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Interactions of the N-Terminal Domain of Apolipoprotein E with a Mimetic Water−Lipid Surface: A Molecular Dynamics Study

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Apolipoprotein E, a protein that is subject to structural changes at a water/lipid interface, is studied by molecular dynamics simulations performed in water and at a water/organic-phase interface. The protein backbone atoms get significantly more hydrated in the interfacial system than in the water simulation and undergo larger positional fluctuations. Larger fluctuations and hydration concur to be more manifest in the interfacial region of the aqueous phase. In this interfacial region, water is more structured and makes, relative to its number of neighbors, more hydrogen bonds than water in the bulk, a picture that has been previously inferred from molecular dynamics simulations of several water−organic liquid interfaces. We propose that the higher degree of protein hydration observed in the interfacial simulation arises from the structural behavior of interfacial water, which needs to make more H bonds and sees the protein as an additional partner. Our results are in agreement with spectroscopic data obtained for another apolipoprotein structurally similar to apolipoprotein E that show an increase in the protein hydration in the presence of a water/lipid interface and suggest that hydration is a factor helping the barrier crossing from the structure in aqueous solution to a partially folded conformation prone to bind to the lipids.

Introduction

Lipid−protein interactions are ubiquitous to living systems. The protein/lipid interface is extremely important, but little is known at this time about the specific interactions at this interface. In particular, the molecular mechanism of apolipoprotein recruitment to lipoprotein surfaces and its related structural alteration are not well understood. Various experiments have proposed that charge−charge[1] and hydrophobic interactions[2−5] play a role in the binding process, hinting that association of a protein with a lipid surface is a complex phenomenon that might involve several types of molecular interactions.

The N-terminal domain of human apolipoprotein E (apoE) and apolipophorin III are exchangeable apolipoproteins which, in absence of lipids, exist as elongated bundles of respectively four and five α-helices. A major conformational change in these proteins occurs upon lipid association, wherein the bundle possibly opens around hinge loop regions to facilitate interaction with lipids by bringing exposure of its hydrophobic interior. In apoE, the conformational change is supported by surface properties measured at the air−water interface[6] and by more recent fluorescence resonance energy transfer experiments[7] and FTIR-ATR spectroscopy data.[8] The opening of the helix bundle in apolipophorin III upon lipid binding has recently been demonstrated by structure-guided disulfide bond engineering.[9]

An understanding of the factors responsible for the protein−lipid association requires their characterization at the molecular level. In this respect, computer simulations can help interpret and rationalize existing experimental data, which often provide indirect evidence of molecular mechanisms. Moreover, the simulation results may guide new experiments. The organic phase has been often used as an alternative to the lipid phase not only in computer simulations[10] but also in experiments.[11] Experiments performed on systems simpler than lipoprotein particles have allowed to gain insight into the interaction of apolipoproteins with lipids.[7−8,11−12] Several of these studies have converged to propose a model in which the preferential interaction of apolipoproteins with the lipids occurs in the following manner: the hydrophobic side chains of the amphipathic helices interact with the lipid acyl side chains. This suggests that a hydrophobic medium could be a reasonably good mimic for the interaction of apolipoproteins with lipids. Moreover, simulations of water/organic solvent biphasic cells have been widely used to study peptide conformation and orientation at interfaces[10,13−17] and pore formation in membranes.[18]

A molecular dynamics (MD) simulation of apoE in water was previously reported, which showed that the protein behaves as a slightly disordered structure,[19] a picture that agrees with that of the structurally homologous apolipophorin III, which at physiological conditions adopts a partially folded state with loose tertiary interactions.[20] In this study, we examine the impact of a water/organic-phase interface on the structure, dynamics, and solvation of apoE by MD simulations. This is done by comparing the structural and dynamical properties of the protein, initially placed on the aqueous side of the system near the interface, to those computed in a trajectory of the protein obtained in water. A biphasic simulation cell with two immiscible liquids, water and an apolar liquid, is used to mimic a water/lipid system as two media of different polarity and H-bond donor/acceptor capabilities.

It is beyond the capability of this work to monitor a conformational change of large-scale amplitude such as the one produced in apoE upon lipid binding. However, we think that the premises of such a motion are within reach of such a study. A particular emphasis of the simulation analysis is on the hydration of the protein, as a previous experimental study[20] showed an increase in hydration of apolipophorin III, which

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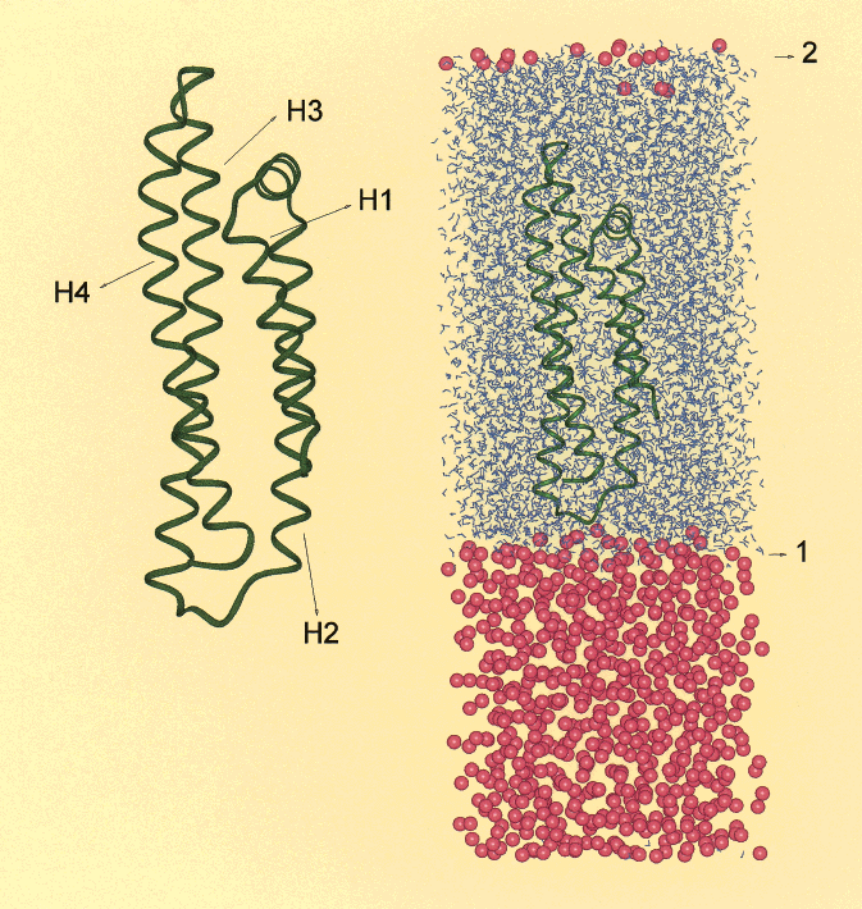

Figure 1. Right: Representation of one snapshot of the periodic interfacial system. The water phase is on top and the organic phase at the bottom. Water molecules are represented as sticks and CCl_4 molecules as spherical balls. The protein is pictured as a ribbon diagram. The location of the interfaces 1 and 2 is noted. Left: Representation of the protein crystal structure oriented as in the initial configuration of the simulation. H1 (24−42 residues), H2 (54−81 residues), H3 (87−125 residues), and H4 (130−164 residues) indicate the four helices forming the bundle.

undergoes a similar opening of its helix bundle upon interaction with a water/lipid interface.

Methods

The simulations were done starting with the high-resolution crystallographic four-helix bundle structure of apoE[21] and were carried out using the CHARMM program.[22] The protein and water interacted via the CHARMM 22 force field, where all protein atoms are explicitly represented[23] and where the water is portrayed by the TIP3P model.[24] The TIP3P water model has the great advantage that it forms a consistent optimized set with the protein parameters in the CHARMM force field.

We used a biphasic cell, composed of water and CCl_4, as a water/lipid interface mimetic. CCl_4 was described by a single Lennard−Jones sphere.[25] This model has been successfully used in simulations of biphasic cells to study the bradykinin antagonist Hoe 140[13] and the peptide hormone lipo-CCK.[26] The same model was also used in simulations of liquid−liquid interfaces to analyze the conformations of the threonine[6]−bradykinin peptide[27] and the monensin antibiotic.[28] The cross interaction term between CCl_4 and the other CHARMM parameters were obtained with the usual combining rules. We do recognize that this Lennard−Jones (LJ) liquid is a very simple model to describe the CCl_4 liquid phase. Simulations of biphasic cells performed with more realistic models for the carbon tetrachloride[17,29,30] should certainly help in improving the properties of the water/CCl_4 interface. However, our objective here is not to focus on CCl_4 as an organic phase with remarkable interfacial properties but to use a simple two-phase system which reasonably describes the water/lipid interface, as reported in the studies mentioned above.

The simulation in water was carried out on a system that consisted of the protein plus 5535 water molecules in a periodic volume with dimensions 62.1/46.575/65.205 Å. Details of this simulation have been given elsewhere.[19] The initial configuration of the interfacial simulation was obtained by joining two previously equilibrated slabs. One consisted of the protein placed in a bath of 5165 water molecules, with dimensions 86.94/46.575/43.73 Å; the other comprised 648 CCl_4 molecules, with dimensions 51.43/46.575/43.47 Å. The application of periodic boundary conditions in the three dimensions resulted in one additional interface (see Figure 1). Bonds connecting hydrogens were constrained using the SHAKE algorithm,[31] which permitted the use of an integration time step of 1 fs.

The relative orientation of the protein with respect to the interface was adjusted so as to place the four-helix bundle axis perpendicular to the interface plane. The loop between helices 2 and 3 was positioned near interface 1, whereas the segments connecting helices 1 and 2 and helices 3 and 4 looked toward interface 2 (see Figure 1). This choice was guided by the view that loops are important for the bundle opening upon lipid binding.[5] Other orientations of the protein relative to the interface should be examined as well in the future.

Long-range interactions were smoothly truncated at 8.5 Å with a shifting function for the electrostatic interaction and a switching function for the van der Waals interaction, the latter

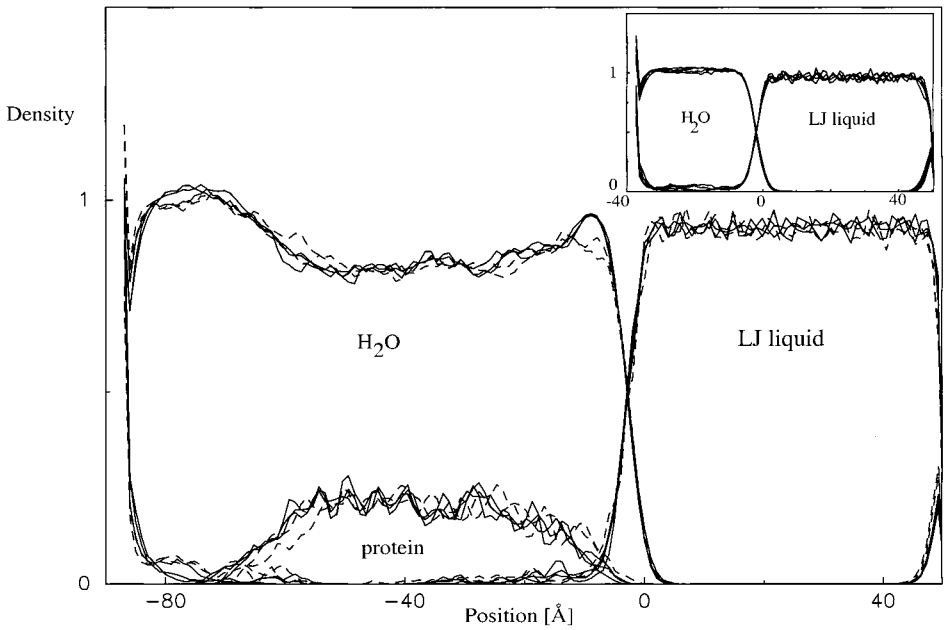

Figure 2. Average density profiles of the various components in the simulated system. The profiles were computed as a time average over all conformations produced every 0.2 ps in successive bins of 300 ps from the 1.5 ns trajectory. A slab size of 1 Å was used. Insert: average density profiles of water and Lennard−Jones (LJ) liquid computed from the 1.5 ns control simulation.

being applied between 7.5 and 8.5 Å. This truncation scheme applied to the calculation of electrostatic interactions was calibrated against the Ewald summation method in simulations of pure liquid water and was shown to perform best with respect to both structural and thermodynamic properties.[32] Moreover, an analysis of the 1.5 ns trajectory of apoE in water monitoring the burial of water molecules within the protein interior revealed that one water molecule buried in the crystal structure exchanges in the course of the simulation with one water molecule initially located in the bulk water, indicating that the balance of protein−protein and protein−solvent interactions, including electrostatic ones, is satisfactory.[33] Ewald summation method has been shown to be a better protocol for evaluating the long-range forces, in particular for polar systems.[34] It has, however, recently been the subject of studies revealing possible limitations when a solvent of low dielectric permittivity is considered and when solute cavities are large compared to the unit cell (for example, see refs 35 and 36), which are two features characterizing our interfacial system. Hence, it is still difficult at that point to transpose the appropriateness of the Ewald method to biphasic systems involving a low dielectric phase, and it seems, to our view, that more interfacial simulations making use of different long-range treatments should be performed to assess the best electrostatic procedure.

The protocol of the interfacial simulation is similar to that followed for the simulation in water. After 15 ps equilibration of the water and CCl_4 structure in the presence of the fixed protein and crystal water molecules, the system was slowly brought up to a temperature of 300 K for 30 ps and equilibrated for 60 ps, after which 1.5 ns of production run was carried out in the microcanonical ensemble. It was shown that simulations performed in this ensemble for an octane/water system produce property values close to those calculated from different thermodynamic ensemble simulations.[37]

Following the same protocol, a control simulation was performed on a water/CCl_4 system that comprises one slab of 2520 water molecules with dimensions 37.3/46.575/43.47 Å joined to a slab of CCl_4 molecules, identical to that used in the protein interfacial simulation.

Results

Density Profiles and Interfacial Width. To establish the stability of the biphasic system, we calculated the average distributions of the various components along the normal to the interface. Figure 2 shows the average density profiles for water, the LJ liquid phase, and protein heavy atoms calculated for bins of 300 ps along the 1.5 ns trajectory. The plots indicate that the interface is quite stable as the profiles are almost unchanged over the 1.5 ns simulation. Using the following definition for the width of the interface, that is, the distance required for the water density to drop from 90% to 10% of its bulk value,[38] we calculated the width of the interface to be 10 Å, a value close to that obtained in a water/hexane system[38] but larger than the width for a water/CCl_4 interface simulated with different parameters.[30] The LJ liquid-phase density profile drops to zero more slowly than the water density. A small number of molecules from the LJ liquid phase are seen to diffuse farther in the water and the protein regions as the simulation time progresses. In contrast, fewer isolated water molecules are found in the organic phase.

A simulation performed on the water/LJ liquid-phase system in absence of the protein was used as a control. The average density profiles computed from this trajectory are very similar to those obtained for the protein simulation (see insert of Figure 2).

Protein Conformational Properties. *Backbone Positional Fluctuations.* An approach for assessing the motions of apoE is to analyze the positional fluctuations that occur in the MD trajectories. The positional fluctuations of the backbone atoms were computed in successive bins of 300 ps along the 1.5 ns trajectory so as to examine the influence of the interface on the dynamical atomic properties of the protein (see Figure 3). In both simulations, one clearly distinguishes the helical bundle regions from the loop regions and from the small helix connecting helices 1 and 2: the root-mean-square fluctuations reach a maximum of about 0.3−0.4 Å in the helical bundle region, while they peak in the loop regions near 1 Å. In the first 300 ps, the magnitude of the fluctuations in the two-phase trajectory is close to that in the single water-phase one.

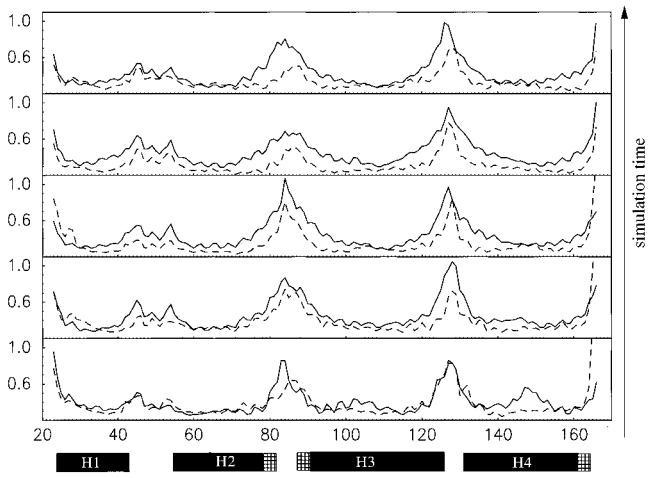

Figure 3. Backbone positional fluctuations of the protein structure around the average conformation computed in successive bins of 300 ps from the 1.5 ns trajectory of the aqueous (dashed line) and interfacial (solid line) simulations. The bars at the bottom indicate the secondary structure elements of the protein. Helix regions located close to interface 1 are indicated by crossed bars. During the simulation, the protein molecule drifts as a whole and also rotates. Thus, average structure and fluctuations were computed after each frame in the trajectory was superimposed onto a reference frame by using only rigid body rotations and translations.

Fluctuations in the interfacial system get larger and depart from those in the water trajectory as the simulation progresses. This is more remarkable in the loops and in the helical regions, extending to about three turns from the loops.

Secondary Structure Content. Average distances for the $i-i+4$ H bonds formed in the bundle helices computed over the 1.5 ns trajectories indicate that the helices, even in their terminal turns, are rather stable as they form in the crystal structure. Only small differences distinguish the distances computed in the interfacial and water simulations (Figure 4). This result agrees with several corroborating experimental data indicating that the helical content of apoE is not affected upon lipid binding.[7−8,11]

ApoE Hydration. Strikingly, apoE in the interfacial simulation, as compared to the simulation in water, evidences a higher number of backbone carbonyl groups hydrogen bonded to water molecules (see Figure 5). The average number of hydrogen bonds with water rarely exceeds one per carbonyl group in the water simulation, while it often reaches two in the interfacial trajectory. The latter also features a higher hydration of the backbone amide groups (see Figure 6).

The marked difference observed in water−backbone hydrogen bonding between the interfacial and bulk protein shows that the interfacial region favors protein hydration (see Figure 5c and 6c). The latter clearly increases for the protein portions located close to the interface. The peptide groups that feature a higher hydration in the interfacial simulation occur chiefly in the last 2−3 turns of helix 2, the first 3−4 turns of helix 3, and the last 2 turns of helix 4, which are helical portions situated close to the interface as shown in Figure 1. The N-terminus of helix 1 is further away from the interface and does not present a higher degree of hydration. A few of these solvated backbone groups are located on the hydrophobic face of the helices, though the same groups elude the interaction with water in the water simulation. To illustrate this finding, we show water molecules hydrogen bonded to protein atoms in the last 300 ps of both trajectories in Figure 7, only for helix 3 for the sake of clarity. This figure shows that the helix portion corresponding to residues on the buried face of the bundle, which is oriented to the right, is more hydrated in the interfacial trajectory up to 3−4 turns from the interface.

This result prompted us to examine whether water molecules have penetrated the bundle interior in both trajectories. Thus, water molecules found to be buried into the protein interior for periods ranging from 200 ps to more than 1 ns have been detected in the two trajectories and are shown in Figure 8. Four water molecules from the five initially buried in the crystal remain buried in the water simulation. One exits the protein interior after about 1 ns. Two water molecules initially in the bulk become buried in the course of the simulation, one of which takes over from the crystal buried water molecule that escapes to bulk water.[33] In the interfacial trajectory, the behavior of the five crystal water molecules is similar: Four remain buried and one, the same as that in the water simulation, leaves the protein interior. However, seven water molecules originally in the bulk penetrate the protein interior in the course of the simulation, one of which replaces the escaping crystal molecule. Thus, six initially bulk water molecules in the interfacial simulation get

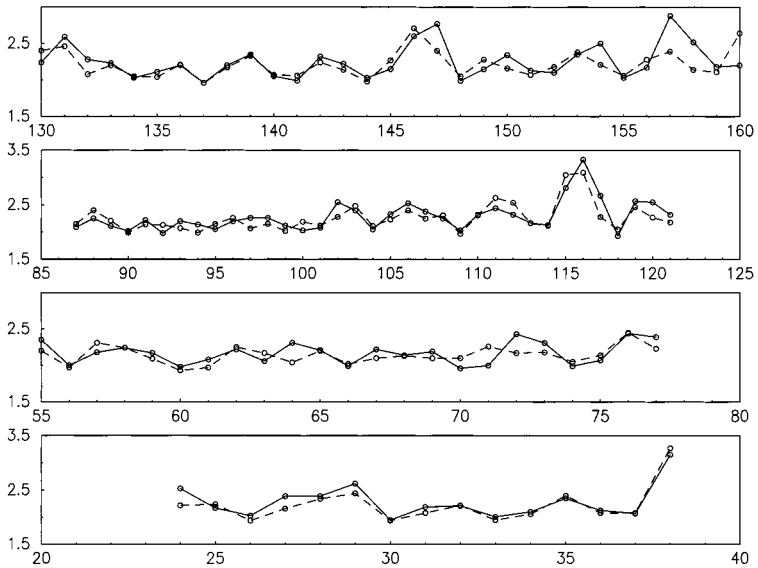

Figure 4. $i-i+4$ H bond distances averaged over the 1.5 ns trajectory of the aqueous (solid line) and interfacial (dashed line) simulations as a function of the residue number in the four bundle helices.

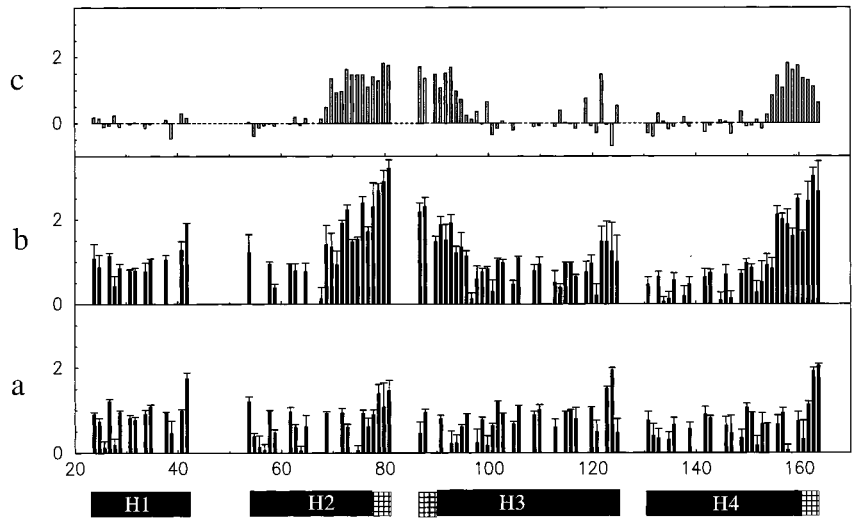

Figure 5. Fraction of H bonds formed by backbone carbonyl groups of apoE with water molecules in the aqueous (a) and interfacial (b) simulations. The fraction is defined as the number of conformations in which the corresponding group makes a hydrogen bond, divided by the total number of conformations in bins of 300 ps of the trajectory. The average values and associated standard errors result from a statistical analysis of 5 successive 300 ps bins. The excess fraction of H bonds formed in the interfacial simulation is shown on top of the figure (c). Hydrogen bonds were identified using the molecular modeling package Brugel [42] with the following criteria: a hydrogen-acceptor distance must be ≤ 2.7 Å, and the donor-hydrogen-acceptor and the hydrogen-acceptor-"from" angles must be $\geq 90°$ ("from" stands for the atom covalently linked to the acceptor atom). The bars at the bottom indicate the secondary structure elements of the protein. Helix regions located close to interface 1 are indicated by crossed bars.

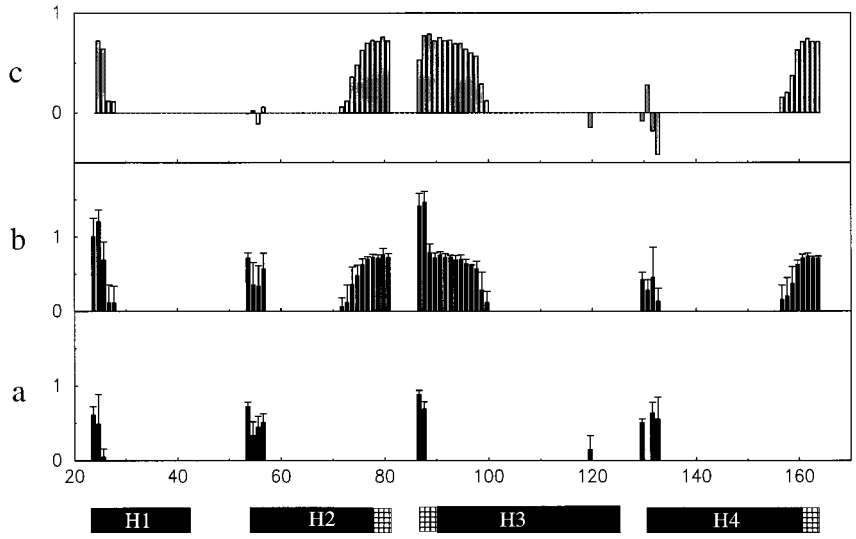

Figure 6. Fraction of H bonds formed by backbone amide groups of apoE with water molecules in the aqueous (a) and interfacial (b) simulations. The fraction is defined as the number of conformations in which the corresponding group makes a hydrogen bond, divided by the total number of conformations in bins of 300 ps of the trajectory. The average values and associated standard errors result from a statistical analysis of 5 successive 300 ps bins. The excess fraction of H bonds formed in the interfacial simulation is shown on top of the figure (c). See Figure 5 for definitions and H bond formation criteria.

buried in protein locations nonhydrated in the crystal structure, while this happens for only one water molecule in the water simulation. (To localize the protein regions that house buried water molecules, one searches for the protein atoms to which these water molecules are hydrogen bonded[33]).

The average interaction energy of the protein with water molecules, calculated from 300 ps bin averages, is equal to -4173 ± 95 and -4467 ± 70 kcal/mol in the water and interfacial simulations, respectively. The water-accessible surface area computed in several conformations along the 1.5 ns trajectories reaches a difference of about 10%. Thus, though the protein in the interfacial simulation is less accessible to water (see Figure 1), its interaction with water is more favorable.

All these results lend support to the beliefs that the protein hydration is more important in the interfacial simulation and that the interfacial region favors protein−water interactions.

Structure of Bulk and Interfacial Water. The observation of a higher degree of protein hydration in our interfacial simulation incited us to investigate the structural behavior of water. The structural properties of bulk and interfacial water in the interfacial trajectory can follow from comparison of their radial distribution functions (RDF). In agreement with previous studies of water−organic and water liquid−vapor interfaces,[38−40] the O−O RDFs computed for 1.5 ns (Figure 9) show increased water structuring in the interfacial region. In particular, the first peak is significantly sharper than that in bulk water, and the second peak is somewhat more pronounced. These behavioral differences have also been observed in our control simulation (see insert of Figure 9).

An analysis of hydrogen bonding of the water molecules in the interfacial zone indicates as one might anticipate that the average number of H bonds per molecule and the average

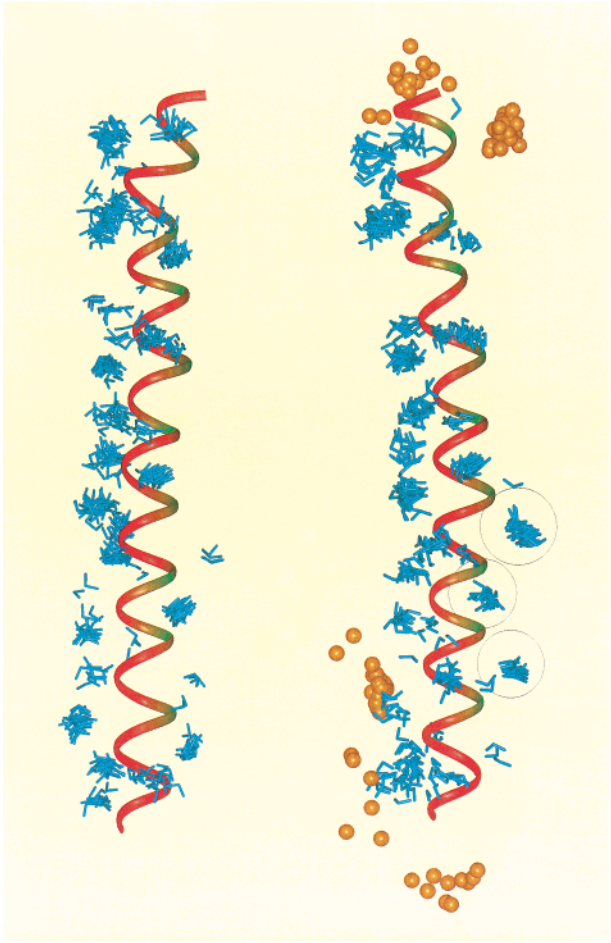

Figure 7. Ribbon diagram of one snapshot structure of helix 3 (residues 87−125) in the aqueous (left) and interfacial (right) trajectories. The portion corresponding to residues on the buried face of the helix is colored in green and oriented to the right. Water molecules hydrogen bonded to protein atoms in the last 300 ps trajectory are shown as blue sticks, while CCl_4 molecules observed within a 5 Å distance of protein atoms in the same trajectory are shown as orange spherical balls. The bottom of the figure faces interface 1. Circles encompass buried helix regions that are more hydrated in the interfacial simulation than in the aqueous simulation.

number of nearest neighbors decrease as one moves from the bulk to the interface. Interestingly however, the relative number of H bonds per water molecule with respect to its coordination number actually increases (Table 1).

Discussion

Apolipoprotein E in water and located at a mimetic water/lipid interface has been investigated by molecular dynamics simulations. A simulation cell with two immiscible liquids, water and CCl_4, was used to simulate the interface. CCl_4 is merely treated as a LJ liquid. However, this biphasic system, designed to describe two media of different polarity and H-bond donor/acceptor capabilities, was shown in several studies to give a satisfactorily representation of a water/lipid interface (see Methods section).

The main goal of this study is to examine the influence of the interface on the structural and dynamic properties of the protein relative to those observed in the single water phase. Before such an issue could be addressed, the preservation of the biphasic cell was verified in the interfacial simulation in the presence of the protein and in a control simulation. The density profiles of both trajectories argue in favor of the

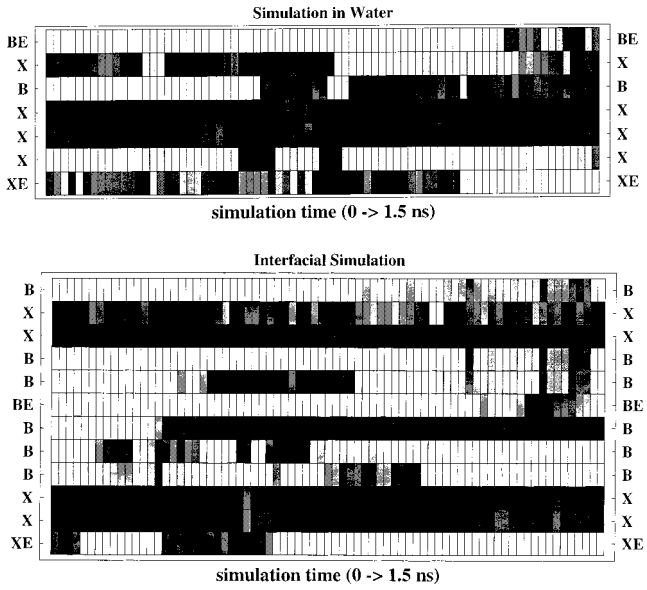

Figure 8. Number of conformations featuring one water molecule buried in the protein interior as a function of the 1.5 ns simulation time in (on top) water and (at the bottom) interfacial trajectories. X indicates the water molecules that are buried in the starting X-ray structure, and B indicates those that are initially in the bulk. The X-ray and bulk water molecules that exchange during the course of the simulation are noted XE and BE. Each rectangle corresponds to a 20 ps bin and is shaded according to the magnitude of the population.

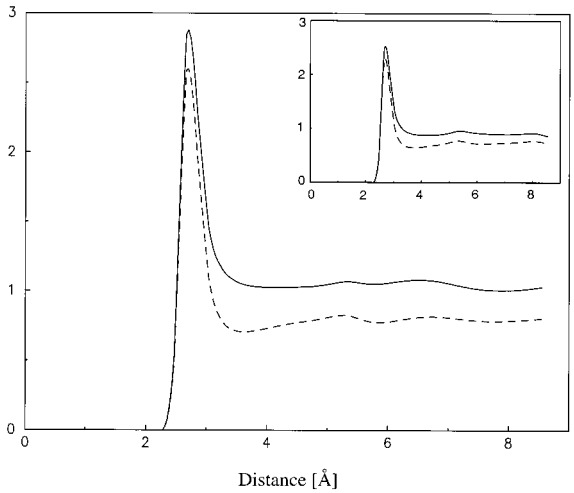

Figure 9. Oxygen−oxygen radial distribution functions for bulk (solid line) and interfacial (dashed line) water in the interfacial simulation and in the control trajectory (insert). The average is over 1.5 ns. Interfacial and bulk water are selected on the basis of a distance criterium. Water molecules within a distance value less than 5 Å from molecules of the organic phase are defined as interfacial water. Those beyond a distance value of 7 Å from molecules of the organic phase and protein atoms are defined as bulk water.

TABLE 1: Hydrogen Bonding Characteristics of Water in the Interfacial Simulation

region	average H bonds	average number of nearest neighbors	relative number of H bonds
bulk	3.2	4.9	0.65
interfacial	1.3	1.5	0.87

persistence of the phase separation. The interfacial width does not substantially vary in the course of the simulations. A larger diffusion of the LJ molecules toward the water phase is observed and disagrees with solubility data on CCl_4.[41] This discrepancy is likely to be due to the simplified description of the organic

liquid and has also been observed in a water/hexane system using a united atom description of the hexane molecule.[38]

Landmarks of protein secondary structure such as H bonds monitored in the two simulations indicate that the secondary structure content of the protein is not affected by the presence of the interface, even in the terminal turns of the bundle helices. This is in agreement with several experimental studies that show the absence of modification of helical content upon interaction with lipid surfaces.[8,11]

The presence of the interface, however, affects the magnitude of the backbone positional fluctuations in the course of the simulation. Analysis of these small-amplitude fluctuations shows that the protein residues, when located near the interface, manifest larger fluctuations than their equivalent in the trajectory in water.

The interfacial region is the locus of not only larger atomic scale fluctuations but also of a much higher degree of protein backbone hydration. Indeed, peptide groups located close to the interface get significantly more hydrated in the interfacial simulation than in the simulation performed in water. Along with a larger number of water−protein hydrogen bonds, the interfacial simulation is also characterized by a higher number of water molecules found to penetrate the protein interior and a more favorable protein−water interaction energy.

The observation that the interfacial region in the biphasic cell fosters favorable interactions between water and protein atoms incited us to peer at the structural behavior of water in the interfacial region. Also, it was previously observed in simulations of flexible interfaces that water in the interfacial region differs from that in the bulk in that it unveils an increased structure. Our interfacial simulations display an identical behavior. Such a behavior has been ascribed to the number of neighboring water molecules smaller in the interfacial area than in the bulk, forcing the water molecules to orient themselves to take better advantage of the existing hydrogen-bonding possibilities from the other water molecules.[38−40] The finding that water molecules in the interfacial region take advantage not only of their neighboring water molecules but also of the hydrogen-bonding potentialities provided by the protein itself is a novel result. One could perhaps have anticipated this finding from the particular behavior of the interfacial water previously observed in simulations of flexible interfaces. To our knowledge, however, none of the numerous simulation studies on solutes and/or peptides interacting with a water/organic solvent interface has estimated the degree of solute hydration and examined the possible implication of this property on the conformation and orientation of these solutes at the interface. One expects the influence of the interfacial water on the protein hydration to be nonspecific of the amino acid sequence. At this stage, additional and much longer simulations are required to gain deeper insight into how protein hydration and small-scale fluctuations could lead to a much more developed conformational change and also on which impact different starting protein orientations would have on the protein behavior observed here. These simulations could also help to measure more accurately the extent of protein hydration we observed in the interfacial region.

These results provide a dynamic perspective of the interactive nature of apoE with the interface that is regulated by the interfacial water whose structural behavior differs from that of bulk water. No experimental data on the hydration of apoE upon lipid binding is available. However, spectroscopic data on another apolipoprotein, the structurally similar apolipophorin III, have shown that a possible contribution to the decrease in the energy barrier allowing the transition from the conformation of the apolipoprotein in the aqueous phase to the open conformation prone to bind the lipids is the internal hydration of the protein.[20]

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