Molecular Dynamics Study of Complexes of Poly(glutamate) and Dodecyltrimethylammonium

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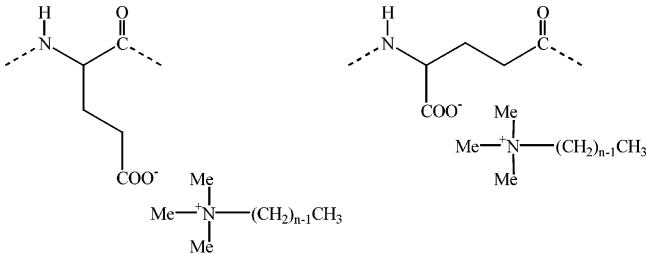
The supramolecular organization of self-assembled stoichiometric complexes formed by dodecyltrimethylammonium cations and ionized poly(γ-glutamic) acid has been investigated in chloroform solution. Atomistic molecular dynamics simulations have been performed considering three different starting conformations for the polyelectrolyte chain, two sets of electrostatic parameters, and two methods for evaluating the electrostatic interactions. Results indicate that the polypeptide chain tends to adopt an α-like helix conformation similar to that obtained in aqueous solution for the un-ionized poly(γ-glutamic) acid. On the other hand, both surfactant···amide and surfactant··· carboxylate interactions were identified in the complex, this multiple pattern being previously observed in other surfactant···polypeptide complexes. Although the influence of the force-field in the results has been found to be negligible, the method used to evaluate the electrostatic interactions affects significantly the dynamics of the system. The more important differences between the results obtained using the spherical cutoff and Particle Mesh Ewald methods are discussed.

Introduction

There are currently two priorities when designing new synthetic complex materials for high performance applications: low cost and simplicity of production. It is immediate to think of the self-assembled complexes as a potential source of new products that would meet both criteria.[1] These materials are characterized by the association of their constituent parts throughout fast kinetics processes and by means of noncovalent interactions.[2] Among this new sort of material, self-assembled complexes formed by charged polymer chains (polyelectrolytes) and oppositely charged molecules have attracted much of the attention due to the simplicity of their assembly process mainly driven by simple electrostatic attraction. Specifically, complexes constituted by peptide polyelectrolytes and amphiphilic counter ions of low molecular weight with different hydrophobic content (surfactants) have centered most recent research efforts.[3−5] In these cases, the association of these components induces microscopic organizations that present different degrees of order depending on the actual conditions of assembling (solvent, temperature, concentration of ions, etc.). Polypeptides can adopt different regular helical conformations depending on their chemical constitution, both in nonaqueous solution and in the solid state.[3a,b] On the other hand, the amphiphilic nature of the surfactants produces arrangements with a spatial separation between the charged head-groups and the aliphatic tails.[3c]

The most studied complex series belonging to this family of materials is the one constituted by ionized poly(α-glutamic) acid (PAGA) and oppositely charged surfactants based on alkyltrimethylammonium (*n*-ATMA; *n* denoting the number of carbon atoms of the alkyl chain, Scheme 1).[3a] In nonpolar solution, the polypeptide chain of *n*-ATMA·PAGA complexes adopts a regular conformation that depends on both the physical nature of the solvent and the structure of the surfactant groups.[4,5] Our recent research has been centered in the investigation of these effects over the self-assembly organization of different types of complexes based on PAGA.[4,5]

Scheme 1. Self-Assembled Complexes Constituted by Alkyltrimethylammonium Surfactant Cations (*n*-ATMA) with Ionized Poly(α-glutamic) Acid (PAGA) and Ionized Poly(γ-glutamic) Acid (PGGA)



n-ATMA·PAGA *n*-ATMA·PGGA

The search for biocompatible materials has made researchers turn to natural polymers as the potential source of raw materials. Thus, the interest raised by biopolymers has largely increased over the last decades, because the cellular machinery can be selectively used as high performance reactors, saving time and money. The raw material of interest is a natural component of cellular systems, which only needs to be extracted after the growth of the producing organism. An excellent example of this polymer manufacturing is the poly(γ-glutamic) acid of bacterial origin (PGGA). This polymer is currently produced at industrial scale,[6] and its biocompatibility has experimentally been proved.[7] Its potential uses as biocompatible fibril material have recently been revised,[8a] and its conformational properties were largely investigated.[9] The raw polymer (PGGA) is soluble in water, and its biosynthesis brings over a block copolymer of different proportions of L and D enantiomers depending on the organism used and the conditions of the culture medium.[10] In aqueous solution, un-ionized PGGA (acidic conditions) forms a stable helical conformation that resembles the α-helix of the α-polypeptides.[9] Additionally, to explore its potential technological use, different esters derived from PGGA have been

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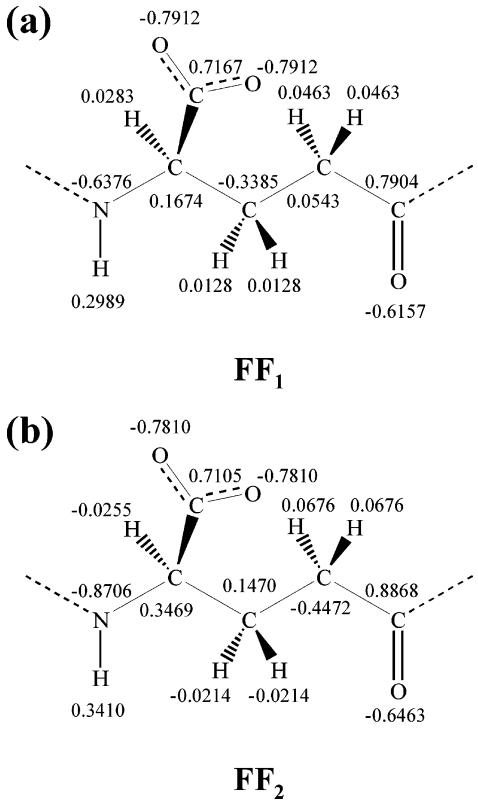

Figure 1. Partial atomic charges derived from ab initio quantum mechanical calculations (see text): (a) FF_1 and (b) FF_2.

obtained by esterification of the biopolymer, and their structural properties have been studied.[8]

The acidic character of PGGA makes it a good potential anionic polyelectrolyte to produce stoichiometric complexes with cationic surfactant molecules. In fact, the production of such kind of materials at lab scale has been recently reported (*n*-ATMA·PGGA).[11,12] However, the experimental data available related to the supramolecular structure and the conformational behavior of the complexes in solution are much lower than those in the PAGA complexes. Unfortunately, there is no clear indication of the organization for the main chain of the polyelectrolyte, even though these complexes are stable in chloroform.[12] In this Article, we report a systematic investigation of the self-assembly organization of the complexes *n*-ATMA·PGGA in chloroform solution, where we studied possible conformations for the polyelectrolyte main chain and the supramolecular organization that derives from each potential polypeptide conformation. Furthermore, the effect of screening of long-range interactions due to discontinuities in the electrostatic potential while performing molecular dynamics (MD) simulations is evaluated by considering different methodological approaches.

Methods

All MD trajectories were generated using the scalable computer program NAMD 2.0.[13] An adapted version of the AMBER94 all-atom force field that includes previously developed parameters for *n*-ATMA·PAGA complexes[4,5a,b,14] was used. All of the bonding and nonbonding terms were extrapolated to the new systems with the exception of the electrostatic parameters of the γ-glutamate residue. In this case, two sets of partial charges were specifically developed for that residue (see Figure 1). For this purpose, geometry optimizations at the ab initio Hartree−Fock quantum mechanical level combined with the 6-31G(d) basis set[15] were performed for a series of representative conformations of the γ-glutamate residue, that is, those related to the helical arrangements of the PGGA and its derived esters.[8,9] For each conformation, the molecular electrostatic potential (MEP) was computed from the wave function of the optimized geometry. Finally, atomic electrostatic parameters were derived for each conformation by fitting the quantum mechanical MEP values into partial charges centered in the nuclei[16] by means of the Levenberg−Marquardt nonlinear optimization procedure. As a result, two different sets of charges were obtained: one corresponding to the conformation of lowest energy, hereafter denoted FF_1, and another derived from the Boltzmann distribution provided by the energies of all of the calculated conformations, denoted FF_2. It should be noted that the parameters provided by the HF/6-31G-(d) level of theory are fully consistent with those included in the AMBER libraries.[14]

Inspection of the charges displayed in Figure 1 reveals that the main difference between FF_1 and FF_2 involves the partial atomic charges located at the backbone carbon atoms. This fact should be attributed to the lowest energy conformation, which only contributes 27% to the parameters FF_2. Interestingly, the atomic charges at the carbon atoms are very different for the considered conformations. This important numerical variation is due to the electronic flux induced by the variation in the backbone dihedral angles but not to the presence of intramolecular interactions, which were not detected in the calculated model compound, that is, the γ-glutamate residue blocked at the N- and C-terminal positions with acetyl and *N*-methylamide groups. To ascertain if the results reached in this work depend on the parametrization procedure, we decided to run the simulations using electrostatic parameters derived from both single- and multiple-conformations. It should be noted that the influence of the procedure used for the electrostatic parametrization was not investigated previously for PGGA and its ester derivatives. All of the quantum mechanical calculations were performed using the Gaussian 03 computer program.[17] Finally, the liquid medium was simulated by using the OPLS model of chloroform,[18] which has been found to provide excellent results when the solute is described with the AMBER force-field.[14]

To explore the conformation of the polypeptide chain, different regular organizations previously reported for PGGA and its derived esters were used as starting points. Thus, three different topologies were built following the conformations described for the three crystal forms of the poly(α-benzyl-γ-glutamate):[8a] the 2/1 helix characteristic of the crystal form I, the 5/2 helix observed in form II, and the helix 17/5 (also denoted α-like helix) of the crystal form III. Moreover, the latter helix was also determined for the PGGA in aqueous solution at low pH.[9] On the other hand, the surfactant molecular cations were initially placed in front of each carboxylate group at a distance close to that determined previously; that is, the distance between the carbon atom of the carboxylate group and the nitrogen atom of the ammonium group was set at 4.04 Å.[19] Therefore, the initial supramolecular organization was not biased by the polypeptide conformation due to the geometrical restrictions imposed by each helical symmetry. Recent extensive work showed that when the polymer conformation was correct, the molecular cations self-organize after a few hundreds of picoseconds.[4,5] In all cases, the target complex was built using: (i) a peptide constituted by 15 γ-glutamate residues and blocked at the N- and C-terminal positions with acetyl and *N*-methylamide groups, respectively; and (ii) 15 independent dodecyltrimethylammonium molecular cations (12-ATMA) with the dodecyl groups arranged in all-trans.

Each complex was placed in the center of a cubic box of 124.7 Å of side length, full of previously equilibrated chloroform molecules. The box dimensions were chosen to avoid biased results due to violation of periodic boundary conditions: a peptide chain arranged in a totally extended conformation would measure at most 30 Å less than the box edge, that is, more than 1.5 times the nonbonding interactions cutoff (see below). The solvent contained 14 509 chloroform molecules and was equilibrated at constant volume and temperature (298 K and 1.48 g/cm^3, the latter being the experimental density). Once the stoichiometric complex was placed inside the box, those solvent molecules that

Table 1. Summary of the MD Simulations in Solution of the 12-ATMA·PGGA Complex

simulation	helix type	set of charges	electrostatic interactions
Ia	17/5	FF_1	spherical cutoff 14 Å
Ib	5/2		
Ic	2/1		
IIa	17/5	FF_2	spherical cutoff 14 Å
IIb	5/2		
IIc	2/1		
IIIa	17/5	FF_1	Ewald (PME)
IIIb	5/2		
IIIc	2/1		

overlapped any complex atom were removed. To equalize the number of solvent molecules of the different systems, the exceeding molecules compared to the system that had the least number were removed. Accordingly, each initial system presented 68 255 explicit atoms including those of $CHCl_3$ molecules. Finally, the density of each system was adjusted to the experimental density value.

In all cases, bond lengths were constrained to their standard values using the shake algorithm.[20] Periodic boundary conditions were applied using the nearest image convention, and the nonbonded pair list was updated every 25 MD steps. Residue based-cutoffs were applied at 14 Å for van der Waals interactions. To avoid discontinuities in the Lennard-Jones potential, a switch function was applied to allow continuum decay of energy when atom pair distances are $\geq$12 Å. For electrostatic interactions, two different approaches were used. First, to estimate the influence of the developed partial charges on the complexes organization and the peptide main-chain conformation, two series of simulations (I and II series, in Table 1) were carried out using a spherical cutoff of 14 Å. Furthermore, additional simulations (III series, in Table 1) were performed computing the none truncated electrostatic potential throughout Ewald Summations. In this approach, the real space term was determined by the van der Waals cutoff (14 Å), while the reciprocal term was estimated by interpolation of the effective charge into a charges mesh with a grid thickness 5 points per volume unit (Particle Mesh Ewalds method; PME).[21] Comparison among the results provided by the spherical cutoff and PME approaches will allow one to evaluate the effects of the electrostatic potential truncation and the total charge screening over the self-assembled organization. Table 1 summarizes the nine simulations described in this work.

Before any MD trajectory was run, 10 000 steps of energy minimization were performed to relax conformational and structural tensions. In all simulations, the numerical integration step was 2 fs. Different consecutive rounds of MD short runs were performed to equilibrate the density of the system, the temperature, and pressure. Thus, once the complex was placed in the center of the simulation box (see above), the solvent structure was first optimized by keeping the stoichiometric complex frozen (belly conditions) and allowing the chloroform molecules to freely move using two rounds of NVT simulation (1 ns at 500 K and a cooling period of 1 ns to equilibrate temperature at 298 K). Next, the optimization of the density was achieved by 1 ns of NPT MD. Finally, the entire system was brought to the simulation conditions through 500 ps of NVT MD (thermal relaxation) followed by 1 ns of isobaric relaxation (NPT conditions), where all of the atoms of the system were allowed to move. Both temperature and pressure were controlled by the weak coupling method, the Berendsen thermobarostat,[22] using a time constant for heat bath coupling and a pressure relaxation time of 1 ps. All of the NPT production simulations (after thermal and isobaric relaxations) were 7 ns long, with trajectories being saved every 500 steps (1 ps interval) for subsequent analysis.

Results

In this work, we have characterized the structural features of the 12-ATMA·PGGA complexes, while evaluating the effect of different methodological aspects related to the classical potentials used to describe surfactant·polypeptide systems. Thus, we will describe not only the supramolecular organization of the complexes but also those microscopic features that depend on how the electrostatic screening affects each particular starting point complex organization.

Main-Chain Conformation. *Conformational Preferences.* Three different possible conformations for the PGGA were studied, as it has been introduced above. The first remarkable feature that can be concluded from these simulation series is that, independently of the methodological approach, the α-like helix, also named as 17/5 helix,[8a,9b] always arises as the preferred regular conformation. Different approaches can be followed to quantify the relative stability between the helical conformations that were taken into account. Classic peptide conformational analysis such as Ramachandran plots is not recommendable for γ-amino acids due to the large number of flexible backbone dihedral angles. In this work, we have used the evolution of the average helical symmetry along the simulated time to investigate the main-chain conformation. Thus, the higher the regularity of the conformation is kept, the more favored the initial conformation can be inferred. As can be observed in Figure 2, only the 17/5 helix was retained in all simulated conditions, maintaining the helix symmetry, that is, the number of residues per turn, steadily. Even when the whole electrostatic contribution is accounted for, the helical symmetry remains almost unvarying around the initial value of 3.4 residues per turn. Furthermore, the intramolecular hydrogen bonds that stabilize this helical conformation are retained for the 10 central residues during the whole trajectories (see Supporting Information).

Inspection of Figure 2a and b suggests that when starting from the 5/2 helix, the organization reached using a truncated electrostatic potential converges into some steady organization. However, examination of the main-chain hydrogen bonds characteristic of this conformation (Figure 3) indicates that no trace of the original helix organization is retained. Moreover, when cutoff is not used, the 5/2-helical symmetry disappears as fast as the pseudo extended 2/1-symmetry (Figure 2c). Thus, the 2/1 helix underwent a rapid loss of any trace of regularity in all simulation conditions.

Effect of the Electronic Screening. The results exposed above revealed an interesting feature: there is a significant dependence between the mobility of the polymer main chain, that is, the conformational flexibility, and the continuity of the electrostatic potential around the polypeptide. Thus, while the final conclusion remains the same, the 17/5 helix is the unique regular conformation favored for the peptide chain, the mobility of both the central segment and the edges of the helix dramatically increases when the electrostatic interactions are accounted without discontinuities at long distance ranges (series III). The average values of the root-mean square-deviation (rmsd; see Supporting Information) and their standard deviations, which are displayed in Table 2, abruptly increase when the PME method is used. Only those trajectories that started from a 5/2 helix model seem to present a certain level of homogeneous behavior independently of the approach employed for computing the electrostatic potential. However, even in this case, the averaged rmsd increases from 3.30 to 5.29 Å when no artificial discontinuities are included in the calculation of electrostatic interactions. On the other hand, it is worth noting that FF_1 and FF_2 provide very similar results from a qualitative point of view, indicating that the procedure used to parametrize the force-field was not essential in this case. This was the reason because FF_1

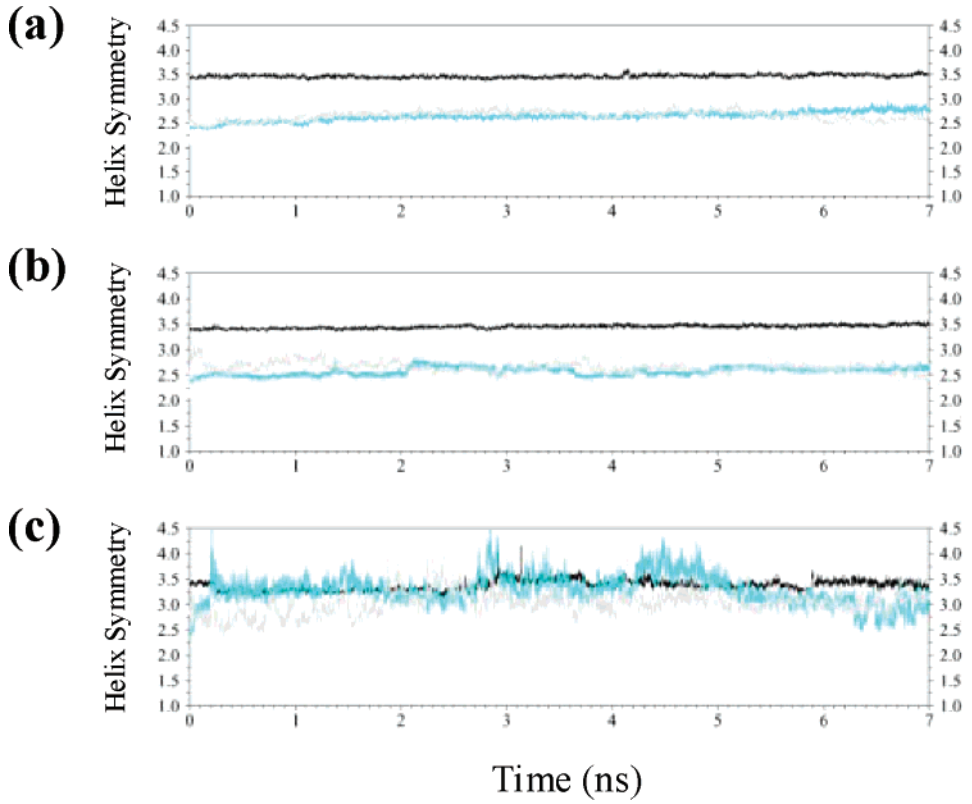

Figure 2. Number of residues per turn for the 17/5 (black), 5/2 (gray), and 2/1 helices (light blue). The number of residues per turn was obtained by averaging the values computed for the nine central residues. From the top to the bottom, the results correspond to: (a) simulation series I; (b) simulation series II; and (c) simulation series III (see Table 1).

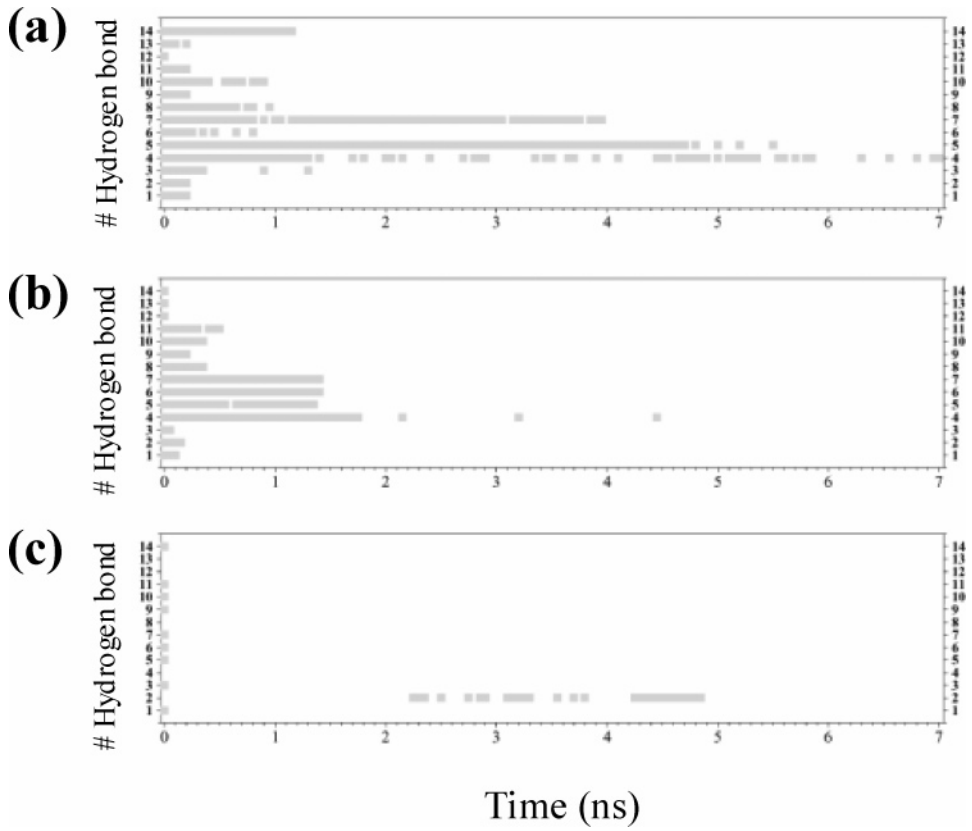

Figure 3. For the simulations starting from the 5/2 helix, spatio-temporal evolution of the C═O(*i*)→H−N(*i*+2) type hydrogen bonds in the PGGA chain. Results correspond to the different approaches used to estimate the electrostatic potential: (a) cutoff 14 Å and FF_1 (simulation Ib); (b) cutoff 14 Å and FF_2 (simulation IIb); and (c) PME and FF_1 (simulation IIIb).

was the only selected for simulations using the PME method, which are very demanding from a computational point of view.

The effect of the electrostatic potential truncation on those conformations that are less favored deserves special interest.

Table 2. Averaged Root-Mean-Square Deviation (rmsd; in Å) after 7 ns Trajectories Computed Only for the Polymer Backbone Atoms (Corresponding Standard Deviations Are Displayed in All Cases)

	MD series		
starting point	I series[a]	II series[b]	III series[c]
17/5 helix	0.72 ± 0.11	0.78 ± 0.09	1.85 ± 0.47
5/2 helix	3.30 ± 0.88	4.60 ± 1.03	5.29 ± 0.61
2/1 helix	4.15 ± 0.86	1.74 ± 0.44	8.23 ± 2.35

[a] Spherical cutoff and atomic charges set FF_1. [b] Spherical cutoff and atomic charges set FF_2. [c] PME and atomic charges set FF_1.

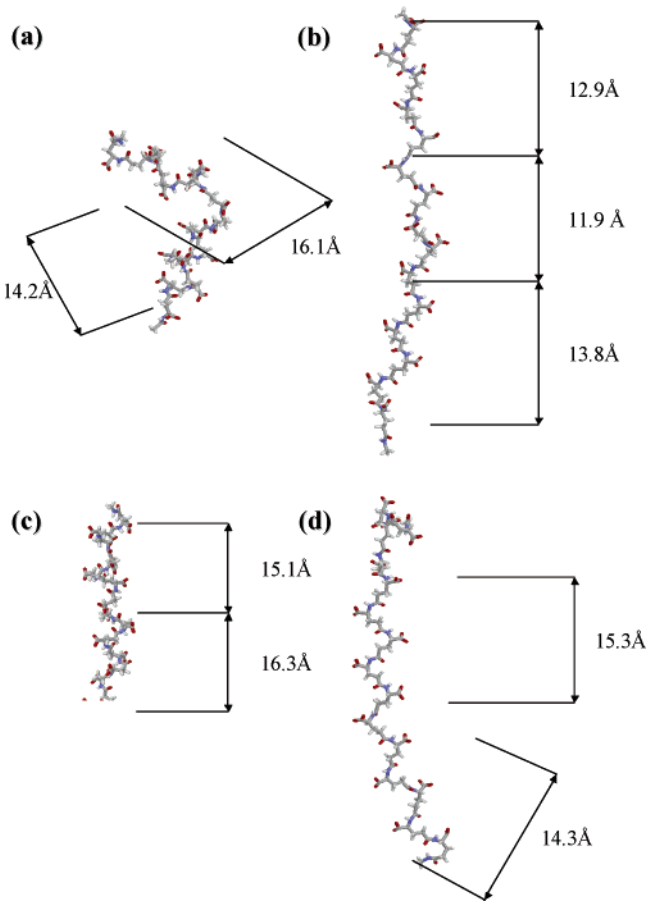

Figure 4. Atomistic representation of the main-chain conformation for the last snapshot corresponding to: the simulation series I starting from the 5/2 helix (a) and the 2/1 helix (b); and the simulation series II starting from 5/2 helix (c) and the 2/1 helix (d). In all cases, the distance associated with the pseudo-repeat period is displayed (see text).

Thus, the 2/1 and 5/2 helices lose their main conformational identity features, more graphically identified later by the absence of hydrogen bonds (Figure 3a and b). However, the final organizations that are observed do not reflect a total break of their characteristic symmetry elements. As is illustrated in Figure 4, the main-chain conformations obtained using cutoff always bring over some degree of three-dimensional order. Independently of the partial charges set, certain types of new helical conformations were achieved after losing the initial regular organizations. This effect becomes significantly marked in those complexes that presented as starting conformation a 2/1 helix (Figure 4b and d).

The new main-chain conformations seem to present some sort of pseudo-repetition period along the helical axis, which ranges from 12 to 16 Å. Even though these results may be interpreted as some significant organization that 12-ATMA·PGGA can adopt in solution, it has been recently reported that force field-based simulations of non-charged molecular species with high dipolar moment, such as the α-helix conformation of polyalanine in solution or the water molecules in liquid phase, provide spurious results when the long-range electrostatic potential is abruptly truncated.[23] Thus, under these conditions, MD trajectories can spontaneously evolve toward some sort of ordered organizations at the edges of the cutoff distances.[23] The behavior found in this work for 2/1- and 5/2-helical conformations seems to mold an analogous scenario, because the pseudoperiodicity noted above is in the order of the cutoff we were using. Thus, although our system is a polyelectrolyte, the uncharged backbone apparently follows such trend. Thus, when the electrostatic screening is limited by a cutoff and the initial conformation is not stable, the polypeptide dynamics apparently becomes partially independent of the electrostatic interactions that occurred with the surfactant molecules. This thesis seems to get more strength by comparing the aforementioned pseudo-ordered conformations with those obtained using the PME method. In the latter case, the polypeptide chain adopts an irregular pseudo extended conformation that cannot be fitted into a helical organization, behaving as a quasi-rigid rod, a typical feature of polymers with high density of charged groups (see Supporting Information).

Self-Assembly and the Electrostatic Screening. A preliminary analysis of the 12-ATMA·PGGA complexes studied in this work showed the general trends already reported for the complexes 12-ATMA·PAGA:[4,5] the association between oppositely charged groups always presents a multiple interaction pattern. This feature is independent of the set of charges used, the methodology followed to compute the electrostatic term, and the initial conformation of the PGGA chain. However, in 12-ATMA·PGGA complexes, the multiple interaction pattern arises not only from the interactions of each surfactant cation with several carboxylate side groups but also from the interaction of the surfactant with the carbonyl group of several close amide moieties.

The final statistics distribution corresponding to the surfactant···carboxylate and surfactant···amide interactions is plotted in Figure 5 for all of the simulations performed (including the time frame simulated per each MD). According to our previous study, these interactions were identified as such when the distance between the nitrogen atom of the molecular cation and the carbon atom of the carboxylate or amide group was smaller than 5.50 Å.[5b] Some remarkable features can be derived from this representation. First, the influence of the approach used to calculate the electrostatic potential on the geometry of the self-assembly is minimal, as we have already advanced. Apparently, the organization of the surfactant with respect to the distribution of carboxylate and amide groups is determined by the starting conformation of the polypeptide chain. Second, there is always a recurrent formation of surfactant···amide interactions. As can be seen in the left plots of Figure 5, the most populated arrangement tends to implicate a maximum of two carboxylate side groups, while previous studies showed that 12-ATMA·PAGA complexes prefer to surround the molecular cation with three carboxylate side groups.[5a,b] However, the triple multiplicity also appears in 12-ATMA·PGGA if the surfactant···amide interactions are added.

Hydrophobic Tails Organization: Conformation and Dynamics. The organization of the aliphatic tails follows in all cases a conformational pattern analogous to that detected in self-assembled complexes formed by PAGA.[5a,b,24] Regardless of the polymer conformation, the conformational properties of the

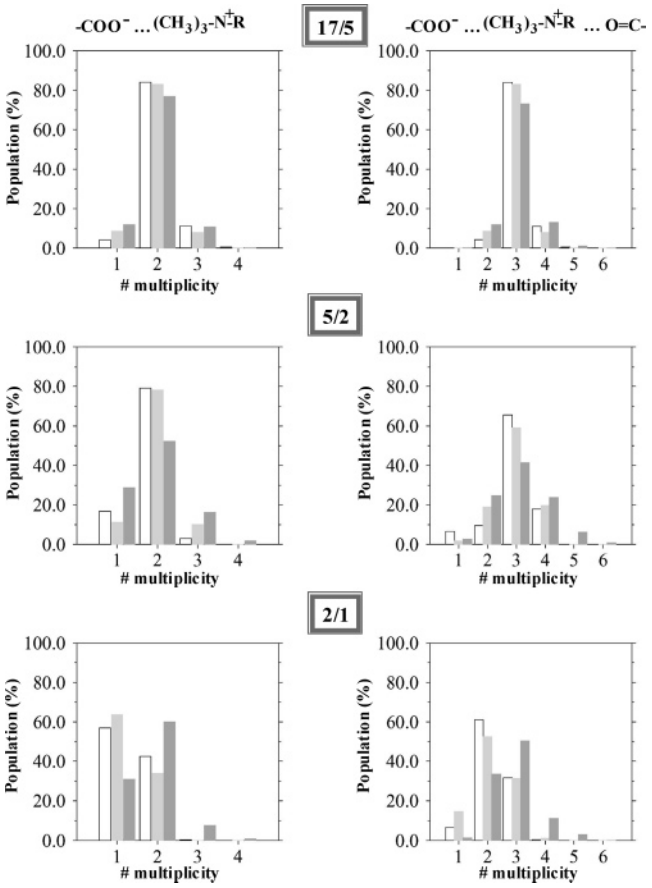

Figure 5. Average number of carboxylate and amide groups that interact with each surfactant molecule (multiplicity) during the simulated time. All results are expressed in percentage populations, averaged over 15 surfactant molecules and 7 ns of trajectory. Each graphic represents the average per each simulation type (see Table 1) depending on the gray scale: white series I simulations, light gray series II simulations, and dark gray series III simulations. From the top to the bottom, the results obtained for the simulations starting from 17/5, 5/2, and 2/1 helices. At the left side, the calculation only includes carboxylate groups, while at the right side, both carboxylate and amide groups are included.

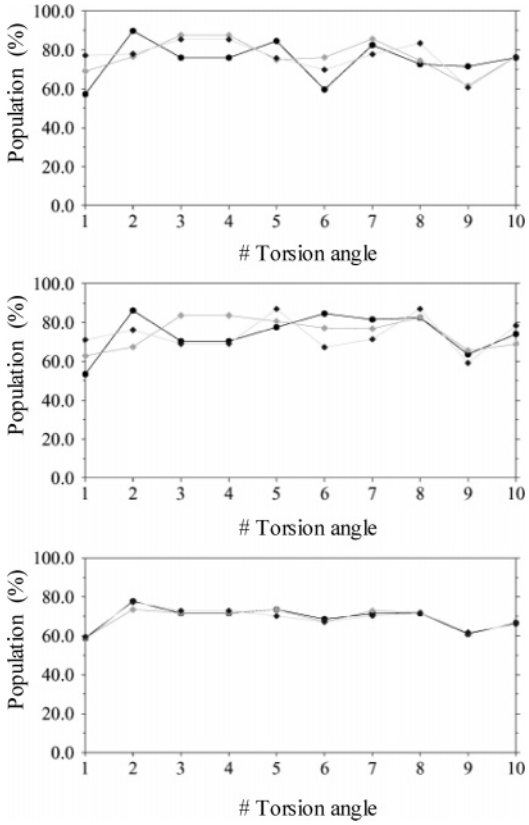

Figure 6. Conformational preferences of the surfactant hydrophobic tails as a function of the force field and the methodological approach used to evaluate the electrostatic interactions. The representation corresponds to the percentage of trans rotamer for each dihedral angle (starting from the positively charged head) derived from MD simulations that used as starting geometry the 17/5 (black circles and black lines), 5/2 (gray diamonds and gray lines), and 2/1 (black diamonds and gray lines) helix. From the top to the bottom (see Table 1): (a) simulation series I; (b) simulation series II; and (c) simulation series III.

dodecyl alkyl chain in 12-ATMA·PGGA complexes under study are similar. Thus, analysis of the hydrophobic tail of the 12-ATMA molecular cations in the simulations of the α-like helix evidenced that the trans is the most populated rotamer for the 10 free rotation dihedral angles (see Supporting Information). Furthermore, the first dihedral angle presents, with respect to the other dihedral angles, a significantly smaller proportion of trans rotamer. This is not surprising because the beginning of the aliphatic tails is anchored at the ammonium group, its conformational freedom being subordinated to the dynamics of the surfactant···polypeptide association. Nevertheless, the rest of the methylenes display the proper independent behavior expected from their complete different chemical nature. Very similar results were obtained for the simulations starting from the 2/1 and 5/2 helices. Thus, the organization of the surfactant dodecyl groups in chloroform solution remains almost identical independently of the polypeptide conformation.

On the other hand, Figure 6 compares the conformational preferences of the paraffin tails provided by the different procedures used to deal with the electrostatic interactions. As can be seen, independently of the accuracy of the algorithm used to compute the electrostatic energy and the set of electrostatic parameters, the hydrophobic segments present undistinguishable conformational profiles. This behavior is fully consistent with the chemical characteristics of the hydrophobic tails. Thus, the dodecyl groups would prefer extended conformations over folded ones because the former organizations guarantee a bigger contact surface, that is, enabling interactions either with other side chains or with the chloroform molecules.

Supramolecular Organization in Solution: Cutoff versus PME. Figure 7 depicts the final organization achieved starting from the α-like helix conformation for each simulation series. The most intuitive feature that can be extracted from these results is that spherical cutoff reduces the effects of thermal agitation in the dynamics of the 12-ATMA·PGGA complexes. Thus, despite preserving the main features that define the 17/5 helix, the outcome of 7 ns of simulation IIIa, that is, computing the electrostatic potential by means of the PME method, results in a distorted conformation that presents a quite marked degree of irregularities (Figure 7c). This is because the use of PME produces an overestimation of surfactant···surfactant interactions with respect to surfactant···solvent interactions. This effect does not contradict the basic conclusions of this investigation: the 17/5 helix is the only suitable regular conformation that this type of stoichiometric complex can present in chloroform. Accordingly, the same conclusion could have been reached only using simulations with spherical cutoff. However, the later method tends to overestimate the relative stability of unreliable organizations by smoothing the conformational flexibility. As we have already mentioned, it would lead to the formation of

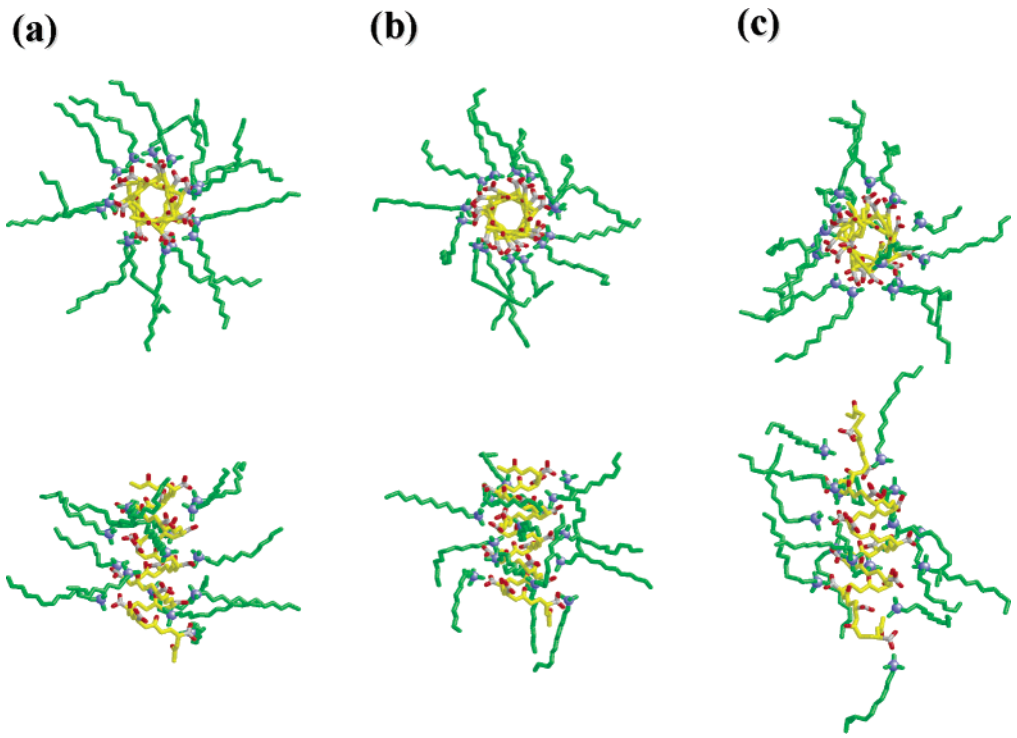

Figure 7. Sticks representation of the final complex organization achieved using the 17/5 helix as starting point (see Table 1): (a) simulation Ia; (b) simulation IIa; and (c) simulation IIIa. All hydrogen atoms have been removed for clarity. All backbone atoms for the glutamic units have been colored in yellow, while all of the surfactant methylene carbons have been colored in green. Nitrogen atoms of the ammonium heads have been depicted with a small blue sphere, while oxygen atoms of the carboxylate side groups are colored in red.

unrealistic transitory regular organizations with repeat periods of the same order of the cutoff.

Discussion

Recent studies revealed that *n*-ATMA·PAGA complexes, which should be considered as constitutional isomers of *n*-ATMA·PGGA, show interesting features: rapid formation in water solution by precipitation,[3a] the association of the constituents is maintained after solvation in nonpolar solvents,[3a,4] and they form biphasic structures in the solid state[3a] upon precipitation. Thus, we would see layers of polypeptide helices and charged surfactant heads separated by a paraffinic pool. This particular sort of condensed phase organization appears to be analogous to the structure observed in the equivalent comb-like polypeptide esters, the poly(α-alkyl glutamate)s bearing long alkyl side chains.[25] Therefore, *n*-ATMA·PAGA self-assembled complexes can be understood as a particular case of comb-like poly(α-alkyl glutamate)s. In very recent studies, we used MD simulations to examine the influence of different chemical and physical factors in the supramolecular structure and organization of different *n*-ATMA·PAGA complexes. These were the surfactant constitution,[5a,b] the temperature,[4] and the polarity of the solvent.[5c]

Interestingly, *n*-ATMA·PGGA complexes present the same classic features observed in complexes derived from PAGA that were already mentioned above; that is, they are soluble in nonpolar solvents, while in water they precipitate forming biphasic structures. Furthermore, un-ionized PGGA adopts in solution a helical conformation similar to that of the polyelectrolyte in *n*-ATMA·PAGA. Thus, atomic-resolution computer simulations combined with experimental information provided by optical rotatory dispersion measurements indicated that the helix of un-ionized PGGA features stabilizing intramolecular hydrogen bonds between amide groups *i* and *i*+3 with a helical symmetry around 3.4 residues per turn.[9] In addition, recent structural studies on different esters derived from PGGA supported the intrinsic tendency of this poly(γ-peptide) to adopt helical conformations.[8] Specifically, the benzyl ester presented different crystal structures depending on the crystallization conditions.[8a] This variety of structural morphologies came along with different main-chain regular conformations, some of them of special interest due to the formation of helices stabilized by intramolecular hydrogen bonds. All of these conformations were determined by combining X-ray diffraction, polarized infrared spectroscopy, and molecular modeling simulations. A partially extended conformation was described in the crystal form I, where interchain hydrogen bonds stabilized the crystal packing. Form II presents a helix of symmetry 5/2, that is, 2.5 residues per turn, stabilized by hydrogen bonds set between amide groups *i* and *i*+2. Finally, form III presents the already characterized α-like helix proper of the un-ionized PGGA. Furthermore, comb-like alkyl esters have also been investigated by combining X-ray diffraction, differential scanning calorimetry, NMR, and molecular modeling simulations.[8b−d] These polymers also presented biphasic organization in the solid state:[8b,d] the polypeptide main chain adopts an α-like helix that packs in compact layers, whereas the aliphatic pool gets separated in a different phase. Despite this, no study about the supramolecular organization of *n*-ATMA·PGGA complexes has been reported yet. Moreover, this is the first detailed investigation that has been done for determining the effect of the chemical constitution of the γ-glutamate monomer over the association architecture, that is, the differences between having long side chain and only two backbone carbon atoms per monomer (α-amino acid) or having the carboxylate close to the amide group and flexible monomers with four backbone carbon atoms (γ-amino acid).

The MD simulations reported in this study indicate that the 17/5 helix is the only stable conformation for the polyelectrolyte backbone of the 12-ATMA·PGGA complex. This conformation

remains regular with the intramolecular hydrogen bonds set at their original positions in all cases, independently of the methodology and the force-field used in the simulations. Conversely, the 2/1 helix disappears very rapidly, no trace of regularity remaining after a few nanoseconds of simulation. However, this is not an unexpected result because this arrangement, which has only been described in the solid state for crystalline fibers of poly(α-benzyl-γ-glutamate), needs intermolecular hydrogen bonds to be stable. This makes the presence of this type of organization in solution improbable. Finally, the 5/2 helix seems to be stable only in the solid state. Thus, although this elongated helix presents intramolecular hydrogen bonds, the van der Waals contacts between interacting residues are less favorable than those in the cylindrical 17/5 helix.

For the past few years, the systematic use of molecular simulations has allowed us to discover unexpected features of the self-assembled complexes constituted by anionic polypeptides and amphiphilic molecular cations such as the anticooperative nature of the electrostatic interactions when more than two charged groups interact[26a] and the influence of the molecular cation constitution in the polymer conformation.[5b] However, the most striking attribute of these complexes was the geometrical architecture of the interaction between charged groups: the association of the charged groups was systematically based upon a multiple mode interaction, in which each molecular cation is surrounded by several oppositely charged carboxylate groups.[4,5,19,26] This feature was proven to be always present with total independence of the theoretical approach used to represent the chemical species, either using model compounds or a more realistic representation of the self-assembled complex. Thus, the multiple mode of interaction is a crucial factor to understand the uncommon thermal stability of the helical conformation mentioned before.[4]

The results obtained in this work for 12-ATMA·PGGA complexes reveal that the ammonium groups of the surfactant chains present a multiple interaction pattern that involves not only surfactant···carboxylate but also surfactant···amide interactions. Surfactant···amide interactions were also detected in stoichiometric complexes formed by PAGA and dodecylammonium molecular cations,[5b] hereafter denoted 12-AA·PAGA complexes. Thus, the replacement of the methyl groups by hydrogen atoms in the molecular surfactants, that is, the transformation of 12-ATMA into 12-AA, produced a reduction of the steric hindrance associated with the positively charged head group allowing the interaction with the main chain. In 12-ATMA·PGGA complexes, the main factor that facilitates the interaction of the surfactants and the amide oxygen atoms of the polypeptide must be found in the chemical constitution of the γ-glutamate monomer: the carboxylate side chain is directly attached to the main chain, while in the α-glutamate monomer the carboxylate side group and the main chain are separated by two methyl groups. Thus, the distance between the main chain and the surfactants head groups is small when the latter interact with the carboxylate side groups, withthermal agitation allowing the amide oxygen atoms to get in contact with the positive charge distribution.

On the other hand, it is worth noting that, independently of the starting point or the final polymer conformation, there are always amide groups contributing to the organization of the supramolecular complex. Interestingly, the intervention of the main-chain polar groups does not seem to alter the PGGA conformation. Thus, as we previously showed, the 17/5 helix remains regular and stable independently of the approach we used to represent the electrostatic potential. The later feature appears as exclusive to complexes based upon PGGA, because in 12-AA·PAGA complexes the helix identity of the main chain mutated toward a new organization when the amide···surfactant interactions appeared.[5b]

In summary, we have shown that the organization of the 12-ATMA·PGGA complexes can be understood as the confluence between the arrangement of the polymeric main chain and the disposition of the charged functional groups around the polymer side chains. It has also been hinted that hydrophobic side chains present an independent organization with respect to the polymer dynamics, only affected by the actual distribution of the surfactant charged heads around the polymer chain. Thus, the final supramolecular organization will only depend on the degree of regularity of the polypeptide main chain; that is, this is the only factor responsible for how well distributed the surfactant molecules will be set around the polyelectrolyte rod.

Conclusions

MD simulations in chloroform solution of 12-ATMA·PGGA allow us to draw the following conclusions.

The polypeptide chain adopts a helical conformation stabilized by intramolecular hydrogen bonds. This conformation, which resembles the α-helix of PAGA, guarantees the homogeneous distribution of surfactant molecules around the peptide chain and enables us to maximize the attractive interactions between oppositely charged groups, while minimizing the repulsion between ions of equal charge.

A multiple interaction pattern has been identified for the polar head group of the surfactant molecules. Thus, in average, each ammonium group interacts with two carboxylate groups and one amide moiety simultaneously. This triple multiplicity does not alter the stability or the identity of the polypeptide conformation.

The conformation of the hydrophobic tails of the surfactant molecules is determined by the interactions with chloroform molecules, no influence of the polypeptide organization being detected. Thus, the dodecyl groups tend to adopt an extended conformation, which allow more favorable interactions with the solvent molecules.

The overall results obtained in this work do not depend on the method used to evaluate the electrostatic interactions or on the force-field.

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Supporting Information Available. Root-mean-square deviations for all of the simulations, spatio-temporal evolution of the intramolecular hydrogen bonds for the simulations that used a 17/5 helix as starting conformations, superposition of snapshots recorded from all of the simulations presented in this work, and conformational distribution of the surfactant hydrophobic tails. This material is available free of charge via the Internet at http://pubs.acs.org.

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