J|A|C|S COMMUNICATIONS

Published on Web 06/16/2009

A Single Bicontinuous Cubic Phase Induced by Fusion Peptides

Marc Fuhrmans,[†] Volker Knecht,[‡] and Siewert J. Marrink*,[†]

Groningen Biomolecular Sciences and Biotechnology Institute & Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, and Max-Planck Institute of Colloids and Interfaces, Department of Theory and Bio-Systems, Research Campus Golm, D-14424 Potsdam, Germany

Received May 1, 2009; E-mail: s.j.marrink@rug.nl

Lipids and other amphiphiles have the unique ability to form lyotropic phases when mixed with water. Peptides are known to modify this phase behavior, altering the phase diagram and facilitating transitions between phases. This modulating effect is of great importance for many membrane related processes *in vivo*, such as fusion and fission, protein segregation, and transport. To understand the physicochemical basis of lipid/peptide interplay, computer simulation studies are becoming increasingly useful.[1] Here, we use molecular dynamics (MD) simulations to study the phase behavior of lipids in the presence of a particular peptide, the Influenza HA fusion peptide. This fusion peptide forms the N-terminal part of the hemagglutinin protein and is inserted in the target membrane during viral infection. *In vitro,* the peptide has been shown to lower the lamellar-to-inverted-hexagonal phase transition temperature, stabilize inverted cubic phases, and cause hemolysis and vesicle-fusion.[2] We report the observation of a single bicontinuous cubic phase that is characterized by a peptide-stabilized stalk/pore complex, providing insight into the possible biological function of this fusion peptide.

In our study we used the recently developed coarse grained MARTINI model,[3] which maps on average four heavy atoms to a coarse grained interaction site. The model has been successfully used to simulate the phase behavior of lipids[4] and to study protein/lipid complexes.[5] The model for the Influenza HA fusion peptide (GLFGAIAGFIENGWEGMIDG) was optimized according to an NMR structure published by Han in 2001[6] (Figure 1). It accurately reproduces the general structure of two helices joined by a linker region at a slightly bent angle. In addition, it successfully mimics the amphiphilic nature of the Influenza HA fusion peptide.

In a spontaneous aggregation approach[7] starting from random mixtures of 256 DOPE lipids, 4 fusion peptides, and water representing levels of hydration between 8 and 12 water molecules per lipid, we find a new phase forming exclusively in the presence of the Influenza HA fusion peptide.[8] The phase forms within the first 2 μs and stays stable for the remainder of the 12 μs simulations.[9] In addition, a larger system obtained by combining 8 copies of the final coordinates of one of the MD simulations was stable for an additional 4 μs simulation.

The new phase can best be characterized by describing the morphology of the aqueous part of the system as a three-dimensional network of water channels lined by the headgroups of the lipids (Figure 2A). At every connection four of these (otherwise unbranched) water channels meet tetrahedrally in a fashion that the network is continuous and periodic in all three dimensions. There are thus no multiple isolated regions of water, and every water bead in the system is connected to every other water bead via the network. The same is true for the lipid part of the system. All lipids

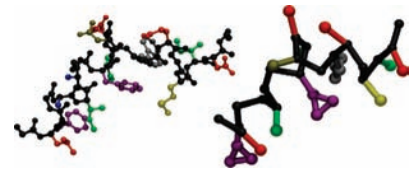

Figure 1. An atomistic model of the Influenza HA fusion peptide (left, hydrogen atoms not shown) and the coarse-grained model used in our simulations (right). The backbone is shown in black and the side chains in colors distinguishing the different amino acids.

in the system are connected in a single network of a morphology identical to the one described for water (Figure 2B). The negative space of one part of the system (lipid or aqueous) thus has the same morphology as the positive space itself. Altogether, the system has a close resemblance to the structure of diamond and is in fact morphologically equivalent to the single diamond cubic phase of symmetry $Fd3m$[10] (Figure 3).

The observation of a single diamond phase is especially significant since it is, to the best of our knowledge, the first observation of a single bicontinuous cubic phase for a lipid system.[11] Bicontinuous cubic phases are believed to generally correspond to specific minimal surfaces that have the peculiar property of having zero mean curvature at every point of the surface as well as being triply periodic. In principle, both single phases, in which the minimal surface corresponds to the interface between the lipid and aqueous parts, and double phases, in which the minimal surface corresponds to the middle of a lipid bilayer that separates the aqueous part of the system into two isolated compartments, are considered possible. So far, however, only double bicontinuous cubic phases have been reported, corresponding to the Schwarz (P), the diamond (D), and the gyroid (G) surface.[12] Morphologically, the single and double phases are very different. Whereas the lipids are still arranged as a curved bilayer in the double phases, the resemblance to a simple bilayer is very hard to see in the single phases. Here the lipids' organization is more similar to an interconnected network of elongated worm-like micelles (cf. Figure 2), probably stabilized by low levels of hydration.

In light of the function of the Influenza HA fusion peptide, it is insightful to describe the single diamond phase in terms of pores and stalks, both of which are involved in the fusion pathway.[13] One finds that there is a tight balance of stalks and pores with the network of stalks defining the pores and vice versa.[14] The single diamond phase thus has more resemblance to the stalk phase of rhombohedral symmetry, which is a regular arrangement of stalks in an otherwise lamellar system, and its inverted counterpart, the mesh-like or pore phase, and can be seen as a combination of these two.

The phenomenon of stalks and pores in close proximity is not uncommon, and it was actually predicted to be energetically

[†] University of Groningen.
[‡] Max-Planck Institute of Colloids and Interfaces.

10.1021/ja903224q CCC: $40.75 © 2009 American Chemical Society

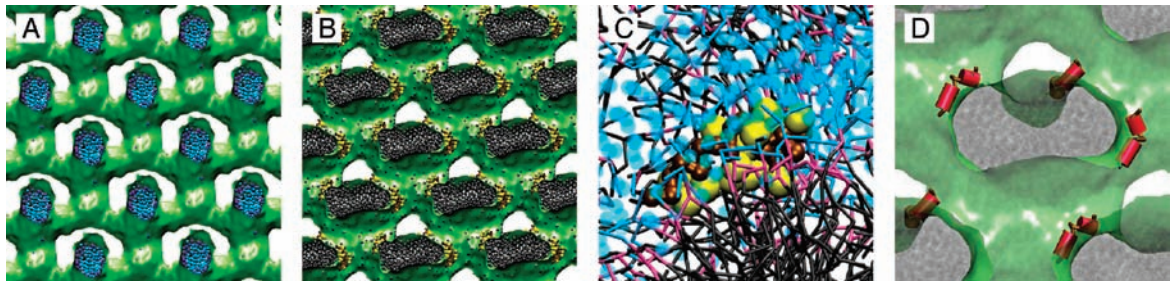

Figure 2. A snapshot of the single diamond phase after 12 μs of simulation. Lipid tail beads are shown in gray, glycerol beads in magenta, water and headgroup beads in blue, and beads corresponding to the backbone and side chains of the Influenza HA fusion peptide in brown and yellow, respectively. In (A), (B), and (D) the surface separating the lipid tail beads from the rest of the system is shown in green, highlighting the three-dimensional networks of the lipid and aqueous components. In (C) a close-up of the peptide embedded in the lipid/water interface is shown. In (D) cylinders highlight helical parts.

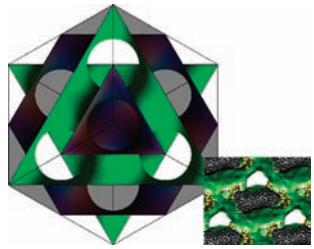

Figure 3. Schematic representation of the triply continuous diamond minimal surface of symmetry *Fd*3*m*. The compartment corresponding to the lipid region has been shaded gray. A detail of the simulated system, a stalk surrounded by three pores, is shown for comparison.

favorable to form a pore in the presence of a stalk in a field theoretic study.[15] In addition, stalk−pore complexes were observed as intermediate states in simulations investigating vesicle fusion[16] and might pose a connection to the function of the Influenza HA fusion peptide.

The fusion peptides, in accordance with their amphiphilic nature, sit on the interface between the lipid and aqueous part with the hydrophobic side chains penetrating slightly into the region of the lipid tails and the more polar residues nested in the headgroup region (Figure 2C). The extent of embedding is similar to what has been observed previously in simulation studies of the binding of this peptide to micelles and bilayers.[17] The peptides' curved structure appears to entail a preference to locate between the bases of two emerging stalks with its helical arms neatly lining the surface, possibly reducing the Gaussian curvature elastic energy associated with the stalk−pore structure[18] (Figure 2D). Given that ability, decreasing the energy costs of forming an intermediate structure of the pathway would facilitate fusion in the presence of the peptides as found by experiments.[2b] In addition, the peptides' seeming ability to stabilize stalks and pores might account for its observed effects on hemolysis and the phase diagram of lipids.[2]

In summary, based on our simulations we predict the existence of single bicontinuous lipid cubic phases induced by fusion peptides, with possible implications for peptide mediated membrane fusion.

Acknowledgment. We thank Ulrich Schwarz for proofreading of the manuscript.

Supporting Information Available: Details of the setup for the simulations and the model for the Influenza HA fusion peptide and an additional figure showing a snapshot of a bigger system. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Marrink, S. J.; de Vries, A. H.; Tieleman, D. P. *Biochem. Biophys. Acta Biomembr.* **2009**, *1788*, 149–168.
(2) (a) Epand, R. M. *Biochim. Biophys. Acta Biomembr.* **2003**, *1614*, 116–121. (b) Han, X.; Tamm, L. K. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13097–13102. (c) Siegel, D. P.; Epand, R. M. *Biochem. Biophys. Acta Biomembr.* **2000**, *1468*, 87–98.
(3) (a) Marrink, S. J.; Risselada, H. J.; Yefimov, S.; Tieleman, D. P.; de Vries, A. H. *J. Phys. Chem. B* **2007**, *111*, 7812–7824. (b) Monticelli, L.; Kandasamy, S. K.; Periole, X.; Larson, R. G.; Tieleman, D. P.; Marrink, S. J. *J. Chem. Theory Comput.* **2008**, *4*, 819–834.
(4) (a) Marrink, S. J.; Mark, A. E. *Biophys. J.* **2004**, *87*, 3894–3900. (b) Risselada, H. J.; Marrink, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17367–17372.
(5) (a) Scott, K. A.; Bond, P. J.; Ivetac, A.; Chetwynd, A. P.; Khalid, S.; Sansom, M. S. P. *Structure* **2008**, *16*, 621–630. (b) Periole, X.; Huber, T.; Marrink, S. J.; Sakmar, T. P. *J. Am. Chem. Soc.* **2007**, *129*, 10126–10132. (c) Yefimov, S.; van der Giessen, E.; Onck, P. R.; Marrink, S. J. *Biophys. J.* **2008**, *94*, 2994–3002.
(6) Han, X.; Bushweller, J. H.; Cafiso, D. S.; Tamm, L. K. *Nat. Struct. Biol.* **2001**, *8*, 715–720.
(7) Marrink, S. J.; Lindahl, E.; Edholm, O.; Mark, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 8638–8639.
(8) In accordance with experiments, in the absence of peptides an inverted hexagonal phase is observed.
(9) Note that due to lower friction in the CG model, interpretation of the dynamics has to be done with care. Based on the diffusion of lipids and water, a conversion factor of four has been applied to provide an approximate time scale (see also ref 3).
(10) In fact a slightly distorted cubic symmetry is observed, with the primitive lattice vectors (9.1/0/0), (0.4/8.8/0), and (2.2/2.3/−5.9).
(11) The phase determination via experimental techniques is often difficult. Diffraction methods are the most reliable way but suffer of an intrinsic low resolution due to the short-range disorder encountered in lyotropic phases. In addition, phases may be metastable. On the other hand, stability is also a problem in simulations and it is hard to differentiate between thermodynamic and kinetic stability.
(12) (a) Seddon, J. M.; Cevc, G. In *Phospholipid Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993;Chapter *12*, pp 403−455. (b) Shearman, C. G.; Ces, O.; Templer, R. H.; Seddon, J. M. *J. Phys.: Condens. Matter* **2006**, *18*, 1105–1124. (c) Schwarz, U. S.; Gompper, G. *Langmuir* **2001**, *17*, 2084–2096. (d) Nazaruk, E.; Bilewicz, R.; Lindblom, G.; Lindholm-Sethson, B. *Anal. Bioanal. Chem.* **2008**, *391*, 1569–1578. (e) Larsson, K.; Tiberg, F. *Curr. Opin. Colloid Interface Sci.* **2005**, *9*, 365–369. (f) Schröder-Turk, G. E.; Fogden, A.; Hyde, S. T. *Eur. Phys. J. B* **2006**, *54*, 509–524.
(13) (a) Chernomordik, L. V.; Kozlov, M. M. *Nat. Struct. Mol. Biol.* **2008**, *15*, 675–683. (b) Siegel, D. P. *Biophys. J.* **1999**, *76*, 291–313.
(14) This relation is illustrated nicely by the fact that the single diamond phase is identical to its own inverted phase.
(15) Katsov, K.; Mueller, M.; Schick, M. *Biophys. J.* **2006**, *90*, 915–926.
(16) (a) Noguchi, H.; Takasu, M. *J. Chem. Phys.* **2001**, *115*, 9547–9551. (b) Smeijers, A. F.; Markvoort, A. J.; Pieterse, K.; Hilbers, P. A. J. *J. Phys. Chem. B* **2006**, *110*, 13212–13219. (c) Knecht, V.; Marrink, S. J. *Biophys. J.* **2007**, *92*, 4254–4261.
(17) (a) Lagüe, P.; Roux, B.; Pastor, R. W. *J. Mol. Biol.* **2005**, *354*, 1129–1141. (b) Huang, Q.; Chen, C. L.; Hermann, A. *Biophys. J.* **2004**, *87*, 14–22.
(18) Siegel, D. P. *Biophys. J.* **2008**, *95*, 5200–5215.

JA903224Q