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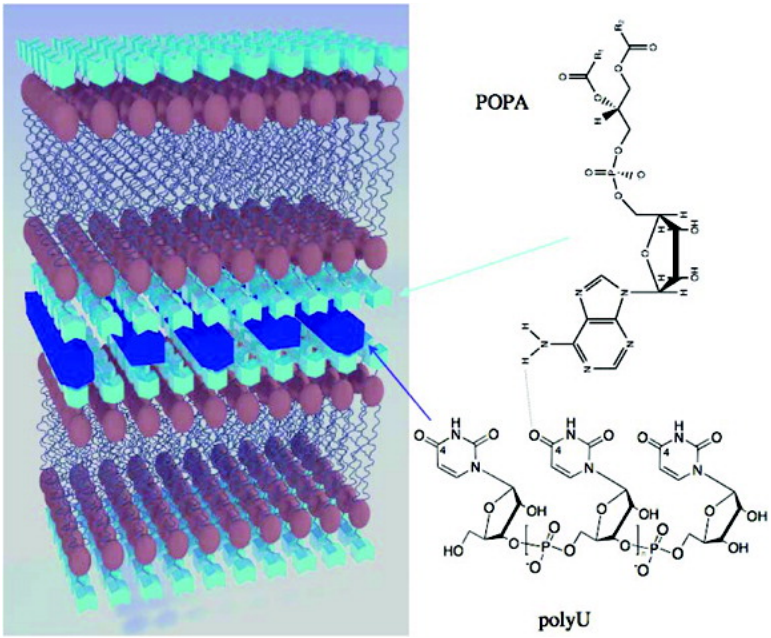
J. Am. Chem. Soc., **2007**, 129 (38), 11664-11665• DOI: 10.1021/ja0714134 • Publication Date (Web): 31 August 2007



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Published on Web 08/31/2007

Nucleolipoplexes: A New Paradigm for Phospholipid Bilayer−Nucleic Acid Interactions

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Most of the current research on synthetic bilayer membrane−nucleic acid complexes (lipoplexes) is fostered by their potential application in the biomedical field as nonviral vectors for gene, antisense oligonucleotides, or si-RNA delivery.[1,2] The vast majority of synthetic nonviral vectors consists of cationic lipid assemblies where nucleic acids bind to cationic liposomes because of the charge interaction between the negative polyelectrolyte and the cationic headgroup of the lipid. A large number of novel cationic lipids so far considered have been designed and assayed in transfection protocols.[3] Structural studies on these lipoplexes have shown that DNA is complexed into lamellar and inverted hexagonal lipid phases.[4−6] The main drawback of this approach is the cytotoxicity, the immunogenic effects associated with some formulations, and the possible binding to serum proteins, mostly negatively charged at physiological pH. Recent studies have exploited that divalent cations can mediate the interaction between DNA and zwitterionic natural phospholipids (i.e., lecithins) leading to the formation of Ca-mediated lipoplexes.[7,8] It is common knowledge that, without divalent bridging, no interaction would occur between DNA and neutral lipid assemblies. A novel approach relies on the design of cationic or neutral lipids with nucleic functionalities to complex and transfect nucleic acids.[9]

More challenging is nucleic acids complexation by anionic self-assemblies.[10] One possible strategy relies on molecular recognition and is directly inspired from nature: in DNA or RNA, two like-charged strands pair thanks to chemical selectivity that overcomes electrostatic repulsion. As a first step toward the engineering of nucleic acid/nucleolipid complexes, we present in this study the evidence of the formation of an ordered lamellar phase of the type L_α^c, without any mediation from divalent cations, where polyuridylic acid (polyU, an RNA homopolymer), spontaneously orders in a lamellar fluid phase formed by the anionic 1-palmitoyl-2-oleoyl-phosphatidyl-adenosine nucleolipid, POPA. This nucleolipid has full biological compatibility and can be enzymatically cleaved by phospholipases[11] once in living organisms to release the polynucleotide.

We have recently reported that polynucleotides bind to small like-charged globular micelles formed by 1,2-dioctanoyl-phosphatidyl-adenosine, we report here the evidence that this behavior is present even in very different suprastructures as bilayer membranes from long-chain derivatives.[12]

Figure 1a shows the SAXD pattern of POPA bilayers swollen with TRIS buffer (0.1M pH 7.5) to reach 50% w/w, corresponding to 47% v/v. The membrane is fully hydrated and in the liquid crystalline phase at room temperature. If heated at 50° for 1 h and then cooled down to room temperature, the smectic period (48 Å) and line shape are fully recovered from the usual lamellar shrinkage owing to the temperature increase.[13,14] From the known lipid volume fraction a membrane thickness, δ_m, of 22.5 Å can be evaluated.

Interestingly the addition of polyU, dissolved in the hydrating buffer in a 1:2 ratio (on a monomer basis with respect to the lipid) to swell the membrane, produces, upon the same thermal treatment as for the pure POPA, a novel lamellar phase (Figure 1b) with a

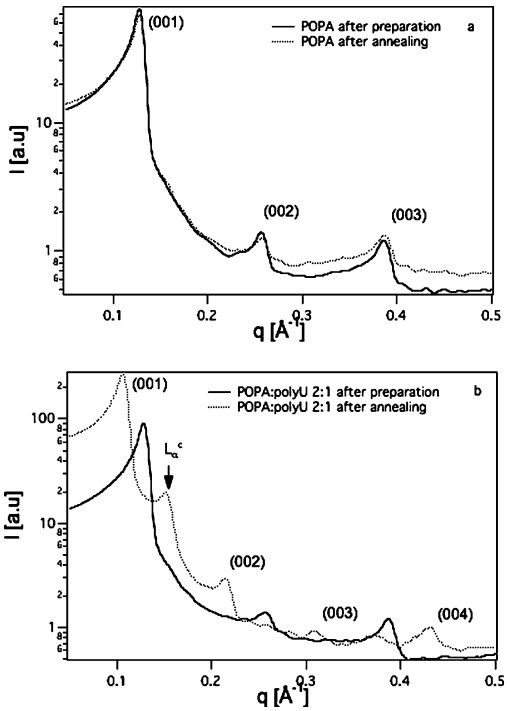

Figure 1. Small-angle X-ray diffraction spectra of pure POPA and mixtures of POPA/PolyU (2:1) bilayers.

smectic period of 57 Å, higher than that found for the pure POPA bilayers (48 Å).

The most remarkable feature of this spectrum is the appearance of a broad peak between the first and the second lamellar Bragg reflections. This behavior has first been observed for DOTAP/DOPC/DNA systems[15,4] and has been attributed to spontaneous ordering of DNA in between the lamellae; the additional peak is due to the formation of a 1D lattice of polynucleotide chains, with a characteristic spacing in our case of 38 Å. To support the assignment of this broad peak, we have varied the lipid/polynucleotide mole ratio, as reported in Figure 2. The correlation distance decreases as the polynucleotide mole ratio is increased supporting that the reflection can be attributed to the ordering of single strands in between nucleolipid membranes, similarly to the "classical lipoplexes".

However, our system substantially differs from conventional lipoplexes, where the driving force leading to the polynucleotide/membrane interaction is mainly of electrostatic nature. In our case the presence of like charges on membranes and on the polyelectrolyte excludes the aspecific charge contribution. Considering that adenosine and uridine are complementary bases in RNA, a possible working hypothesis is the presence of molecular recognition[16,17] between the bases decorating the anionic bilayer and the complementary bases on the strand that contribute to the formation of a novel mesostructure. This is strengthened by the fact that the

10.1021/ja0714134 CCC: $37.00

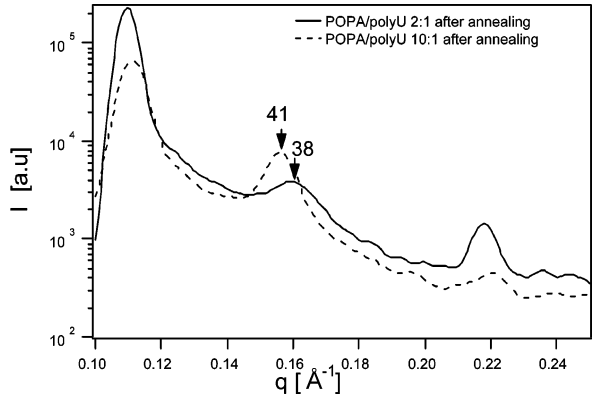

Figure 2. Comparison of SAXS spectra of POPA/polyU complexes at different molar ratios. The arrows indicate the broad peak assigned to polynucleotide ordering between the bilayers.

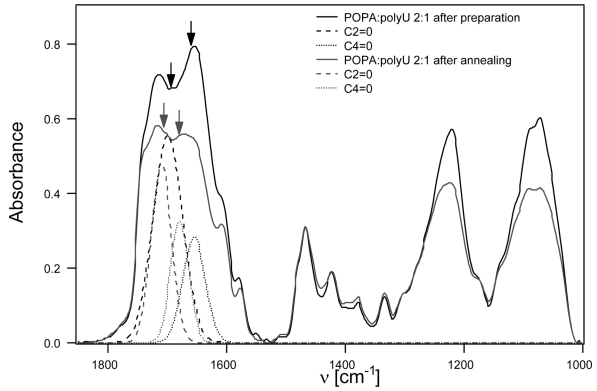

Figure 3. FTIR spectra of POPA/polyU/TRIS buffer (50% w/w) after preparation (black line) and POPA/polyU/TRIS buffer (50% w/w) after annealing (gray line). C_4═O and C_2═O carbonyl stretching bands (sketched lines) are the deconvolved bands by Gaussians.

addition of the noncomplementary polyA to POPA bilayers does not produce structural evolution upon annealing (see Supporting Information).

To infer direct evidence of the interactions between the complementary bases, we have performed FTIR spectroscopy on the lamellar phase hosting the polynucleotide, Figure 3.

The assignments of the broad band between 1800 and 1600 cm^{-1} have been obtained by comparison with classical nucleic acid literature[18,19] using a convolution of Gaussian absorptions (see Supporting Information). The evolution of the ternary system during the annealing procedure, which yields the structural transformation observed with SAXD, can be illuminating. In the freshly prepared sample, characteristic bands, due to C_4═O and C_2═O stretching of the uridine moiety, can be noticed at 1660 and 1690 cm^{-1}, respectively (black arrows Figure 3); after the annealing, these bands, that are diagnostic for H-bonding with adenosine, shift to 1675 and 1712 cm^{-1}, that is, in the direction expected for the formation of a Watson−Crick pair (gray arrows in Figure 3). Evidence of excess base stacking, due to insertion of polyU in the bilayer, has been also obtained through UV spectroscopy (see the Supporting Information section). UV spectra for the sample POPA/polyU show the characteristic 260-centered band and a shoulder centered at 330 nm due to the absorption of retinol, which has been included in the membrane as an internal standard.[20] It is noteworthy that sample annealing is associated to a meaningful hypochromism, i.e., the signature for base stacking. These observations support that inclusion of polynucleotide single strands between anionic POPA membranes, observed through SAXD, occurs via molecular recognition between complementary base pairs and overcomes electrostatic repulsion. Figure 4 reports a descriptive model for the suprastructure.

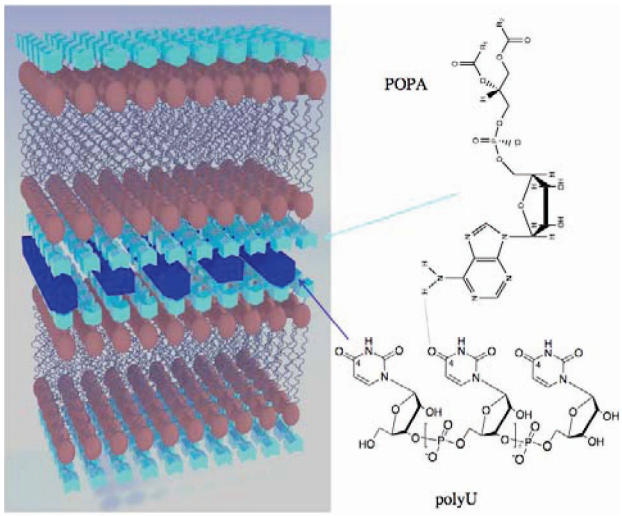

Figure 4. Illustrative cartoon for a possible polyU arrangement in a nucleolipoplex. For the sake of clarity polyU is here represented as a rigid rod.

The above finding opens up new perspectives in the fabrication of lipid/nucleic acid complexes up to now obtained by favorable columbic interactions or the presence of divalent cations. The proof of principle that chemical design based on molecular recognition can be the driving force for bioconjugate constructs establishes a new paradigm with potentially important impact both in a biomedical field and in supramolecular chemistry.

Acknowledgment. Financial support from EU-FP6 (AMNA NMP4-CT-2004-013575), CSGI/CNR-FUSINT and MUR (PRIN-2006) is acknowledged.

Supporting Information Available: Details of synthesis, sample preparation, experimental methods and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Smyth-Templeton, N.; Lasic, D. D. *Therapeutic Mechanisms and Strategies*; Marcel Dekker Inc.: New York, 2000.
(2) Mahato, R. I.; Kim, S. W. *Pharmaceutical Perspectives of Nucleic Acid-Based Therapeutics*; Taylor and Francis: London and New York, 2002.
(3) Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci.* **1987**, *84*, 7413−7417.
(4) Radler, O. J.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810−813.
(5) Caracciolo, G.; Pozzi, D.; Amenitsch, H.; Caminiti, R. *Langmuir* **2005**, *21*, 11582−11587.
(6) Dias, S. R.; Lindman, B.; Miguel, G. M. *J. Phys. Chem. B* **2002**, *106*, 12600−12607.
(7) McManus, J. J.; Radler, O. J.; Kenneth, A.; Dawson, A. *J. Am. Chem. Soc.* **2004**, *126*, 15966−15967.
(8) Hongjun, L.; Harries, D.; Wong, C. L. G. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11173−11178.
(9) Chabaud, P.; Camplo, M.; Payet, D.; Serin, G.; Moreau, L.; Barthelemy, P.; Grinstaff, W. M. *Bioconjugate Chem.* **2006**, *17*, 466−472.
(10) Iwaura, R.; Yoshida, K.; Masuda, M.; Ohnishi-Kameyama, M.; Yoshida, M.; Shimizu, T. *Angew. Chem.* **2003**, *42*, 1009−1012.
(11) Shuto, S.; Ito, H.; Ueda, S.; Imamura, S.; Fukukawa, K.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 209.
(12) Banchelli, M.; Berti, D.; Baglioni, P. *Angew. Chem.* **2007**, *46*, 3070−3073.
(13) Milani, S.; Baldelli Bombelli, F.; Berti, D.; Dante, S.; Hauss, T.; Baglioni, P. *Biophys. J.* **2006**, *90*, 1260−1269.
(14) Cevc, G. *Phospholipids Handbook*; Marcel Dekker: New York, 1993.
(15) Salditt, T.; Koltover, I.; Radler, O. J.; Safinya, C. R. *Phys. Rev. Lett.* **1997**, *79*, 2582−2585.
(16) Berti, D.; Barbaro, P.; Bucci, I.; Baglioni, P. *J. Phys. Chem. B* **1999**, *103*, 4916−4922.
(17) Berti, D.; Baglioni, P. *Curr. Opin. Colloid Interface Sci.* **2006**, *110*, 74−78.
(18) Banyay, M.; Sarkar, M.; Graslund, A. *Biophys. Chem.* **2003**, *104*, 477−488.
(19) Miles, H. T.; Frazier, J. *Biochemistry* **1978**, *17*, 2920−2927.
(20) Rajendra, J.; Damianoglou, A.; M.; H.; Booth, P.; Marck Rodger, P.; Rodger. A. *Chem. Phys.* **2006**, *326*, 210−220.

JA0714134