Association and Phase Behavior of Cholic Acid–Modified Dextran and Phosphatidylcholine Liposomes

ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · FEBRUARY 2010

Impact Factor: 7.46 · DOI: 10.1021/jz100087k

3 AUTHORS:

Guangyue Bai
Henan Normal University
42 PUBLICATIONS **859** CITATIONS
SEE PROFILE

Marieta Nichifor
Petru Poni Institute of Macromolecular Che…
62 PUBLICATIONS **979** CITATIONS
SEE PROFILE

Margarida Bastos
University of Porto
70 PUBLICATIONS **1,315** CITATIONS
SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY Letters

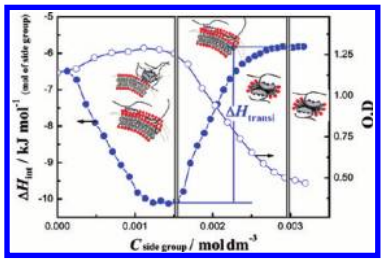
pubs.acs.org/JPCL

Association and Phase Behavior of Cholic Acid-Modified Dextran and Phosphatidylcholine Liposomes

Guangyue Bai,[†] Marieta Nichifor,[‡] and Margarida Bastos*[,†]

[†]CIQ (UP), Department of Chemistry, Faculty of Sciences, University of Porto, R. Campo Alegre, 687, P-4169-007 Porto, Portugal, and [‡]"Petru Poni" Institute of Macromolecular Chemistry, 700487 Iasi, Romania

ABSTRACT The interaction between liposomes (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)) and a hydrophobically modified water-soluble polymer (HMP; a bile acid-modified dextran) has been investigated by isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC), combined with turbidity measurement and cryogenic scanning electron microscopy (cryo-SEM). The thermodynamic information on the association (enthalpy of interaction, enthalpy of transition of mixed vesicles to mixed micelle-like aggregates) was obtained from ITC. Further, the phase behavior for the system could be derived from the ITC measurements, and be confirmed by turbidity and cryo-SEM. The effect of cholic acid (CA) side groups on the ordered arrangement of DMPC bilayers was studied by DSC, by following the changes they induce in the gel-to-liquid crystalline liposome phase transition. The DSC results were in excellent agreement with the interpretation proposed for the ITC results. The morphology of the aggregates, as characterized by cryo-SEM, is in line with the proposed aggregate morphologies.



SECTION Surfactants, Membranes

In recent years, the modification of liposomes with polymers has been an important topic of reasearch.[1−3] Amphiphilic polymers can interact with the liposome surface by internalization of the hydrophobic moiety into the outer half of the bilayer and orienting the hydrophilic portion toward the aqueous bulk solution. The interaction is stronger than that of hydrophilic polymers and results in a lower damage of the vesicle membrane.[4] In particular, hydrophobically modified water-soluble polymers (HMPs) are believed to act as reliable anchors into the lipid bilayer, and therefore have a great potential as drug delivery systems.[5−12] The incorporation of hydrophobic groups of HMPs into the bilayer modifies the characteristics of the liposomes, in a way that depends on the structural characteristics of the added HMPs, such as flexibility of backbone, properties of the grafted hydrophobic groups and degree of substitution (DS), polymer concentration, and side group concentration. The interaction in such systems is of biological relevance to mimic biomembrane/biopolymers interactions because lipid vesicles are frequently perceived as model membranes, and amphiphilic polymers represent models for self-organized lipoproteins.[13,14]

In order to obtain new insight into the properties of useful liposomes/HMP systems, we chose a system with good biocompatibility: phophatidylcholine liposomes (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)) as large unilamellar vesicles (LUVs, with the size 100 nm) and a newly synthesized hydrophobically modified dextran containing a bile acid, cholic acid (CA; DS = 4 mol %, or 0.224 mmol CA/g polymer) as the pendent group (Dex-CA) (Figure 1). This polymer belongs to a series that was for the first time synthesized by Nichifor and Carpov[15] by covalent attachment of bile acids to the dextran backbone through ester links.

The modified polymer can self-associate to form aggregates of different sizes, depending on the concentration and bile acid type, as demonstrated by different fluorescence and light scattering techniques.[16] The bile acid-modified dextrans have the advantage of a better compatibility with biological systems, thus exhibiting potential biomedical applications. Biocompatibility is a property of both the polysaccharde backbone and the grafted bile acid. Polysaccharides (dextran) are natural biocompatible and nontoxic polymers, with potential for drug site targeting.[17] However, in their native form, they interact only weakly (or not at all) with liposome membranes. Bile acids are biological surfactants with a rigid steroid skeleton and facial amphiphilicity. They act as solubilizers and emulsifiers for cholesterol and lipids in the intestine[18] and help their transport across biological membranes (they are permeation enhancers).[19] The interaction of bile acids with liposome and the induced transition from vesicle to micelles are well documented.[20−22] The interaction of bile acids-modified dextran with liposomes should be stronger than that with unmodified dextran, and similar to free bile acids, as the pending bile acids can act as reliable anchors for the polymer into the lipid bilayer. To the best of our knowledge, this is the

Received Date: January 21, 2010
Accepted Date: February 17, 2010

DOI: 10.1021/jz100087k | *J. Phys. Chem. Lett.* **2010**, *1*, 932–936

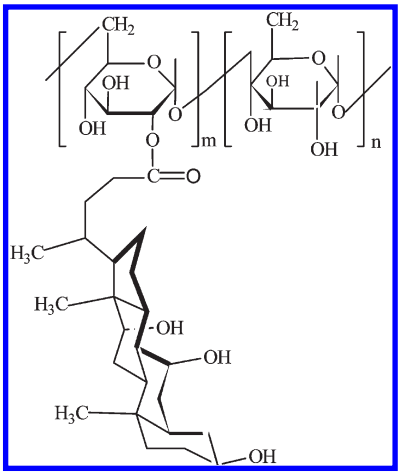


Figure 1. Chemical structure of dextran modified with CA, according to the synthetic methods previously described.[15] Dextran with MW 40 KDa and polydispersity index PI = 1.12 was used, and a DS of 4 mol % was obtained (DS = 100 [$m/(m+n)$], mol %).

first report on the interaction of liposomes with bile acid-containing polymers.

In the present work, we were able to observe the transition between vesicles and micelles, showing that the type of HMP studied here, depending on the side group concentrations, can partition into the lipid bilayer or solubilize the membrane to form mixed micelles. The use of isothermal titration calorimetry (ITC) to determine the phase boundaries of oppositely charged polyelectrolyte/surfactant systems, as well as the corresponding interaction enthalpies, is very recent and is proving to be an excellent technique.[23−25] This technique has already been used to study the interaction between bile acid surfactants and lipid vesicles.[22]

ITC information proved to be crucial for a detailed mapping of the interactions as a function of relative concentrations. The interaction process was described as free vesicles + polymer → polymer−vesicle aggregate (mixed aggregates). The interaction enthalpies per mole of added CA side group (ΔH_{int}) were obtained from the differences between the observed enthalpies for titration of Dex-CA into DMPC liposomes and into buffer. Figure 2 shows the results obtained from ITC and turbidity measurements for such an experiment, when a polymer concentrated solution ($C_{polymer}$ = 5% (w/w), i.e., $C_{side\ group}$ = 0.011 mol·dm^{-3}) was added into a DMPC vesicle suspension (C_{DMPC} = 0.016 mol·dm^{-3}). The change in ΔH_{int} and optical dispersion (OD) are plotted as a function of the polymer's pendent group (CA) final concentration ($C_{side\ group}$), due to the controlling role of these groups on the system's behavior.[26] Three regions can be observed.

In region I, an exothermic event is observed, which increases in absolute magnitude as $C_{side\ group}$ concentration increases, until the $C_{side\ group} \approx 1.6 \times 10^{-3}$ mol·dm^{-3}. In this concentration region (corresponding to $C_{polymer}$ = 0.06−0.7% (w/w)) the polymer still exists in an aggregated state (critical aggregation concentration (cac) = ∼0.06% (w/w) for Dex-CA, (DS = 4 mol %)),[16] although with small aggregation numbers, due to the limitation imposed by the dextran backbone, i.e., the hydrophobic microdomains consist of several

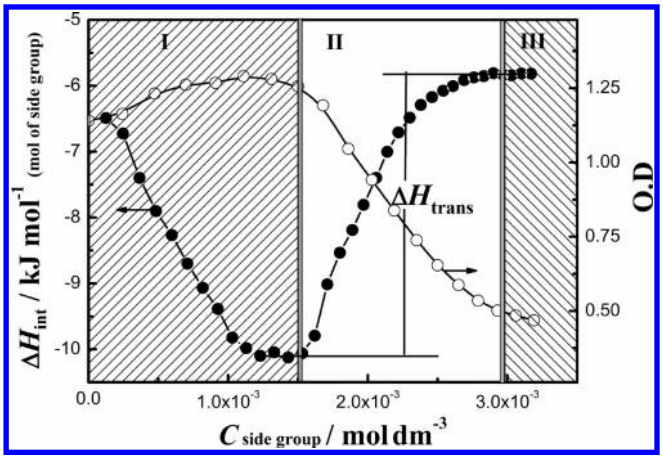


Figure 2. Results from ITC and turbidity measurements at 308.15 K for the titration of a 5% (w/w) Dex-CA solution ($C_{side\ group}$ = 0.011 mol·dm^{-3}) into a 0.016 mol·dm^{-3} DMPC suspension (see Supporting Information (SI)). Solid circles correspond to the curve of ΔH_{int} versus $C_{side\ group}$, and open circles correspond to the curve of OD versus $C_{side\ group}$. Region I: coexistence of vesicles, mixed DMPC−polymer vesicles; region II: coexistence of mixed DMPC−polymer vesicles and mixed micelle-like aggregates; region III: mixed micelle-like aggregates and polymer aggregates.

CA pendant groups from different polymer chains. When the polymer is added into the vesicle suspension, the exothermic process is caused by the dilution process of the pure polymer aggregates, adsorption of polymer aggregates on the vesicle surface, and the partition of the CA side groups into the membrane. The dilution enthalpies were found to be negligible when compared to the total heat effect, and thus the exothermic effect results only from two types of favorable interactions: (i) adsorption of CA−polymer aggregates on the vesicle surface and/or intercalation of the CA pendant groups between polar lipid head groups (hydrophilic face); (ii) hydrophobic interaction between alkyl chains of DMPC and hydrophobic surface of the CA pendant groups. The contributions (endothermic) from polymer demicellization and from the change in vesicle shape are much smaller than the exothermic enthalpies from (i) and (ii) above, and therefore the observed total enthalpy of interaction is exothermic. Further, it increases linearly with the $C_{side\ group}$, from −6.86 kJ·mol^{-1} to −10 kJ·mol^{-1}. This region corresponds to the formation of mixed DMPC−polymer vesicles. When the polymer side group concentration reaches ∼1.0×10^{-3} mol·dm^{-3}, most of the free vesicles have formed mixed DMPC−polymer vesicles (Figure 2). The maximum absolute value of the exothermic interaction enthalpy (approximately −10 kJ/mol side chain) reaches a plateau region for $C_{side\ group}$ = (1.0−1.6) × 10^{-3} mol·dm^{-3}). We interpret this constant value as reflecting the existence of CA-saturated mixed vesicles. In region I, the incorporation of the side group CA occurs in the outer layer of the vesicles. Due to the steric effects of CA and dextran backbone, the anchored polymers direct the shape and size changes of the vesicles,[1] which results in a modest OD (Figure 2) increase as the polymer concentration increases. The cryogenic scanning electron microscopy (cryo-SEM) micrographs of pure liposome suspension (average size 600 nm, see SI) and a Dex-CA/liposome mixture with a DMPC-to-side

DOI: 10.1021/jz100087k | J. Phys. Chem. Lett. 2010, 1, 932–936

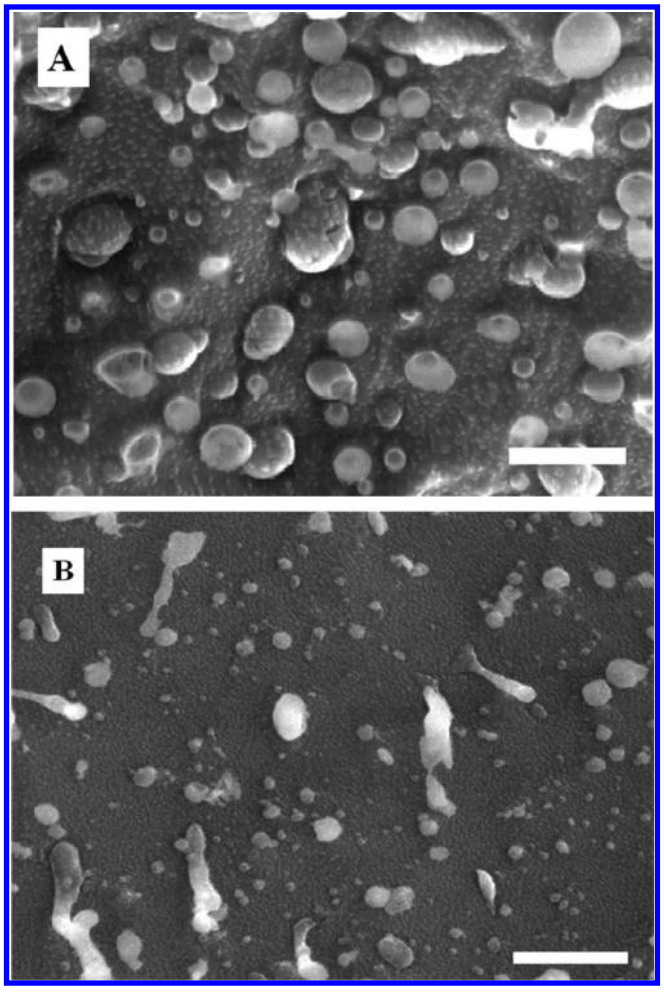


Figure 3. Cryo-SEM in region I. (A) Pure DMPC vesicles in water (average size 600 nm, C_{DMPC} = 0.035 mol·dm^{-3}); bar represents 1 μm. (B) Mixture of DMPC (600 nm) and Dex-CA (n_{DMPC} / $n_{side\ group}$ = 57; C_{DMPC} = 0.033 mol·dm^{-3}; $C_{side\ group}$ = 5.8 × 10^{-4} mol·dm^{-3}) in water; bar represents 1 μm. The method of liposome preparation is described in the SI.

group molar ratio of $n_{DMPC}/n_{side\ group}$ = 57 (inside region I, which spans ratios $n_{DMPC}/n_{side\ group}$ = 9−129) are shown in Figure 3. Before polymer addition, the DMPC vesicles exist as spherical structures (Figure 3A). With polymer addition, the size and shape (Figure 3B) of some vesicles change due to the incorporation of CA side groups and/or intercalation of CA molecules between liposome polar groups. As a result, mixed DMPC−polymer vesicles and/or vesicle clusters (Figure 3B) are formed.

In region II ($C_{side\ group}$ = (1.6−3.0) × 10^{-3} mol·dm^{-3}), the absolute value of the interaction enthalpy decreases, and the corresponding OD values decrease dramatically (Figure 2) with the increase of polymer side group concentration. In this region, the mixed lipid/polymer vesicles start to disintegrate to mixed micelle-like aggregates, and the two type of aggregates coexist.

In region III ($C_{side\ group}$ > 3.0 × 10^{-3} mol·dm^{-3}), the interaction enthalpy is constant at ca. −6.2 kJ/mol, corresponding to the complete disintegration of the vesicles into

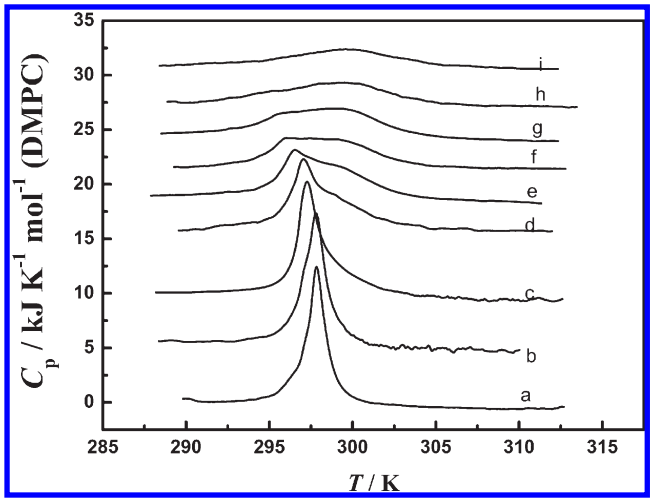


Figure 4. DSC curves for pure DMPC LUVs (0.016 mol·dm^{-3}) and for their mixtures with Dex-CA with different polymer (side group) concentrations (see SI). Concentrations of the side group ($C_{side\ group}$/mol·dm^{-3}) are (a) 0; (b) 0.1 × 10^{-3}; (c) 0.6 × 10^{-3}; (d) 0.9 × 10^{-3}; (e) 1.0 × 10^{-3}; (f) 1.4 × 10^{-3}; (g) 1.6 × 10^{-3}; (h) 2.0 × 10^{-3}; and (i) 2.9 × 10^{-3}.

mixed micelle-like aggregates. We can therefore obtain the enthalpy of transition of mixed vesicles into mixed micelle-like aggregates (ΔH_{trans}) from the difference between the two linear segments that occur before and after the steep break (see Figure 2). ΔH_{trans} is endothermic, and the calculated value is 3.8 kJ/mol of side group. This value reflects mostly the transfer of CA side groups and lipid molecules from mixed bilayers to mixed micelle-like aggregates. Since the process is endothermic, we can also say that it is entropy driven.

The known influence of included hydrophobic species on the liposome's thermotropic behavior (gel-to-liquid crystalline phase transition, at ∼25 °C for DMPC liposomes) led us to anticipate that the addition of a HMP would be reflected in a change in transition profile.[27] In order to further characterize the information obtained by ITC and to understand the effects of CA side groups on the ordered arrangement of DMPC bilayers, we performed differential scanning calorimetry (DSC) measurements for the studied system of Dex-CA/DMPC liposomes. In Figure 4, a series of C_p versus T curves are plotted for pure DMPC LUVs (C_{DMPC} = 0.016 mol·dm^{-3}) and DMPC liposomes in the presence of different amounts of Dex-CA.

The change in profile of the C_p versus T curves induced by the presence of the polymer gives very important information about the interaction process. For pure DMPC in Hepes buffer, the phase transition occurs at T_m = 23.7 ± 0.5 °C and ΔH_m = 22.1 ± 1.0 kJ·mol^{-1}. For the pure polymer, the DSC scan shows no phase transition at the studied concentration and temperature ranges (result not shown). From Figure 4 it is clearly seen that increasing the amount of polymer initially induces a decrease in T_m. This is ascribed to a destabilization of the gel phase due to the insertion of CA side groups in the hydrophobic part of the membrane, and a better compatibility of CA with the liquid crystalline phase. We also observe a decrease in cooperativity, showing that the CA moieties are inserted in the membrane core, affecting the alkyl chain

DOI: 10.1021/jz100087k | J. Phys. Chem. Lett. 2010, 1, 932–936

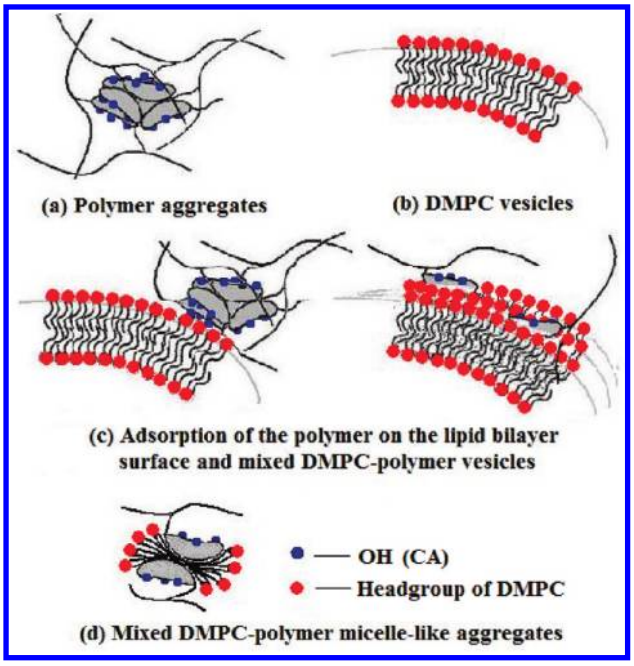


Figure 5. A schematic representation for the interaction between DMPC vesicles and Dex-CA.

organization and interaction. We can also see that the curve starts to be distorted with increasing polymer concentration, and starting at curve d ($c_{\text{side group}} \sim 0.9 \times 10^{-3}$ mol·dm^{-3}) we observe a curve splitting. Increasing the side group concentration up to $C_{\text{side group}} = 1.6 \times 10^{-3}$ mol·dm^{-3}, the phase transition becomes much broader, but two peaks still exist (g). This is in agreement with the results in region I obtained from ITC and turbidity (Figure 2). When $C_{\text{side group}} > 1.6 \times 10^{-3}$ mol·dm^{-3}, only a single, very broad transition peak is apparent (i and h in Figure 4). If we compare these results to the ones obtained by ITC, we observe that these two concentrations are in region II. This broad transition reflects a highly noncooperative event to occur in this region.[28,29] The complete disappearance of the DSC peaks occurs when $C_{\text{side group}} > 3.0 \times 10^{-3}$ mol·dm^{-3}, across the phase boundary between region II and region III. The DSC results are therefore in excellent agreement with the interpretation we gave above of our ITC results. The DSC curves for d−g can be deconvoluted into the component curves, and the relative contributions to the total transition can be quantitatively calculated (to be published).

All information as obtained from calorimetry (ITC and DSC), supported by turbidity and microscopy, discloses a much clearer picture of the interaction mechanism of the Dex-CA/DMPC mixed system, unraveling the details of the effect of the polymer side group concentration on the interaction with DMPC liposomes. Some aggregated morphologies are proposed, with a schematic representation shown in Figure 5.

Figure 5a,b represents the aggregate morphologies for the polymer and DMPC, respectively, before the start of the titration.

In dilute polymer concentration, two possible aggregates appear, from adsorption of polymer−CA micelles on the vesicle surface and from incorporation of CA moieties into the vesicle outlayer (Figure 5c). When the concentration range rises to $(1.6-3) \times 10^{-3}$ mol·dm^{-3}, a phase transition occurs, from mixed vesicles to mixed micelle-like aggregates. In addition, more CA groups are inserted in the membrane, which eventually causes bilayer disruption and the lipids and CA groups to rearrange into mixed micelle-like aggregates. This transition occurs at a $C_{\text{side group}} \sim 3.0 \times 10^{-3}$ mol·dm^{-3}, or at a molar ratio of DMPC/side group of about 4 (Figure 5d).

The various measurements performed allowed us to understand the effect of the polymer on liposome characteristics and integrity, and to map the phase bounderies from the ITC measurements. Further, the DSC results supported our interpretation of the phase boundaries as obtained from ITC. Finally, the electron microscopy micrographs are in line with the proposed aggregate morphologies.

SUPPORTING INFORMATION AVAILABLE

Preparation of liposomes and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author:

*To whom correspondence should be addressed. Tel.+351 22 0402511; fax: +351 22 0402659. E-mail address: mbastos@fc.up.pt.

ACKNOWLEDGMENT

Thanks are due to FCT for financial support to CIQ(UP), Unidade de Investigação 81, and for a Post-Doc grant to G.B. (SFRH/BPD/41407/2007).

REFERENCES

(1) Antunes, F. E.; Marques, E. F.; Miguel, M. G.; Lindman, B. Polymer−Vesicle Association. *Adv. Colloid Interface Sci.* **2009,** *147−148*, 18–35.
(2) Dong, C.; Rogers, J. A. Polymer-Coated Liposomes: Stability and Release of ASA from Carboxymethyl Chitin-Coated Liposomes. *J. Controlled Release* **1991,** *17*, 217–224.
(3) Amado, E.; Blume, A.; Kressler, J. Novel Non-ionic Block Copolymers Tailored for Interactions with Phospholipids. *React. Funct. Polym.* **2009,** *69*, 450–456.
(4) Zhang, L.; Peng, T.; Cheng, S.; Zhuo, R. Destabilization of Liposomes by Uncharged Hydrophilic and Amphiphilic Polymers. *J. Phys. Chem. B* **2004,** *108*, 7763–7770.
(5) Polozova, A.; Winnik, F. M. Mechanism of the Interaction of Hydrophobically-Modified Poly-(*N*-isopropylacrylamides) with Liposomes. *Biochim. Biophys. Acta* **1997,** *1326*, 213–224.
(6) Auguste, D. T.; Prud'homme, R. K.; Ahl, P. L.; Meers, P.; Kohn, J. Association of Hydrophobically-Modified Poly(ethylene glycol) with Fusogenic Liposomes. *Biochim. Biophys. Acta* **2003,** *1616*, 184–195.
(7) Wang, Y. J.; Winnik, F. M.; Clarke, R. J. Interaction between DMPC Liposomes and HM-PNIPAM Polymer. *Biophys. Chem.* **2003,** *104*, 449–458.
(8) Deme, B.; Dubois, M.; Zemb, T.; Cabane, B. Coexistence of Two Lyotropic Lamellar Phases Induced by a Polymer in a Phospholipid−Water System. *Colloids Surf. A: Physicochem. Eng. Aspects* **1997,** *121*, 135–143.

DOI: 10.1021/jz100087k | *J. Phys. Chem. Lett.* **2010,** *1*, 932–936

(9) Polozova, A.; Yamazaki, A.; Brash, J. L.; Winnik, F. M. Effect of Polymer Architecture on the Interactions of Hydrophobically Modified Poly-(*N*-isopropylamides) and Liposomes. *Colloids Surf. A: Physicochem. Eng. Aspects* **1999,** *147*, 17–25.
(10) Shi, X.; Li, J.; Sun, C.; Wu, S. The Aggregation and Phase Separation Behavior of a Hydrophobically Modified Poly(*N*-isopropylacrylamide). *Colloids Surf. A: Physicochem. Eng. Aspects* **2000,** *175*, 41–49.
(11) Okumura, Y.; Mizushima, H.; Fujinaga, K.; Sunamoto, J. Immobilization of Liposomes on Hydrophobically Modified Polymer Gel Particles in Batch Mode Interaction. *Colloids Surf. B: Biointerfaces* **2007,** *55*, 235–240.
(12) Auguste, D. T.; Kirkwood, J.; Kohn, J.; Fuller, G. G.; Prud'homme, R. K. Surface Rheology of Hydrophobically Modified PEG Polymers Associating with a Phospholipid Monolayer at the Air−Water Interface. *Langmuir* **2008,** *24*, 4056–4064.
(13) Yaroslavov, A. A.; Melik-Nubarov, N. S.; Menger, F. M. Polymer-Induced Flip-Flop in Biomembranes. *Acc. Chem. Res.* **2006,** *39*, 702–710.
(14) Quemeneur, F.; Rinaudo, M.; Pepin-Donat, B. Influence of Polyelectrolyte Chemical Structure on their Interaction with Lipid Membrane of Zwitterionic Liposomes. *Biomacromolecules* **2008,** *9*, 2237–2243.
(15) Nichifor, M.; Carpov, A. Bile Acids Covalently Bound to Polysaccharides. 1. Esters of Bile Acids with Dextran. *Eur. Polym. J.* **1999,** *35*, 2125–2129.
(16) Nichifor, M.; Lopes, A.; Carpov, A.; Melo, E. Aggregation in Water of Dextran Hydrophobically Modified with Bile Acids. *Macromolecules* **1999,** *32*, 7078–7085.
(17) Mehvar, R. Dextran for Targeted and Sustained Delivery of Therapeutic and Imaging Agents. *J. Controlled Release* **2000,** *69*, 1–25.
(18) *The Bile Acids. Chemistry, Physiology, and Metabolism: Chemistry*; Nair, P. P., Kritchevsky, D., Eds.; Plenum Press: New York, 1971; Vol. 1.
(19) Ross, B. P.; Braddy, A. C.; McGeary, R. P.; Blanchfield, J. T.; Prokai, L.; Toth, I. Micellar Aggregation and Membrane Partitioning of Bile Salts, Fatty Acids, Sodium Dodecyl Sulfate, and Sugar-Conjugated Fatty Acids: Correlation with Hemolytic Potency and Implications for Drug Delivery. *Mol. Pharmaceutics* **2004,** *1*, 233–245.
(20) Lee, E.-O.; Kim, J.-G.; Kim, J. D. Induction of Vesicle-to-Micelle Transition by Bile Salts for DOPE Vesicles Incorporating Immunoglobulin G^1. *J. Biochem.* **1992,** *112*, 671–676.
(21) Long, M. A.; Kaler, E. W.; Lee, S. P. Structural Characterization of the Micelle−Vesicle Transition in Lecithin−Bile Salt Solutions. *Biophys. J.* **1994,** *67*, 1733–1742.
(22) Hildebrand, A.; Neubert, R.; Garidel, P.; Blume, A. Bile Salt Induced Solubilization of Synthetic Phosphatidylcholine Vesicles Studied by Isothermal Titration Calorimetry. *Langmuir* **2002,** *18*, 2836–2847.
(23) Bai, G.; Santos, L. M. B. F.; Nichifor, M.; Lopes, A.; Bastos, M. Thermodynamics of Interaction between Hydrophobically Modified Polyelectrolyte and Sodium Dodecyl Sulfate in Aqueous Solution. *J. Phys. Chem. B* **2004,** *108*, 405–413.
(24) Bai, G.; Nichifor, M.; Lopes, A.; Bastos, M. Thermodynamic Characterization of the Interaction Behaviour of a Hydrophobically Modified Polyelectrolyte and Oppositely Charged Surfactants in Aqueous Solution: Effect of the Surfactant Alkyl Chain Length. *J. Phys. Chem. B* **2005,** *109*, 518–525.
(25) Bai, G.; Nichifor, M.; Lopes, A.; Bastos, M. Thermodynamics of Self- Assembling of Hydrophobically Modified Cationic Polysaccharides and Their Mixtures with Oppositely Charged Surfactants in Aqueous Solution. *J. Phys. Chem. B* **2005,** *109*, 21681–21689.
(26) Piculell, L.; Guillemet, F.; Thuresson, K.; Shubin, V.; Ericsson, O. Binding of Surfactants to Hydrophobically Modified Polymers. *Adv. Colloid Interface Sci.* **1996,** *63*, 1–21.
(27) Abrunhosa, F.; Faria, S.; Gomes, P.; Tomaz, I.; Pessoa, J. C.; Andreu, D.; Bastos, M. Interaction and Lipid-Induced Conformation of Two Cecropin−Melittin Hybrid Peptides Depend on Peptide and Membrane Composition. *J. Phys. Chem. B* **2005,** *109*, 17311–17319.
(28) Firestone, M. A.; Seifert, S. Interaction of Nonionic PEO−PPO Diblock Copolymers with Lipid Bilayers. *Biomacromolecules* **2005,** *6*, 2678–2687.
(29) Firestone, M. A.; Thiyagarajan, P.; Tiede, D. M. Structure and Optical Properties of a Thermoresponsive Polymer-Grafted, Lipid-Based Complex Fluid. *Langmuir* **1998,** *14*, 4688–4698.

DOI: 10.1021/jz100087k | *J. Phys. Chem. Lett.* **2010,** *1*, 932–936