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Characterization of Nonbiological Antimicrobial Polymers in Aqueous Solution and at Water−Lipid Interfaces from All-Atom Molecular Dynamics

Ivaylo Ivanov,*,† Satyavani Vemparala,‡ Vojislava Pophristic,§ Kenichi Kuroda,‡,|| William F. DeGrado,|| J. Andrew McCammon,† and Michael L. Klein‡

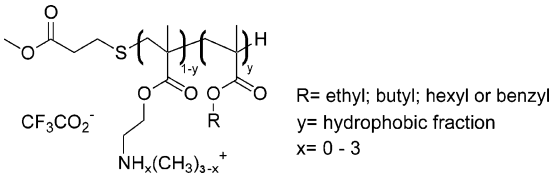
Department of Chemistry and Biochemistry, University of California—San Diego, La Jolla, California 92093, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104, Department of Chemistry and Biochemistry, University of the Sciences in Philadelphia, Philadelphia, Pennsylvania 19104, and Department of Biophysics and School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received September 20, 2005; E-mail: iivanov@mccammon.ucsd.edu

Nonbiological amphiphilic polymers have been used as chemical disinfectants or biocides.[1−8] The origin of their antimicrobial activity lies in the ability to disrupt bacterial cell membranes. The mechanism is thought to be analogous to the one employed by natural antibacterial peptides[9,10] and toxins, such as maganin, defensin, or melittin. The generic nature of the mechanism opens up the possibility of designing antimicrobial agents with very broad antibacterial spectrum and low susceptibility to bacterial resistance. Design of such polymers for biomedical applications, however, requires not only activity comparable to that of natural peptides but more importantly specificity.[4,8] Both activity and specificity are critically dependent on the interplay of several factors—backbone conformational rigidity, the chemical nature of hydrophobic units, and presence of charged units.

In this paper, we characterize a class of recently designed nonhemolytic antimicrobial polymethacrylate copolymers[1] (see Scheme 1). Among the features that make them interesting from a design perspective are (i) high capability to form amphiphilic structures; (ii) composition and length can be controlled; (iii) enormous sequence and structural diversity; (iv) inexpensive to produce. We have applied molecular dynamics to investigate the structural properties and activity of these recently synthesized amphiphilic random copolymers, designed to mimic the antimicrobial activity of natural peptides. The composition, molecular weight, and hydrophobicity (ratio of hydrophobic and cationic units) of these short copolymers can be modulated to achieve structural diversity, which is crucial in controlling the antimicrobial activity. Thirty-four copolymer sequences were set up for classical molecular dynamics (details provided in Supporting Information Table 1). The systems differed by composition (i.e., ratio of hydrophobic to charged units), length (8, 10, or 20 monomer units), and sequence (alternating vs block copolymers). The polymer molecules (originally set up in extended configurations) were either simulated in aqueous solution or inserted in the aqueous phase above a pre-equilibrated dioleoyl phosphatidylcholine (DOPC) bilayer patch (containing 72 or 85 lipids). Counterions were added to achieve overall neutrality. The models were minimized to remove unfavorable contacts, brought to 303 K by velocity rescaling, and equilibrated for 1 ns. The production runs were carried out in the NPT ensemble (1 atm and 303 K) for 7.5−9.5 ns for the systems at the lipid/water interface and 3 ns for the systems set up in pure solvent. The long-range electrostatic interactions were treated using the smooth particle mesh Ewald method. For the nonbonded short-range interactions, we employed a cutoff of 12 Å. The integration time step was 1.5 fs. All simulations were performed with the program NAMD[11] using parameters adapted from the CHARMM force field.[12] Two independent clustering strategies[13] based on analysis of root-mean-square (RMS) deviation matrices were employed to identify dominant shapes from the simulation trajectories: (i) the linkage algorithm (0.4 Å RMSD cutoff criterion); and (ii) the Jarvis−Patrick method (parameters M and P set to 15 and 4, respectively).

Scheme 1. Composition of Designed Random Polymethacrylate Copolymers (see Table 1 in the Supporting Information for details)



Recognition of the relationship between amphiphilic character and antimicrobial function in biological systems has led to the advancement of several design paradigms in the case of their synthetic biomimetic counterparts. The first is achieving amphiphilicity by introduction of hydrophobic and hydrophilic groups. Second, control over backbone flexibility of the polymer is deemed crucial because a rigid backbone allows pre-built facial amphiphilicity to be maintained. Third, highly repetitive structures are thought to be beneficial and expected to enhance activity though multiple identical interactions with the lipid membranes. The biomimetics in this study depart in some ways from these established views. A flexible backbone is apparently not an impediment to optimal activity.[1,10] Herein, we provide an explanation by showing that the polymers adopt well-defined shapes in solution and at bilayer interfaces.

Chains consisting solely of charged units adopted extended helical shapes (for simplicity, all polymers were set up to be stereochemically regular). This can be easily explained by the electrostatic repulsion of the charged side chains, which space themselves as far apart as possible (see panel a of Figure 1). Alternating copolymers of hydrophobic and charged units generally form crescent shapes or segments of a helical turn (see panel c of Figure 1) and tend to be less extended. Their shapes are not very different between the water and lipid−water environment (typical heavy atom RMSDs of ∼5−7 Å). Block copolymers take on more varied conformations and display wider variation with respect to the environment (see Figure 1, panels b and d). They also tend to be more compact (see Figure 1 in Supporting Information). The triblock copolymer in panel b adopts an almost circular conformation, with the two hydrophobic blocks coming together and the middle hydrophilic block forming an arch between.

Furthermore, clustering analysis shows that although the number of clusters can vary depending on sequence, in the majority of cases, very few dominant structures are observable during the trajectories. Figure 2 depicts the clustering results for four representative systems for the backbone carbon atoms only (the side chain orientations can display considerable disorder). The two clustering algorithms produce a different total number of clusters depending on the choice of parameters, but they do agree very well on the number and weight of dominant conformations.

† University of California—San Diego.
‡ Department of Chemistry, University of Pennsylvania.
§ University of the Sciences in Philadelphia.
|| Department of Biophysics and School of Medicine, University of Pennsylvania.

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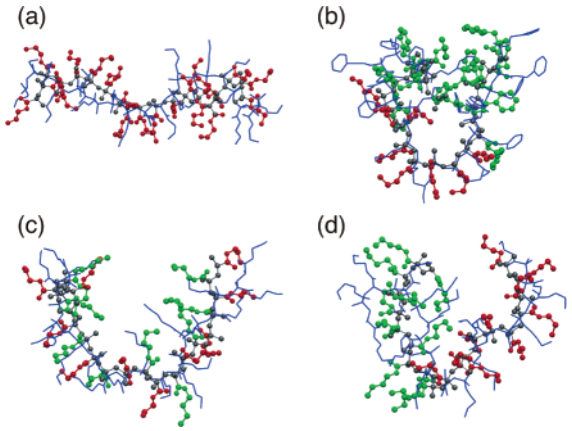

Figure 1. Shapes adopted in aqueous solution (stick representation) and at the bilayer interface (ball-and-stick representation) for four polymer sequences (a−d; see Table 1 in the Supporting Information for details) with 20 unit length. Hydrophobic side chains are colored in green, hydrophilic in red, backbone atoms in gray, aqueous structure is in blue. Hydrogen atoms were omitted for clarity.

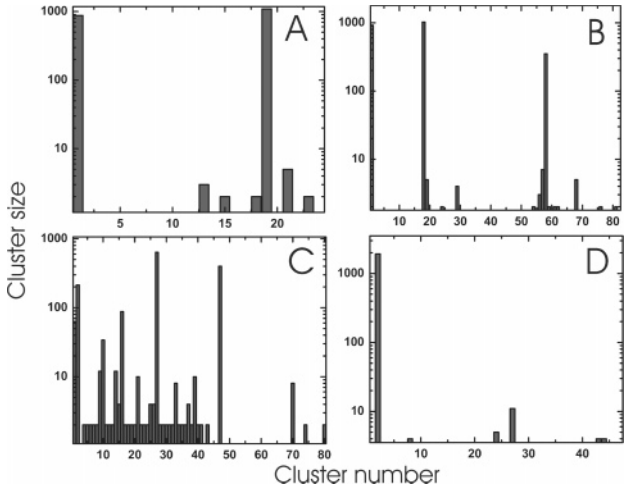

Figure 2. Results from cluster analysis with the linkage method based on backbone RMSD for four copolymer chains (length ten) in the presence of the lipid bilayer (see SI for details). Cluster size is defined as the number of structures belonging to a particular cluster.

Finally, for two of our more hydrophobic sequences (with six butyl or four benzyl units), we observe partial insertion into the bilayer even on the nanosecond time scale of classical molecular dynamics. Figure 3 presents snapshots from the trajectory of a butyl block copolymer during such an insertion event. Insertion occurs with the polymer in an almost vertical orientation, despite the fact that originally it was set up in a fully extended conformation parallel to the bilayer surface. The amine groups appear to play a role during the initial phase of the simulation when the polymer is tumbling on the surface, by forming contacts with the phosphates of the lipid headgroups. For tertiary amines when no such contacts could be formed, we observe a quick departure of the molecule into the aqueous phase, where it remains until the end of the simulation. However, the primary driving force for insertion appears to be the hydrophobic effect. Initially, one of the butyl side chains finds its way into the tail region of the bilayer. By interacting closely with it, the other side chains in the hydrophobic block follow through the thus formed narrow opening in the headgroup region. Finally, when the insertion is complete, the hydrophobic chains spread out and interact favorably with the lipid tails, while all charged hydrophilic units remain in the aqueous region. Briefly, the polymer conformation adapts to the amphiphilic bilayer environment by partitioning the hydrophobic units into the lipid tail region and the charged units into the aqueous phase.

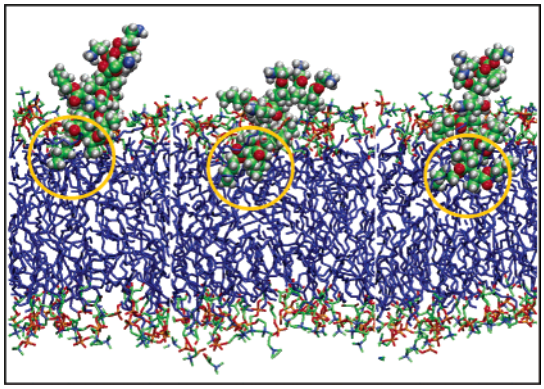

Figure 3. Snapshots taken from the trajectory for a block copolymer with six butyl and four charged units during the process of insertion into the DOPC bilayer. The atoms are colored as follows: carbon in green, oxygen in red, nitrogen in blue, hydrogen in silver, phosphorus in orange, the lipid tail carbon atoms in dark blue.

In this context, dynamic flexibility might actually be beneficial. Again, the sequence of units is important; for the benzyl polymer, only the alternating variant inserts (see Figure 2 in the Supporting Information), whereas for the block copolymer, the consecutive benzene rings stack together to minimize contacts with the aqueous phase. The resulting stacked structure does not seem to be able to pass though the headgroup region.

In conclusion, our calculations confirm that the structural properties of a class of recently synthesized polymethacrylate polymers sensitively depend on composition. Consequently, the activity and selectivity of these novel antimicrobial agents can be fine-tuned by controlling their overall hydrophobicity, the chemical nature of the side chains, or their sequential order. Overall, these findings are in agreement with experimental observations[1] that hydrophobicity is the primary determinant of activity, whereas the presence of charged amine groups is important for selectivity.

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Supporting Information Available: Details on the composition of all investigated systems, radii of gyration, clustering results, and the complete ref 12 have been made available. This material is available free of charge via the Internet at http://pubs.acs.org.

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