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Thermoresponsive Nanostructures by Self-Assembly of a Poly(*N*-isopropylacrylamide)−Lipid Conjugate

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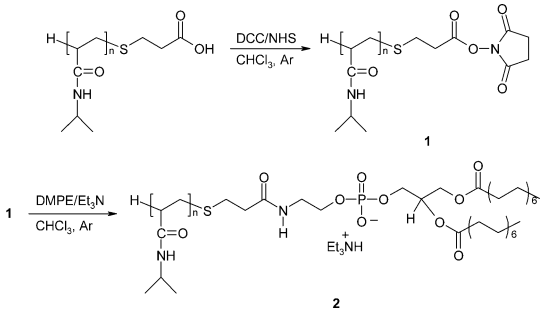
Self-assembly represents an attractive route for engineering stimuli-responsive materials of controlled nanostructure. Incorporating external control over structure and physical properties offers the possibility of constructing systems capable of both tunable transformation and controlled transmission of energy or information. PEG-grafted, lipid-based complex fluids comprising a mixture of a phospholipid, a PEG−lipid (dimyristoylphosphatidylethanolamine, DMPE) conjugate, and a zwitterionic cosurfactant in water, for example, undergo a thermoreversible phase transition, converting between an optically birefringent, elastic solid (gel) lamellar phase (Lαg) and a nonbirefringent, low-viscosity, 2D hexagonally ordered array of prolate micelles (H_1).[1] Both phases feature lattice dimensions on the order of hundreds of nanometers that are tunable over a wide range simply by changing either the water content or the number of repeat units on the appended PEG chains.

Soft nanostructures such as the PEG-grafted, lipid-based complex fluids can serve as scaffolds for the encapsulation of inorganic nanoparticles. Furthermore, the collective optical (electronic) properties of the particles can be modified simply by adjusting the dimensions of the water channel, thereby altering their packing arrangements.[2] Previously, this was achieved by using PEG−lipid conjugates of varying PEG molecular weight. A more elegant approach would be to control the water channel dimensions by application of an external stimulus, such as a change in temperature. In addition to its obvious fundamental interest, external control of confined water in these materials may have practical significance as well. For example, it could provide a convenient mode by which to "expel" substances for controlled drug delivery or biocatalysis.

With this in mind, we have sought to prepare stimulus-responsive complex fluids by replacing the tethered PEG component with poly(*N*-isopropylacrylamide), PNIPAM, a polymer well-known to undergo dramatic, temperature-induced changes in chain conformation. PNIPAM, along with its copolymers, is in fact, among the most widely studied "smart" polymers exhibiting significant thermoresponsiveness.[3] Numerous reports describing the synthesis and use of PNIPAM and various copolymers thereof in such diverse areas as separations,[4] sensors,[5] biocatalysis,[6] and biomedical device modification and drug delivery[7] have appeared. Less work has appeared, however, regarding the synthesis of terminally grafted PNIPAM,[8] in particular, the synthesis of phospholipid−PNIPAM conjugates and their potential in the development of thermoresponsive nanostructures. In this report, we describe the synthesis of PNIPAM terminally grafted to a phospholipid headgroup and present initial results suggesting that this material will prove useful in creating a new family of complex fluids, one with greater temperature-dependent tunability.

Amphiphilic PNIPAM (used to ensure integration into the lipid bilayer) was prepared in a two-step procedure. First, dicyclohexylcarbodiimide, DCC, was used to prepare the active ester of the carboxy-terminated PNIPAM, **1** (Scheme 1), using *N*-hydroxsuccinimide, NHS.[9] The success of this step was indicated by the appearance of several new vibrational modes in the infrared spectrum, at 1209 cm^{-1} (ν C−O, ester or ν C−N−C succinimidyl), 1739 cm^{-1} (ν C═O ester), 1785 cm^{-1} (ν C═O succinimidyl, *as*),[4] and 1816 cm^{-1} (ν C═O succinimidyl, *sy*), consistent with formation of the NHS ester.[10] Formation of the ester was also confirmed by a UV spectroscopic assay (λ max at 259 nm).[4,11]

Scheme 1. Synthesis of PNIPAM−DMPE Conjugate



The activated polymer was coupled to the available primary amine of DMPE via amide bond formation between the polymer and the lipid (Scheme 1). The presence of the PNIPAM−DMPE conjugate, **2**, was verified by ^{31}P NMR, which showed a strong isotropic resonance at 1.33 ppm. A second indication of successful formation of the conjugate was provided by the magnitude of the downward shift observed for the product, which compared well to that observed for PEG−DMPE conjugates (δ = 1.09 ppm in CD_3-OD) relative to the DMPE (δ = 0.31 ppm in 2:1 (v/v) $CDCl_3/CD_3$-OD). Also consistent with the formation of the product was the absence of modes attributable to the activated polymer from the infrared spectrum. Finally, because the polymer contained a sulfur atom, the presence of sulfur and phosphorus in an approximate 1:1 ratio could be used as a "marker" to identify the conjugate (%P 0.132 ± 0.005; %S 0.0976 ± 0.0109). In contrast to the results for the pure MeOH fractions from the column chromatographic run (which contained the product), the MeOH−$CHCl_3$ fractions did not yield this ratio by elemental analysis (%P < lod; %S 0.0926 ± 0.0402). In addition, these fractions lacked a phosphorus resonance in the ^{31}P NMR. The combined yield for the activation and coupling reactions was determined to be 24%. Comparable yields were observed for similar polymer conjugation reactions and were attributed to the well-documented inefficiency of the reaction of single sites on high molecular weight polymers.[12] In addition, some (small) fraction of the PNIPAM did not contain the carboxy terminus.[4]

Optical microscopy and SAXS were used to evaluate the effect of replacing the PEG−DMPE conjugate with the newly synthesized PNIPAM−DMPE. The complex fluids were prepared by dispersing DMPC (dimyristoylphosphatidylcholine), PNIPAM−DMPE, and LDAO (lauryldimethylamino-*N*-oxide) in water, while maintaining a 4:1 (w%) ratio of water to organic components and a polymer−

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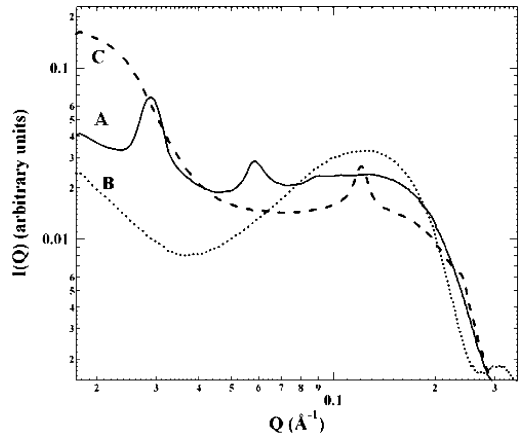

Figure 1. Scattered X-ray intensity, $I(q)$, as a function of momentum transfer, q, for (A) quarternary phase at 22 °C, (B) 11 °C, and (C) above the LCST of PNIAM, 60 °C.

lipid conjugate concentration of 10 mol %. This mixture was found to yield a material that spontaneously self-organized to form a stable, macroscopically homogeneous, optically transparent gel at room temperature (22 °C). Upon a reduction in temperature (15 °C), the gel converted to a transparent, free-flowing fluid; an increase in temperature (32 °C) converted the sample to an opaque, free-flowing fluid.

Temperature-dependent polarized optical microscopy was used as a convenient way of characterizing the phase transitions in the quaternary phase. The room-temperature optical birefringence pattern of a sample confined in a capillary displayed strong birefringence, indicative of a liquid crystalline lamellar phase.[1] Cooling of the sample was accompanied by a disappearance of the birefringence, indicating conversion of the material into an optically isotropic phase. In contrast, warming the sample to the LCST of PNIPAM (32 °C) or above led to only a diminution, rather than a complete disappearance, of birefringence (difficult to observe since the sample becomes opaque). These simple optical birefringence experiments delineated two possible phase transitions. To more fully characterize these structural states, temperature-dependent SAXS measurements were performed.

The SAXS curve for the room temperature (22 °C) gel phase is shown in Figure 1A. The diffraction pattern shows three resolvable peaks with a d spacing ratio of integral order ($q = 0.0288$, 0.0584, 0.0884 Å^{-1}). Such a diffraction pattern suggests a lamellar structure composed of alternating layers of water and bimolecular layers of amphiphiles. The Bragg peaks corresponded to stacking along one dimension of the lamellae with periodicity of 218 Å. Decreasing the sample temperature to 11 °C produced a scattering profile featuring a very broad peak centered at $q = 0.124$ Å (Figure 1B). The breadth of the diffraction peak indicates poor coherence and is suggestive of formation of a nonlamellar phase. (Full structural elucidation awaits complementary neutron scattering experiments.[1]) The observed structural transformation at 11 °C may have arisen from polymer chain expansion with improving solvent quality.[13] Prior studies of lipopolymer aggregates in solution showed that the bilayer stress induced by chain repulsion will be relaxed by a transformation into a micellar phase.[1] As the temperature was increased toward the LCST of PNIPAM, the three peaks present in the room-temperature SAXS pattern were replaced by a pair of well-resolved Bragg peaks in the high q region. At 45 °C (data not shown), the recorded scattering curve contained features consistent with a mixed phase, while at 60 °C, the scattering profile was consistent with complete conversion to a collapsed lamellar structure ($q = 0.119$, 0.239 Å^{-1}) with a lattice spacing of 53 Å (Figure 1C). Such a dimension was similar to that observed in a simple DMPC−water dispersion, and most likely arose from the temperature-triggered collapse of the lipid-grafted NIPAM chains to the globular state.[3] Each of the temperature-induced states were completely reversible after cycling above the LCST. The dramatic structural variations produced in these materials by simple introduction of the hydrophobically modified PNIPAM stand in contrast to the results of prior studies in which the extent of interaction of surfactants terminally grafted to PNIPAM or hydrophobically modified PNIPAM copolymers coating phospholipid vesicles yielded temperature-dependent changes in the release of entrapped dyes in liposomes.[8,14,15] Only one such study has demonstrated the use of a PNIPAM copolymer-modified lipid vesicle to control the lamellar to hexagonal (H_{II}) phase transition of PE (phosphatidylethanolamine) liposomes.[15] In contrast to these prior systems, the introduction of the new lipid−PNIPAM conjugate into a lipid mesophase produces dramatic, yet controllable (reversible), changes in the nanostructure.

In summary, a thermoresponsive, noncovalent self-assembled aggregate featuring two distinct temperature-triggered phase transitions has been prepared. These phase transitions are believed to arise from temperature-induced changes in PNIPAM chain conformation and the confinement of the polymer in nanosized water channels. This work demonstrates the significant potential of externally controlling the structure of self-assembled materials and lays the groundwork for the development of "active" nanostructures.

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Supporting Information Available: Synthesis of compounds **1** and **2**, complex fluids, and SAX experiments; and polarized optical micrographs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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