Membrane–Based Functions in the Origin of Cellular Life

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Performance Report

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Introduction

Even the simplest protocell must have had the capabilities to catalyze the chemical reactions needed for its survival and growth, and to communicate with its environment. Furthermore, these functions must have been accomplished by simple molecules that were present in protobiotic milieu. One such group of potential early catalysts and signaling molecules are peptides. Peptides are short polymers of amino acid molecules, are are possibly the precursors of the the enzymes and receptors in contemporary cells. Unfortunately, short peptides typically have disordered structures in aqueous solution and, therefore, do not appear to be suitable for the desired cellular functions. However, many of these peptides can acquire a broad range of well–defined secondary structures, such as α–helices, β–strands or β–turns, at water–membrane, water–oil or water–air interfaces. The secondary structures adopted by the peptide depends upon the sequence of amino acids along the polymer backbone. A crucial, common characteristic of these interfaces is that a nonpolar phase is adjacent to an aqueous phase.

Previously, we studied interfacial behavior of very short, model peptides and longer peptides composed of both polar and nonpolar amino acids. All these peptides exhibit interfacial activity, *i.e.* they tend to accumulate at aqueous interfaces. Recently, we extended these studies to the undecamer of poly–L–leucine at the water-hexane interface. Since this peptide consists only of nonpolar residues, it is not expected to be interfacially active but, instead, to partition into the nonpolar phase. Thus, our calculations consider a simple model of peptide insertion into the interior of a membrane, a phenomenon fundamental to

the transduction of signals and the transport of material between the cell interior and the environment.

Methods

All the MD simulations were carried out in the (N,V,E) ensemble. The system consisted of a lamella of water forming an interface with a lamella of hexane and a single solute molecule. The environment of the undecamer of poly–L–leucine consisted of 1380 water molecules and 409 hexane molecules. The x,y–dimensions, parallel to the interface, were 42×42 Å. Periodic boundary conditions were applied in all three directions. The equation of motions were solved using the Verlet algorithm with time steps of 2 or 2.5 fs. The temperature was maintained at 300 K. Water molecules were described by the TIP4P model. Hexane molecules were represented by the OPLS potential functions. The nonbonded and the intramolecular parameters for the peptides were taken from the AMBER force field of Cornell. Intermolecular interactions involving water molecules and/or small, electrically neutral groups of the solute and hexane molecules were smoothly truncated between 7.5 and 8.0 Å.

Organization of Peptides at Membrane Interfaces

It is expected that most peptides composed of mixtures of polar and nonpolar residues will adopt amphiphilic, interfacially active structures. Whether these structures are ordered or not depends on the sequence of amino acids. In contrast, nonpolar peptides are expected to partition from water to a nonpolar medium. In aqueous solution, such peptides are disordered, whereas in a nonpolar environment, they most likely exist as α–helices. They must, therefore, fold during transfer across the interface. This is clearly relevant to the formation and insertion of transmembrane helices. Jacobs and White proposed a hypothetical scheme for this process whereby integral peptides first accumulate at the water–bilayer interface and form the helix. Once folded, the peptides penetrate the bilayer *via* the nonpolar regions of the α–helices. Alternative schemes, in which penetration precedes, or is coupled to, folding can also be envisioned.

We investigated these possibilities in the example of poly–L–leucine. First, we tested the predictions about its stability in water and hexane. When the undecamer was placed in water as a β–strand, it rapidly unfolded into a random coil — a family of disordered conformations observed during 6.2 ns of a molecular dynamics trajectory. The final structure was used in subsequent simulations as a model for the random coil. In contrast, when the peptide was place in hexane in a β–strand or a random coil conformation, it refolded into an α–helical structure within 3.0 and 3.7 ns, respectively. In both cases, the folding proceeded sequentially from the C–terminus.

To simulate poly–L–leucine at the water–hexane interface, the peptide, in a random coil conformation, was placed in water such that its center of mass was *ca.* 11 Å from the interface. Initially, the undecamer moved rapidly towards hexane, reaching the interface within 1 ns. This indicates that a strong average force attracts a nonpolar peptide to the nonpolar phase. Once the peptide reached the interface, it started slowly folding into an α–helix and, simultaneously, partitioning into hexane. These two processes appear to be closely coupled: some penetration into hexane is needed to initiate folding. Then, as the helical structure started forming, some hydrophilic atoms of the backbone became shielded from the aqueous environment. This made the peptide structure more hydrophobic, allowing for more penetration into the nonpolar phase and promoting further folding. In the present study, folding was not sequential. First, six consecutive residues, from the N–terminus, formed a helical fragment. Subsequently, four residues, from the C–terminus, folded into a helix. Here, the term "helix" denotes the portion of the Ramachandran map, for which $-180 \leq \phi \leq 0$ and $-90 \leq \psi \leq 0$. After 34 ns, the completely formed helix was immersed in hexane, just next to the interface.

Folding of the peptide proceeded through an intermediate, a 3_{10}-helix. During the simulations, this helix persisted for only 2 ns before converting to the α-helix. The two helices differ in the pattern of hydrogen bonding along the peptide backbone. In an α-helix, each hydrogen bond involves residues separated by three other residues, whereas in a 3_{10}-helix the participating residues are separated by only two other residues. Occasional formation of the 3_{10}-helix was also observed for the folded peptide. This indicates that this helix not only

mediates folding but remains in equilibrium with the α-helix once this process is completed. A very similar conclusions was reached from experimental studies of alanine-based peptides in aqueous solution.

The orientation of the peptide, defined by the direction of the end–to–end vector, is nearly parallel to that interface. However, orientations nearly perpendicular to the interface with the N–terminus buried in hexane are also probable. In contrast, perpendicular orientations with the C–terminus in hexane are highly unfavorable since they require dehydration of the three carbonyl group at this end of the peptide which are not involved in intramolecular hydrogen bonding. Most transmembrane proteins exhibit similar preferences and incorporate into the membrane through the N-terminus.

Since nonpolar peptides appear to adopt helical structures in nonpolar environments, independently of their specific sequence, we propose that inserting such peptides into nonpolar liquids and membranes requires simultaneous folding and penetration. Although results from a single trajectory are insufficient to draw general conclusions about the mechanism of folding, we note that nonsequential pathways are, at least, probable. Determining whether they are also the most preferred, and how equilibrium free energy and kinetic effects contribute to the selection of folding pathways, requires further studies.

Conclusions

If peptides consist of nonpolar residues only, they become inserted into the nonpolar phase. As demonstrated by the example of the leucine undecamer, such peptides fold into an α–helix as they partition into the nonpolar medium. The folding proceeds through an intermediate, called the 3_{10}-helix, which remains in equilibrium with the α–helix. This process represents a simple, protobiologically relevant example of environmentally–mediated self–organization of biological molecules. Once in the nonpolar environment, the peptides can readily change their orientation with respect to the interface from parallel to perpendicular, especially in response to local electric fields. The ability of nonpolar peptides to modify both the structure and orientation with changing external conditions may have provided a simple mechanism of transmitting signals from the environment to the interior of a protocell.

Publications

1. "Structure and Dynamics of Small Peptides at Aqueous Interfaces. A Multi-nanosecond Molecular Dynamics Study," C. Chipot and A. Pohorille, *J. Mol. Struct. (THEOCHEM)*, in press.

2. "Adsorption and Solvation of Ethanol at the water liquid–vapor interface," M. A. Wilson and A. Pohorille, *J. Phys. Chem.*, **101**, 3130 (1997).

3. "Phenomena at Aqueous Interfaces," A. Pohorille, *Encyclopedia of Computation Computing*, (in press).

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Year 03						From 10/1/97	Through 9/30/98
Personnel			%	Annual		Fringe	
NAME	ROLE IN PROJECT	Hours	Effort	Rate	Salary	Benefits	Totals
Michael Wilson	Principal Investigator	1,500	75%	47,270	35,453	6,382	41,835
Academic fringe benefit rate @		18%					
					35,453	6,382	41,835
Consultant Costs							
Equipment							
Supplies							
Computer Supplies				750			750
Travel							
PI to Gordon Conference on Interfacial Structure: Destination: Plymouth, NH Duration: 6 days Airfare: $500 Transportation: $100 Per Diem: $500				1,100			1,100
Other Expenses							
Computer Maintenance				4,000			4,000
DIRECT COSTS							47,685
INDIRECT COSTS @ 26%							12,398
TOTAL COSTS							60,083