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Thermal Stability of the Secondary Structure of Poly(α,L-glutamate) in Self-Assembled Complexes as Studied by Molecular Dynamics in Chloroform Solution

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Unlike neutral polypeptides, the understanding of many aspects of electrically charged polypeptides (polyelectrolites) is still rather poor. These polyions are able to form self-assembled complexes when they interact through electrostatic attraction with oppositely charged small amphiphilic molecules (surfactants) consisting of a polar headgroup and a nonpolar tail. Anionic poly(α,L-glutamate) (PALG) is among the most important charged polypeptides because it forms a well-defined α-helix upon addition of cationic alkyltrimethylammonium surfactants (*n*-ATMA, where *n* indicates the number of carbon atoms of the alkyl group).[1−4] The conformational properties of stoichiometric *n*-ATMA·PALG complexes were examined in dilute chloroform solution and in the solid state by circular dichroism, infrared spectroscopy, and X-ray diffraction.[1−3]

We have recently modeled the supramolecular structure of self-assembled complexes using molecular dynamics (MD) simulations at 298 K in dilute chloroform solution.[4] Results, which were fully consistent with available experimental data, allowed us to gain new insights about the microscopic organization of stoichiometric *n*-ATMA·PALG (with $n = 6$, 8, and 12) complexes. Among them, the most noticeable was the asymmetric arrangement of the surfactant ions around the polypeptide α-helix, which is a consequence of a multiple interaction pattern. Thus, each *n*-ATMA cation was preferentially bound to more than one carboxylate group at the same time. We report here a new MD study about the α-helix of *n*-ATMA·PALG and its temperature dependence in dilute chloroform solution.

MD simulations of a 15-residue 12-ATMA·PALG complex solvated by explicit chloroform molecules were performed at 300, 350, 400, 450, and 500 K.[5] Figure 1 shows as a function of the time the root-mean-square difference (RMSD) in backbone atom positions between the α-helix ($\varphi,\psi = -60.5°, -44.2°$) modeled for poly(α,L-glutamic acid)[6] (PALGH) and the conformations sampled for the polyanion during the simulations at 300 and 500 K, as well as the radius of gyration (R_G) at the same temperatures. Despite the distance RMSD was, on average, 0.11 Å lower at 300 than at 500 K (Table 1), it remained noticeably small throughout the trajectories at the five temperatures considered. The R_G is around the value expected for a canonical α-helix (7.25 Å) in the whole range of temperatures. Furthermore, no significant fluctuation was detected for R_G during the simulations, indicating that those RMSDs are basically related with local conformational rearrangements in which the elongated shape of the molecule is not disturbed.

Table 1 shows the variation of the average fractional native helix content, $<n_\alpha>$, with the temperature. The content of α-helix was

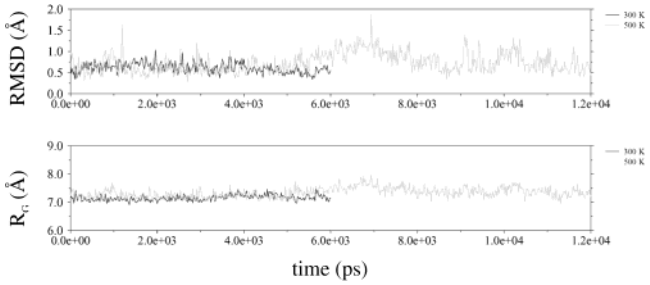

Figure 1. Backbone RMSD of the PALG atom positions from the α-helix modeled for the PALGH and R_G of the polypeptide chain at 300 and 500 K (6 and 12 ns trajectories, respectively).

Table 1. Average Values of the More Significant Parameters at Different Temperatures[a]

T (K)	*t* (ns)	RMSD (Å)	R_G (Å)	$\langle n_\alpha \rangle$	$\langle n_{310} \rangle$
300	6	0.61 ± 0.11	7.15 ± 0.01	0.85 ± 0.08	0.01 ± 0.03
350	6	0.48 ± 0.09	7.14 ± 0.07	0.91 ± 0.08	0.01 ± 0.03
400	6	0.56 ± 0.17	7.23 ± 0.12	0.84 ± 0.11	0.03 ± 0.05
450	6	0.61 ± 0.19	7.26 ± 0.12	0.81 ± 0.11	0.05 ± 0.06
500	18	0.72 ± 0.23	7.34 ± 0.14	0.72 ± 0.13	0.07 ± 0.07
600	4	1.08 ± 0.42	7.45 ± 0.20	0.64 ± 0.13	0.08 ± 0.06
700	4	2.36 ± 1.19	7.89 ± 0.75	0.40 ± 0.21	0.04 ± 0.06
800	2	4.05 ± 1.64	9.76 ± 1.66	0.11 ± 0.18	0.03 ± 0.04

[a] The length of the trajectories is indicated.

measured as the number of C═O(i) ← H−N(i + 4) hydrogen bonds,[6] i.e., d(O···H) < 2.5 Å and −N−H···O > 135°. As can be seen, $<n_\alpha>$ decreases by ∼0.1 when the temperature increases from 300 to 500 K, although even at the latter temperature it is large enough to reflect the presence of an α-helix conformation. Our recent studies[4] on *n*-ATMA·PALG complexes revealed that C═O(i) ← H−N(i + 3) hydrogen bonds, which are characteristic of the 3_{10}-helix,[7] can be occasionally formed at the extremes of the helix. Thus, small rearrangements from the α- to the 3_{10}-helix were found as a consequence of the extremes frying. The average fractional 3_{10}-helical content, $<n_{310}>$, is also listed in Table 1. It is worth noting that the tendency of the extremes to adopt a 3_{10}-helix increases with the temperature, even although $<n_{310}>$ is always considerably smaller than $<n_\alpha>$. This observation is fully consistent with the general trends discussed above for the RMDS and R_G.

The α-helix predicted at room temperature for the polymer chain of 12-ATMA·PALG is supported by the CD spectra registered at the same temperature.[1,3] It should be emphasized that PALGH adopts random coils when the side chains are charged at pH 8.0, i.e., the polyacid transforms into PALG and monomeric helices when the side chains are mostly neutral at pH 4.9.[8] The repulsive

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interactions between the carboxylate side groups, which are minimized in the coil, are responsible for the helix unfolding at basic pH.[6] However, the α-helix obtained for the polyanion in the self-assembled complex is almost identical to that modeled for the PALGH. This indicates that cationic surfactants reduce the impact of the repulsive interactions between the carboxylate groups on the secondary structure of the polypeptide, forming strong attractive electrostatic interactions.

The results presented in this communication predict an extraordinary thermal stability for the helix of stoichiometric 12-ATMA·PALG, since it does not unfold even above the (normal pressure) boiling point of the solvent. To provide a rough estimation of the upper bound of the melting temperature for the helix, we carried out additional simulations at 600 and 700 K. The polypeptide conserves the helical conformation at the former temperature, whereas at the highest temperature we could observe a substantial helix rupture after 3.2 ns. This limit was confirmed by a new simulation at 800 K, a complete transformation of the helix into a coil-like state being detected very rapidly (∼1 ns).

The origin of this thermal stability probably resides in both the length of the dodecyl group, which precludes the contact between the polypeptide and the solvent molecules, and the strength of the noncovalent link between the surfactants and the polyanion, which remains essentially unaltered by the temperature. Analyses of the trajectories indicate that the spatial disposition of the dodecyl chains changes with the temperature. At room temperature, the alkyl groups adopt an extended (all-trans) conformation and are distributed homogeneously around the PALG helix. At high temperature, the dodecyl chains are reoriented as a consequence of the rotation of some bonds, even although the trans is still the most populated conformation. The alkyl groups tend to wrap the helix maintaining their protecting role against the solvent. On the other hand, interaction between the surfactants and the polypeptide is retained at high temperatures. Thus, the strength of these electrostatic interactions allows preservation of the surfactant molecules anchored around the polypeptide, which is essential to avoid the contact with the chloroform molecules. To confirm that the above-mentioned factors are responsible of the thermal stability of the complex under study, a MD simulation was performed at 450 K for a system constituted by a PALG helix with dodecyl chains covalently linked to the side ester groups. Results were similar to those obtained for 12-ATMA·PALG. Thus, the helix was retained throughout the trajectory, with the RMSD and $<n_\alpha>$ being 0.64 ± 0.14 and 0.83 ± 0.10, respectively.

A recent MD study of a 15-residue poly(L-alanine) in aqueous solution showed that at 450 K the α-helix folds and unfolds very rapidly, i.e., three times in 8.5 ns.[9] Thus, the folding−unfolding time scale at 450 K (∼3 ns) is about 30 times faster than at 300 K, which was predicted to be about 100 ns by the same authors. We have also performed MD simulations of the same polypeptide in chloroform solution at different temperature. Results indicate that the helicity clearly decreases with the temperature, i.e., structures with less than 40% of the initial hydrogen bonds are detected at 400 K. Unfortunately, no MD study on the thermal stability of the polypeptide secondary structure in self-assembled complexes has been reported previously.

The impact of the temperature in the structure of *n*-ATMA·PALG complexes has been investigated in the solid state.[2b] The lamellar organization of these complexes was described as a layered arrangement of polypeptide α-helices separated by layers of surfactant molecules. These structures were shown to be stable in the range 293−423 K.[2b]

In conclusion, this MD study predicts a very remarkable thermal stability for *n*-ATMA·PALG stoichiometric complexes in dilute chloroform solution. These results make self-assembled surfactant-polypeptide materials particularly promising.

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Supporting Information Available: Backbone atom positional RMSD, R_G, percentages of hydrogen bond, Ramachandran plots of the PALG chain, distribution of the number of carboxylate groups that interact with each surfactant and atomistic models of the complex, and RMSD and percentages of hydrogen bonds for the polyalanine chain (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) MD simulations of 12-ATMA·PALG were performed at 300, 350, 400, 450, and 500 K, two independent simulations being performed at the two highest temperatures. Periodic boundary conditions were applied using the nearest image convention. In all cases, the initial structure consisted of an α-helix for the PALG chain surrounded by the surfactant molecules, which were arranged in the following way: (i) a fully extended conformation was considered for the dodecyl side group and (ii) the polar headgroups were oriented facing a carboxylate moiety of the polypeptide chain. The complex was solvated with 2200 chloroform molecules (ρ = 1.46 g/cm^3), which were previously equilibrated in a cubic box. The molecular system was constructed by centering the complex in the solvent box and deleting the overlapping solvent molecules. Simulations at 300, 350, and 400 K were of constant pressure and temperature (*NPT*) type. In these cases, the system was brought to the desired temperature by *m* ps of constant volume and temperature (*NVT*) MD, where *m* ranges from 750 to 1100, with the hydrogen-bonding distances of the α-helix constrained at 2 Å. After this, the system was equilibrated at P = 1 atm by performing 100 ps of *NPT* MD, in which no constraint was applied. The resulting structures were used as starting points of *NPT* MD simulations, each one consisting of a total of 6 ns. For the simulations at 450 and 500 K, we used as a starting point the complex equilibrated at 400 K, the temperature being gradually increased along 100−250 ps of *NVT* MD. Then, the complexes were equilibrated for an additional 200 ps using *NPT* MD. Two independent systems were prepared at each temperature, which differed only in the length of the trajectory used for the last heating. Accordingly, our production runs at these temperatures consisted of four independent *NVT* MD, which were 6-ns long. However, one of the simulations at 500 K was enlarged to 12 ns to improve the sampling. To estimate the melting temperature, additional *NVT* simulations were performed using the same protocol at 600, 700, and 800 K, which were as much 4-ns long. Parameter settings not commented on here and the force field were as in ref 4a. MD simulations (6-ns long) were also done for a PALG helix with dodecyl chains covalently linked to the ester groups (450 K) and for helical polyalanine (300, 350, and 400 K), 15 residues being considered in both cases. The heating and the equilibration protocols were similar to those displayed above for the complex. Force-field parameters for the PALG derivative were derived using the same procedure as in ref 4a, while parameters for polyalanine were directly taken from Amber libraries.
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