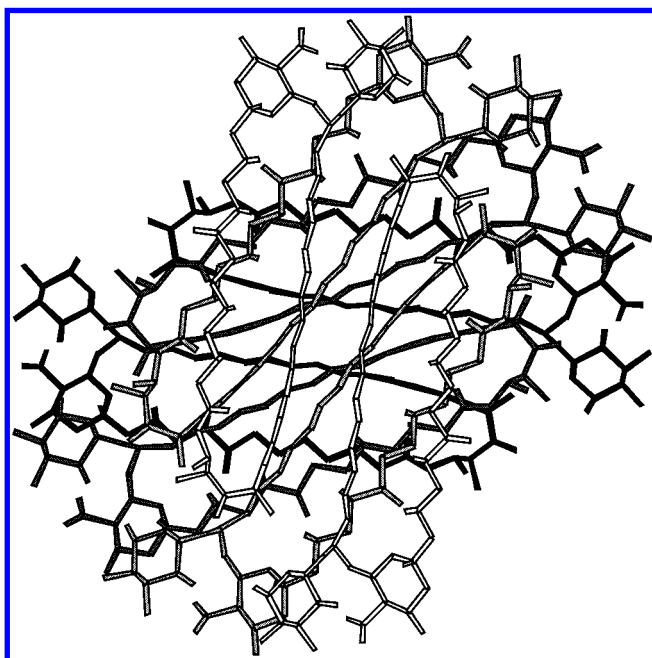


Figure 5. AmB double helicoidal oligomer (four unit cells) optimized in water.

important ordering of water around this part of molecule, the water molecules forming a network of water...water hydrogen bonds. When two monomers are associated into a dimer (by van der Waals forces between the hydrophobic parts for instance), some water molecules are expelled from this part of the molecule and released into the bulk water, leading to (i) a loss of H-bonds energy between the water molecules, $\Delta(\Delta H_{\text{solv}})$ thus positive and (ii) an increase of disorder, $\Delta(\Delta S_{\text{solv}})$ positive. (3) As a whole, taking into account both association and solvation (eq 2), one obtains: $(\Delta(\Delta H_{\text{tot}}))$ as -0.7 kcal/mol, $(\Delta(\Delta S_{\text{tot}}))$ as $+12.3$ cal/mol/K and $(\Delta(\Delta G_{\text{tot}}))$ as -4.1 kcal/mol. Thus the association in water of two AmB into an *in-plane* dimer is a favorable process proceeding from a very subtle balance between AmB...AmB and AmB...water interactions (enthalpy

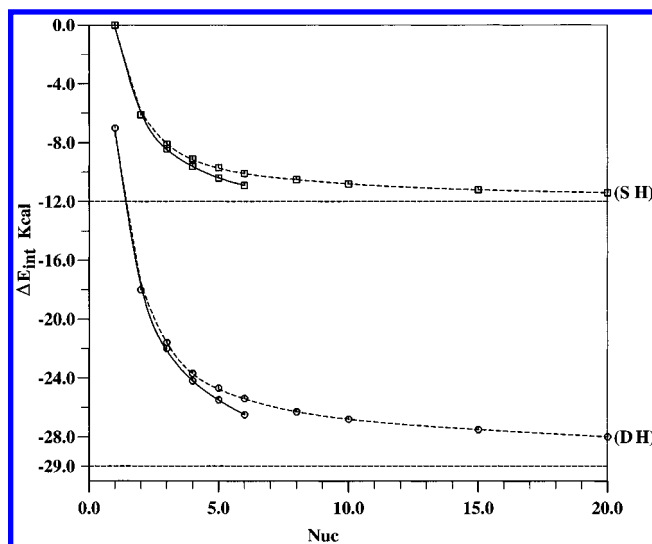


Figure 6. Evolution of E_{int} as a function of the number of unit cells Nuc in both *single* and *double* helicoidal oligomers. Continuous plots concern the results obtained when taking into account *long-range* effects in the calculations of E_{int} for respectively *single* (SH) and *double* (DH) helicoidal oligomers. Dashed plots (concerning respectively *single* and *double* helices) illustrate results obtained using eqs 9 and 10.

effect) and creation of order during association and of disorder during solvation (entropy effect).

At this level of work, we can reasonably assume that the here-above discussion can be generalized to the stabilization in water of double helices, since these structures mainly proceed from infinite stacking of *in-plane* dimers.

1.3.2. Single and Double Helices. Energy optimizations in water have been performed by considering some *single* and *double* helicoidal oligomers containing 2–6 unit cells (thus respectively 2–6 and 4–12 AmB molecules). In this section, both structural and energy results will be given.

1.3.2.1. Geometrical Structure. Considering a given helicoidal oligomer (*single* or *double*), we have obtained the same geometrical parameters for any value of the number of unit cells, Nuc, thus our results may be extended to infinite *single* or *double* helices. (1) A *single helix* illustrated by Figure 4 is characterized by (i) an helicoidal angle $\theta_{\text{ox}} = 42^\circ$ and (ii) an intercell distance $R = 5.7$ Å. (2) A *double helix* illustrated by Figure 5 is characterized by (i) an helicoidal angle $\theta_{\text{ox}} = 30^\circ$ and (ii) an intercell distance $R = 5.8$ Å. The geometrical parameters defining an optimized *in-plane* dimer used as a unit cell are intermolecular distance $D = 4.9$ Å and the translation parameter $\Delta Z = 2.4$ Å.

1.3.2.2. Thermodynamics of Association in Water. At this level of work, our final goal being to analyze the relative stability (in water at room temperature) of infinite *single* and *double* helices, we have analyzed the evolution of thermodynamical quantities of association and solvation as a function of Nuc. For a given value of Nuc, the number of AmB molecules involved in a *single* and *double* helicoidal oligomers being different all results are given *per AmB* molecule.

(A) Self-Association. Our calculations has led to results which are similar (from a qualitative point of view) to the ones here-above-discussed for AmB dimers, namely, (i) a negative value of $\Delta(\Delta H_{\text{ass}})$ dominated by the intermolecular interaction energy E_{int} and (ii) a negative entropy change $\Delta(\Delta S_{\text{ass}})$ with quite identical values for both *single* and *double* helices.

As an outcome of these two last results it appears that the relative stability of the two studied helicoidal structures proceeds from the value of the intermolecular interaction energy E_{int} . A

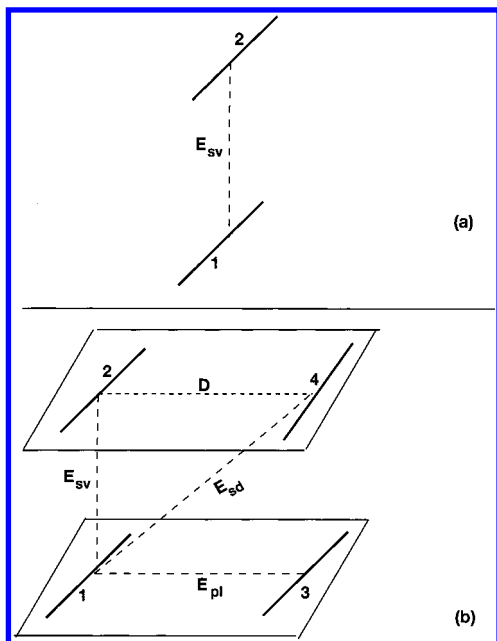


Figure 7. Illustration of the main intermolecular interactions occurring in the smallest *single helicoidal* oligomer (a) and in the smallest *double helicoidal* one (b).

study of the evolution of E_{int} per AmB molecule ($(1/N)(E_{\text{int}})$) as a function of Nuc the number of unit cells (illustrated by Figure 6) shows that, in the oligomers under study, E_{int} favors the *double* helix with regards to the *single* one. Consequently, both $\Delta(\Delta H_{\text{ass}})$ and $\Delta(\Delta G_{\text{ass}})$ favor the *double* helix at an isolated molecular state. At this step, the greatest stabilization of a double helix may be understood in light of Figure 7 illustrating the different interactions occurring in the smallest *single* helicoidal oligomer (two AmB molecules) and in the smallest *double* helicoidal oligomer (four AmB molecules).

In the *first* case, E_{int} only involves one vertical stacking interaction, denoted E_{sv} . Using the geometrical parameters here-above-defined, we obtained E_{int} as -12.1 kcal/mol (thus -6 kcal/mol per AmB).

In the *second* case, E_{int} involves (i) two *in-plane* interactions (denoted E_{pi}) between the two molecules forming the unit cell, (ii) two vertical stacking interactions (E_{sv}), and (iii) two diagonal stacking interactions (denoted E_{sd}) between the unit cells. The sum $2 E_{\text{sv}} + 2 E_{\text{sd}}$ (denoted E_{st}) is negative, since both E_{sv} and E_{sd} contributions are attractive. Taking into account the structural geometrical parameters here-above given, we obtained E_{pi} as -14.0 kcal/mol, E_{st} as -44.0 kcal/mol, thus, E_{int} as -72 kcal/mol (-18 kcal/mol per AmB).

The generalization of this discussion to infinite *single* or *double* helices is quite easy when neglecting long-range effects which are not very important in our complexes as clearly shown in Figure 6.

Expressions for the total *intermolecular interaction* energy per AmB of a *single* helix (E_{intSH}) (eq 10) or a *double* helix (E_{intDH}) (eq 11), both of them involving N AmB molecules, as

$$E_{\text{intSH}} = \frac{(N-1)}{N} E_{\text{sv}} \quad (9)$$

$$E_{\text{intDH}} = \frac{1}{N} \left[\left(\frac{N}{2} - 1 \right) E_{\text{st}} + \frac{N}{2} E_{\text{pi}} \right] \quad (10)$$

When N tends to infinity, E_{intSH} tends to E_{sv} while E_{intDH} tends to

$$(1/2)E_{\text{st}} + (1/2)E_{\text{pi}} = E_{\text{sv}} + E_{\text{sd}} + (1/2)E_{\text{pi}} \quad (11)$$

Using the geometrical parameters optimized for both *double* and *single* regular helicoidal oligomers, E_{intSH} and E_{intDH} have been calculated for the infinite *Double* and *Single* helices respectively as: -30.0 and -12.0 kcal/mol per AmB. The *intermolecular interaction* energy favors the *double* infinite helix by 18 kcal/mol per AmB.

(B) Solvent Effects. The calculations performed for the different helicoidal oligomers have once again led to same qualitative results than the ones hereabove-discussed for AmB dimers. Being interested in the relative stability of the *single* and *double* infinite helicoidal structures, we have studied the evolution of $\Delta(\Delta H_{\text{solv}})$, $\Delta(\Delta G_{\text{solv}})$, and $T\Delta(\Delta S_{\text{solv}})$ (values given per AmB) as a function of the number of unit cells (Nuc), in both *single* and *double* helicoidal oligomers; see illustrations in Figure 8a and b. It may be noticed that (1) The values of $\Delta(\Delta H_{\text{solv}})$ and $\Delta(\Delta S_{\text{solv}})$ increase with Nuc and tend to a positive constant value when Nuc tends to infinity. We have respectively obtained, for *single* or *double* helicoidal infinite aggregates, (i) $\Delta(\Delta H_{\text{solv}})_{\text{Nuc} \rightarrow \infty}$ respectively calculated as 7.0 and 22.0 kcal/mol per AmB. (ii) $\Delta(\Delta S_{\text{solv}})_{\text{Nuc} \rightarrow \infty}$ respectively calculated as 36.0 and 86.0 cal/mol/Kelvin per AmB. (2) The values of $\Delta(\Delta G_{\text{solv}})$ (negative values) decrease with Nuc tending to a negative constant value. The value of $\Delta(\Delta G_{\text{solv}})_{\text{Nuc} \rightarrow \infty}$ which is quite similar (namely, as -4.0 kcal/mol per AmB) for both *single* and *double* infinite aggregates.

(C) Total Association Energy in Water. Accounting for results concerning the intermolecular energy and solvation effect, it appears that (i) $\Delta(\Delta H_{\text{tot}})_{\text{Nuc} \rightarrow \infty}$ favors the *Double* infinite helicoidal aggregate over the *single* one by 3.0 kcal/mol per AmB. Such a stabilization mainly proceeds from the very important van der Waals forces which in the *double* helix prevail against the destabilizing solvation enthalpic effects ($\Delta(\Delta H_{\text{solv}})$). (ii) $\Delta(\Delta S_{\text{tot}})_{\text{Nuc} \rightarrow \infty}$ favors the *Double* infinite helicoidal aggregate over the *single* one by 50 cal/mol/K per AmB. (iii) $\Delta(\Delta G_{\text{tot}})_{\text{Nuc} \rightarrow \infty}$ favors the infinite *double* helicoidal aggregate over the *single* one by 18 kcal/mol per AmB. It has to be noticed that the entropy factor plays a non negligible role in the stabilization of the *double* helix. At the light of the here-above results the stabilization of the infinite *double* helix over the *single* helix proceeds from a very subtle balance between several effects. Energy effects dominated by van der Waal forces and entropy effects dominated by the creation of disorder proceeding from expulsion of water molecules toward the bulk liquid.

II. Aggregates Spectroscopy

Several different effects render the spectrum of molecular self-associations different from the one of its monomeric constituents. Among them, one may cite the excitonic effect (arising from the interaction between the N molecular residues involved in the complex) which splits each monomer transition into N bands usually all but a few having no intensity. The theoretical exciton splitting is calculated within the framework of the "Excitonic Method" which rests on the concept of *perturbation* with respect to the isolated monomers.

II.1. Survey of the Method. Essential to the perturbation theory is the evaluation of the total Hamiltonian of an aggregate involving N molecules, as $H = H_0 + V$. (1) H_0 , the unperturbed Hamiltonian, is evaluated as the sum of the Hamiltonians $H_{(n)}$ of each of the N isolated molecules involved in the aggregate ($n = 1, N$). The eigenfunctions of H_0 are the products of the eigenfunctions of $H_{(n)}$, namely, for any excited state i , for instance, $|\psi_{i(n)}\rangle = \Phi_i^{(n)} \prod_{m \neq n} \Phi_0^{(m)}$. (2) V is perturbation operator

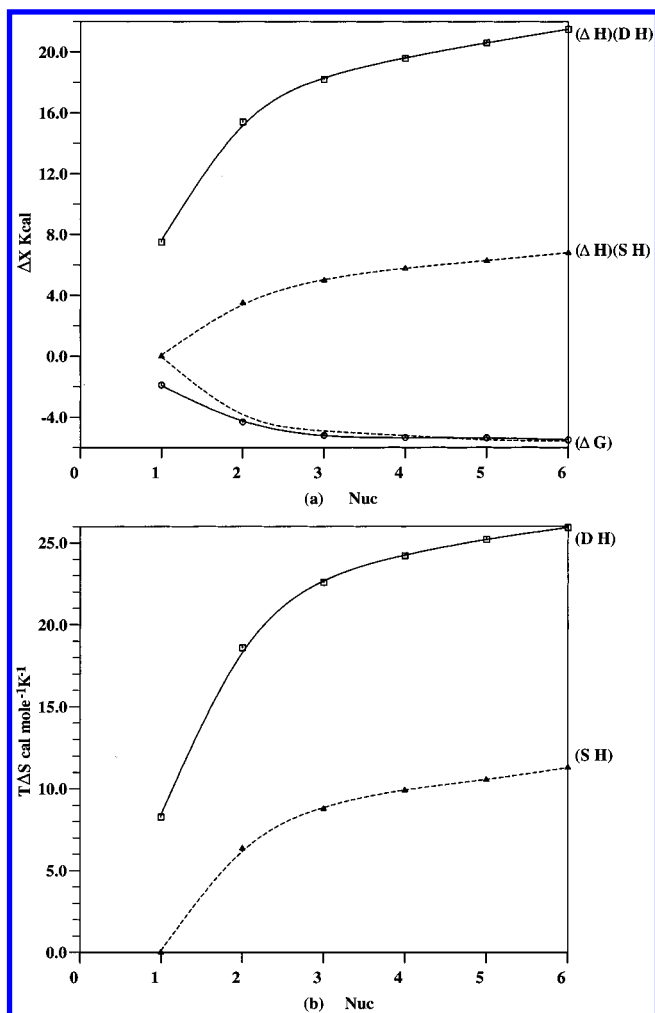


Figure 8. (a) Evolution of $\Delta(\Delta H_{\text{solv}})$ and $\Delta(\Delta G_{\text{solv}})$ in kcal/mol per AmB as a function of the number of unit cells (Nuc) in both *single* (dashed plots, SH) and *double* (continuous plots DH) helicoidal oligomers. (b) Evolution of $T\Delta(\Delta S_{\text{solv}})$ in kcal/mol per AmB as a function of the number of unit cells (Nuc) in both *single* (dashed plots SH) and *double* (continuous plots DH) helicoidal oligomers.

which can be expressed following Longuet-Higgins³⁵ as a local charge density operator, $\rho^{(n)}(\vec{r}^{(n)})$ associated with each molecule

$$V = \iint \frac{\rho^{(n)}(\vec{r}^{(n)})\rho^{(m)}(\vec{r}^{(m)})}{|\vec{r}^{(n)} - \vec{r}^{(m)}|} d\vec{r}^{(n)} d\vec{r}^{(m)} \quad (12)$$

Each operator $\rho^{(n)}(\vec{r}^{(n)})$, depending only on the coordinates of the subsystem, the separation of these variables for the two subsystems being realized in the integrand and any general matrix element of \mathbf{V} , $\langle \Phi_i(n) \Phi_j(m) | V | \Phi_k(n) \Phi_l(m) \rangle$, may be written as

$$\iint \frac{\langle \Phi_i^{(n)} | \rho^{(n)} \vec{r} | \Phi_k^{(n)} \rangle \langle \Phi_j^{(m)} | \rho^{(m)} \vec{r} | \Phi_l^{(m)} \rangle}{|\vec{r}^{(n)} - \vec{r}^{(m)}|} d\vec{r}^{(n)} d\vec{r}^{(m)} \quad (13)$$

This represents the electrostatic interactions between appropriate charge distributions, more precisely in our case between *transition charge* distribution; for more details see the paper of Claverie.¹⁴ (3) H is the total Hamiltonian, in the basis of eigenfunctions of H_0 . The diagonalization of this matrix, leads to N eigenstates $\psi_k = \sum_i \sum_n C_k^{i,n} \psi_{i(n)}$. The value of the coefficients $C_k^{i(n)}$ is related to the concept of localization or

delocalization of a given excited state. Such a concept has two meanings, namely, (i) localization on a given excited state i of the monomer or delocalization among several excited states i and j and (ii) localization on a given molecule n , or delocalization among several molecules involved in the aggregate.

II.2. Computational Procedure. For spectroscopy calculations within *Excitonic* methodology, one has to consider only the heptaenic chain of AmB molecule since it is the only part of AmB accounting for the UV absorption spectrum. Emphasis has to be put that it is not the coordinates of an heptaenic chain which have been used in these calculations, but the ones of atoms belonging to the heptaenic chain calculated from the whole AmB association (dimer or aggregate) with the origin point O of the Cartesian coordinates axis localized on the AmB mass center (as it was precedently defined for *energy* calculations) and not on the one of the heptaenic chain. The crucial problem in the determination of *exciton splitting* in the framework of the excitonic model is the computational time, thus some simplifications have to be made. Among them, we have retained the three which are more extensively used: the first one is related to the *transition dipole* determination and the evaluation of the different matrix elements of \mathbf{V} , the second one concerns the size of the excitonic matrix, and the third one deals with the structure of the excitonic matrix.

II.2.1. Evaluation of the Matrix Elements. *Ab initio* evaluations of the matrix elements of \mathbf{V} are not possible owing to the size of AmB molecule. Thus, taking advantage of the Longuet-Higgins representation of \mathbf{V} (eq 12 and ref 35), it is possible to (i) define the charge distribution of each molecule independently of the others and (ii) to replace the continuous (electronic) part of the charge density by an appropriate multipole expansion around one or several centers of the molecule,^{14,19} the crucial problem being the determination of (a) the number of molecular centers and (b) the multipole order at which this expansion can be truncated. The widely used approximation is the *monocentric transition dipole* which can be very simply obtained from the experimental absorption spectrum of monomeric species following the general procedure described in litterature. In fact, as is well-known,¹⁴ there is a serious defect in using such an approximation when the intermolecular distance R is in the range of values of the molecular size and when the molecular shape is quite different from a sphere: in the case of AmB, R has been evaluated as 6.0 Å and the molecular length along the long axis is 17 Å. Thus, a *multicentric transition charge* distribution (transition charges being located on each atom) has been retained in this work for the calculation of the matrix elements of \mathbf{V} .

To obtain such a distribution, a CI calculation including all singly excited configurations has been first performed within the framework of the semiempirical CI-INDO method (Momicchioli et al.³⁶) Then a multicentric multipolar (up to dipole) expansion of the INDO *transition charge* distribution has been performed. *Atomic transition charges* and *atomic transition dipoles* localized on the carbon atoms of the heptaenic chain have been obtained. The calculated value (13.5 D) of the *transition dipole* between the ground-state and the first excited state (mainly directed along the long axis OZ) is in quite good agreement with the estimated experimental value (12.5 D). It has been verified that the total calculated value (13.5 D) of the *transition dipole* mainly proceeds (by 98.5%) from *atomic transition charge* located on carbon atoms, the contribution of the *atomic transition dipoles* located on carbon atoms can be neglected in the calculations of spectral shift due to AmB associations.

II.2.2. Size of the Excitonic Matrix. To reduce the size of the excitonic matrix, one can build it with only states of interest for the problem under study. In this work, being concerned by the value of the hypsochromic shift of the first UV absorption band, H could be built in the basis of all degenerated excited states $|\Psi_{1(n)}\rangle$ (with $n = 1, N$), leaving out all other excited states. Such a simplification can be retained after having checked out the smallness of the ratio $V_{nm}^{1,i}/\Delta E$, with ΔE as the energy difference between states $|\Psi_{1(n)}\rangle$ and $|\Psi_{i(n)}\rangle$.³⁷ From our CI-INDO calculations, a 9000 cm^{-1} energy difference has been calculated between the second ($i=2$) and first excited ($i=1$) states, this value is only twice the one expected for the hypsochromic shift experimentally observed for AmB associations in water, but we have verified that these two states (with orthogonal transition dipoles) do not interact.

II.2.3. Structure of the Matrix Elements. When the studied association involves more than two molecules i.e. in the calculations concerning helicoidal aggregates, we could use an Hückel-like excitonic matrix structure in which only $V_{n,n+1}$ matrix elements were calculated but preliminary calculations have shown that, when neglecting of long-range terms, one underestimates the calculated value $\Delta\nu_+$ by nearly 40%. Thus, in this present work, we have favored a *not*-Hückel-like excitonic matrix structure in which all matrix elements $V_{n,m}$ have been calculated. At this level of work, it appears that long-range effects can be neglected for the calculation of interaction energy in the groundstate (see section I.3.2.2 and Figure 6) but have to be taken into account in spectroscopy calculations. Such an assertion is not peculiar when recalling that the total *intermolecular interaction* energy (E_{int}) in most of stacked aggregates mainly proceeds from the "Dispersion" energy, a contribution which varies with the intermolecular distance R as R^{-6} , while the excitonic coupling terms $V_{n,m}$ can be evaluated as R^{-3} at long distances. Thus $V_{n,m}$ varies more slowly with R than the dispersion contribution of E_{int} .

II.3. Results. All results presented in this section concerning the values of $\Delta\nu_+$ are calculated in the framework of the precedently justified simplifications, namely, using a multicentric *atomic transition charge* distribution within a *not*-Hückel-like *excitonic* matrix structure involving only all degenerated excited states $|\Psi_{1(n)}\rangle$ (with $n = 1, N$). Section II.3.1 refers to fully optimized HH and HT *stacked* dimers and *in-plane* dimers while section II.3.2 refers to fully optimized *single* and *double* helicoidal oligomers.

II.3.1. Dimers. (1) In agreement with theory both HH and HT optimized dimers (Figure 2a and b) lead to an hypsochromic shift $\Delta\nu_+$ of 480 cm^{-1} and 610 cm^{-1} , respectively, but these values of $\Delta\nu_+$ cannot account for the hypsochromic shift experimentally observed in water. A stacked HT dimer (with intermolecular distance R as 6.0 Å) was earlier proposed⁷ to account for spectroscopy experiments but this result proceeds from the use of the crude approximation of a *monocentric transition dipole*. We verified that calculations using this approximation with our optimized geometries led to $\Delta\nu_+$ of 3550 cm^{-1} .

(2) The value of $\Delta\nu_+$ proceeding for the optimized *in-plane* dimer has been calculated as (750 cm^{-1}). So neither one of these dimers gave a $\Delta\nu_+$ in agreement with the experiments.

II.3.2. Single and Double Helices. In part I of this paper, we have shown that all energy results obtained for any helicoidal aggregate can be extended to infinite aggregates. Thus it has appeared interesting to give an evaluation of the hypsochromic shift value for infinite *single* and *double* helices. So, as a preliminary task, we have studied the evolution of $\Delta\nu_+$ as a

function of the number of unit cells (Nuc) for both *single* and *double* helices. Following our results, it appears that the value of $\Delta\nu_+$ increases with Nuc and tends to a limit value for Nuc = 60. The results obtained for fully optimized *single* and *double* helices are the following ones: (i) *single* infinite helix, $\Delta\nu_+ = 1010 \text{ cm}^{-1}$, with the oscillator strength as 1042 and (ii) *double* infinite helix, $\Delta\nu_+ = 3950 \text{ cm}^{-1}$, with the oscillator strength as 5500. So the *double* infinite helix can account for the experimental observed value of the hypsochromic shift observed in the UV spectrum of AmB in water. Such an aggregation model (which accounts for spectroscopy experiment) is not new, since it has earlier been proposed by Hemenger et al.¹¹ with the following geometrical parameters: helicoidal angle θ_{ox} as 45.7°, intercell distance R as 2.9 Å, and intracell distance between polyene as 4.9 Å. It immediately appears that the intercell distance is very different from the one given in this work; such a value could not be obtained in any optimization energy in the case of AmB. In fact, it should be recalled that Hemenger has adjusted its model (without any energy calculations) to fit with spectroscopic data, in particular, he has estimated the value of R at the light of results generally determined for some biological aggregates without any bulky groups (DNA for instance).

Conclusion

This work which represents a theoretical study of both energy and spectroscopy of AmB self-associations has emphasized several important results, namely, (1) AmB Self-association is a favorable process in water, stable aggregates (from the dimers to the infinite double helices) can be obtained from an energetical point of view, the degree of association depending on the AmB concentration. (2) But if some dimer structures (mainly *stacked* and *in-plane* ones) can exist in water, they do not account for the hypsochromic UV absorption frequency shift, when calculations are performed within the *excitonic* model used at a high degree of accuracy. In fact, when the AmB concentration increases, these dimers are the basis of bigger aggregates. (3) The most stable helicoidal structure in water is a *double* helix characterized by (i) an intracell distance $D = 4.9 \text{ Å}$, (ii) an helicoidal angle $\theta_{\text{ox}} = 30^\circ$, and (iii) nearest neighbor intercell distance R as 5.8 Å (thus a pitch of 62.7 Å). (4) This double helicoidal aggregate (which ensures a minimal exposure of AmB hydrophobic parts to water and a good hydration of the polar heads) satisfies both thermodynamics and spectroscopy: the value of the hypsochromic frequency $\Delta\nu_+$ has been calculated as 3950 cm^{-1} and is in quite good agreement with the experimental value, $\Delta\nu_+ \approx 4368 \text{ cm}^{-1}$. Before ending this paper, emphasis has to be put on the fact that the interest of this work goes beyond an interpretation by theory of spectroscopic experimental results obtained with AmB solutions. Endeavor has been made in order to settle the suitable strategy to clear up the problem of association of molecules in a given medium in relation with a given experimental observable (in this work, the UV absorption spectrum). (1) From a *methodological* point of view both *energy* and *spectroscopy* calculations had to be performed in concert, but first of all, a great attention has to be paid to all approximations used to simplify the calculations: the use of quite sophisticate intermolecular potential (for intermolecular interactions) and multicentric *atomic charge transition* distribution (for calculations of $\Delta\nu_+$) could be recommended in order to obtain reliable results. (2) From a *physical* point of view, the stabilization of a self-associated structure against another one cannot be a-priori guessed, several physical parameters have to be considered (intermolecular

interactions, temperature, solvent effects, etc.) each of them having a specific effect. In fact the balance of the whole physical process can be very subtle, witness the value total entropic factor calculated as the sum of the unfavorable association entropy (order creation) and favorable solvation entropy (disorder creation).

References and Notes

- (1) Kotler Brajtburg, J.; Medoff, J.; Kobayaski, S.; Bogg, S.; Schlessinger, D. *Antimicrob. Agents Chemother.* **1979**, *15*, 716.
- (2) Bolard, J. *Biochim. Biophys. Acta* **1986**, *864*, 257.
- (3) Lambing, H. E.; Wolf, B. D.; Hartsel, S. *Biochim. Biophys. Acta* **1993**, *1152*, 185.
- (4) Bolard, J.; Legrand, R.; Heitz, F.; Cybulska A. *Biochemistry* **1991**, *30*, 5707.
- (5) Legrand, P.; Ramos, H.; Cohen, B. E.; Bolard, J. *Antimicrob. Agents Chemother.* **1992**, *36*, 2518.
- (6) Gaboriau, F.; Chéron, M.; Leroy, L.; Bolard J. *Biophysical Chemistry* **1997**, *66*, 1.
- (7) Ernst, C.; Grange, J.; Rinnert, H.; Dupont; Lematre, J. *Biopolymers* **1981**, *20*, 1575.
- (8) Rinnert, H.; Maigret, B. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 853.
- (9) Barwicz, J.; Gruzecki, W.; Gruda, I. *J. Colloid Interface Sci.* **1993**, *158*, 71.
- (10) Bolard, J.; Seigneuret, M.; Boudet, G. *Biochim. Biophys. Acta* **1980**, *599*, 280.
- (11) Hemenger, R. P.; Kaplan, T.; Gray, L. J. *Biopolymers* **1983**, *22*, 911.
- (12) Mazerski, J.; Bolard, J.; Borowski, E. *Biochim. Biophys. Acta* **1982**, *719*, 11.
- (13) Kasha, M. *Radiation Res.* **1963**, *20*, 55.
- (14) Claverie, P. *Intermolecular Interactions. From Diatomic to Biopolymers*; Pullman, B., Ed.; Wiley: New York, 1978; pp 69–395.
- (15) Claverie, P. *Application to the theory of Intermolecular Interactions in molecules in Physics, Chemistry and Biology*; Kluwer Academic Publisher: 1988; Vol. II, pp 393–415.
- (16) Langlet, J.; Claverie, P.; Caron, F.; Boeue, J. C. *Int. J. Quantum Chem.* **1981**, *20*, 289.
- (17) Hess, O.; Caffarel, M.; Langlet, J.; Caillet, J.; Huizoon, C.; Claverie, P. *Stud. Phys. Theor. Chem.* **1990**, *71*, 323.
- (18) Langlet, J.; Caillet, J.; Caffarel, M. *J. Chem. Phys.* **1995**, *103*, 8043.
- (19) Vigné-Maeder, F.; Claverie, P. *J. Chem. Phys.* **1988**, *88*, 4934.
- (20) Langlet, J.; Bergès, J.; Caillet, J.; Démaret, J. P. *Biochim. Biophys. Acta* **1994**, *1191*, 79.
- (21) Caillet, J.; Bergès, J.; Langlet, J. *Biochim. Biophys. Acta* **1995**, *1240*, 179.
- (22) Bergès J.; Caillet, J.; Langlet, J.; Gresh, N.; Hervé, M.; Gary-Bobo, C. M. *Stud. Phys. Theor. Chem.* **1990**, *71*, 253.
- (23) Huron, M. J.; Claverie, P. *J. Phys. Chem.* **1972**, *76*, 2123.
- (24) Huron, M. J.; Claverie, P. *J. Phys. Chem.* **1974**, *78*, 1853.
- (25) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
- (26) Langlet, J.; Claverie, P.; Caillet, J.; Pullman, A. *J. Phys. Chem.* **1988**, *92*, 1617.
- (27) Ganis, P.; Avitabile, G.; Mechlini, W.; Schaffner, C. P. *J. Am. Chem. Soc.* **1971**, *93*, 4560.
- (28) Mazerski, J.; Borowski, E. *Biophys. Chem.* **1995**, *54*, 49.
- (29) Baginski, M.; Resat H.; McCammon J. A. *Mol. Pharm.* **1997**, *52*, 560.
- (30) Balakrishnan, A. R.; Bansal, M.; Easwaran, K. R. K.; Nambodri, K.; Gaber, B. P. *J. Biomol. Struct. Dyn.* **1995**, *12*, 957.
- (31) Balakrishnan, A. R.; Easwaran, K. R. K. *Biochim. Biophys. Acta* **1993**, *1148*, 269.
- (32) Evans, D. F.; Ninham, B. W. *J. Phys. Chem.* **1985**, *90*, 226–234.
- (33) Privalov, P. L.; Gill, S. *Pure Appl. Chem.* **1989**, *6*, 1097–1104.
- (34) Wimley, W. C.; Whitea, S. H. *Biochemistry* **1993**, *32*, 6307–6312.
- (35) Longuet-Higgins, H. C. *Proc. R. Soc. (London)* **1956**, *A235*, 537.
- (36) Momicchioli, F.; Baraldi, I.; Bruni, M. C. *Chem. Phys.* **1995**, *70*, 161.
- (37) Ecoffet, C.; Markovitsi, D.; Millie, P.; Lemaistre, J. P. *Chem. Phys.* **1993**, *177*, 629.