

Tailoring Phospholipid Micellar Shape for Drug and Protein Delivery Systems

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Introduction

Phospholipid micelles are relatively new drug delivery systems. They are thermodynamically stable or metastable, self-assembling systems [1]. Their mean hydrodynamic diameter (~16 nm) allows them to extravasate to the tissue compartments where the drug action is required. Phospholipid micelles have been shown to be nontoxic and acceptable for intravenous administration, and they appear to be more stable than other surfactant micelles upon aqueous dilution.

This phospholipid-based drug delivery system requires a clear understanding of which particle shapes are most effective in delivering a particular drug and how to achieve them. The particle shape in self-assembling micelle systems is derived from the local curvature, which is determined from the component surfactant shape and interactions [2, 3]. The additional degree of freedom introduced by mixing two or more surfactants of different intrinsic curvature allows for considerable latitude in controlling particle shape. The extensive literature on mixed surfactant systems bears this notion out [4].

Egg yolk phosphatidylcholine (EYPC) has zero intrinsic curvature. Distearoyl phosphatidyl ethanolamine modified with polyethylene glycol (DSPE-PEG) of increasing molecular weight has correspondingly increasing positive curvatures and solubilities. Thus, mixtures of EYPC with different proportions of DSPE-PEG with PEG of various molecular weights will provide the latitude to control particle shape. The hypothesis that we tested was that when EYPC is incorporated into simple DSPE-PEG micelles, the particle curvature and shape will change to provide larger hydrophobic core volumes. Therefore, the solubilization potential of phospholipid micelles will increase. Structural information from this work will be compared with drug solubilization data on the same systems.

Another important clinical application of “PEGylated” phospholipids is in protein therapeutics. Proteins pose challenging stability issues, as they are complex macromolecules that undergo physical degradation by aggregation and conformational and chemical changes. These PEGylated phospholipids are attractive candidates for stabilizing protein therapeutics in their active form. Testing the efficacy of this approach requires measuring

the size and shape of the protein-DSPE-PEG 5000 complex and assessing the changes in protein structure that occur upon phospholipid association.

Our goal was to characterize the morphology of the micelles as a function of EYPC and DSPE-PEG composition and PEG molecular weight, gain knowledge about how to control micelle morphology, determine how proteins associate with PEGylated phospholipids, and determine how this interaction modulates the morphology of the resulting assembly.

Methods and Materials

Various molar ratios of DSPE-PEG 2000 and 5000 and phosphatidylcholine (PC) (100:0, 85:15, 75:25, 60:40, 50:50, and 30:70) were dissolved in methanol. The mixtures were coprecipitated under vacuum by using a rotary evaporator to form dry films, then dried overnight to remove traces of solvent. The films were then rehydrated to a 1 mM lipid concentration with an isotonic 0.01 M HEPES buffer at pH 7.4 and equilibrated in the dark under argon at room temperature.

Protein solutions were mixed with DSPE-PEG 5000 micellar dispersions in buffer at the isoelectric point of the specific protein and allowed to equilibrate at room temperature for predetermined amounts of time. We also compared the interaction of the protein with polysorbate 80 (a standard pharmaceutical excipient used to stabilize proteins) to our PEGylated phospholipid-protein system. In addition, we assessed cytokine interactions with sodium dodecyl sulfate (SDS, a well-characterized surfactant used to stabilize proteins at low concentrations and to denature them at high concentrations). Finally, we used two other proteins (enzyme and enzyme inhibitor) for comparison in our PEGylated phospholipid system.

Small-angle x-ray scattering (SAXS) experiments were performed at BESSRC beamline 12-ID at the APS. The scattering data were measured on a 2-D charge-coupled device (CCD) detector. A q -range ($q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the x-ray beam) from 0.065 nm^{-1} to 2.5 nm^{-1} was covered within a single setting. The resolution effects are negligible on this instrument. The data were radial-averaged by using the standard software of the facility

and rebinned into 200 points evenly distributed on a log q -scale. The absolute scale of the scattering intensity was determined by measuring the incoherent scattering from pure water. Indirect Fourier transform analysis was performed on these scattering data, and the detailed structures of the lipid systems were determined from a modeling approach for analyzing scattering data.

Results and Discussion

DSPE-PEG:EYPC Micelles

Figure 1 shows SAXS data taken for DSPE-PEG2000 (Fig. 1a) and DSPE-PEG5000 (Fig. 1b) mixed in different mole fractions with EYPC. A model for the micelles with a hydrophobic core, surrounded by a dense hydrophilic layer that is covered by PEG chains in the form of Gaussian random coils attached to the outer surface (Fig. 2), is in good agreement with the scattering data (Fig. 1). The micelles are never perfectly spherical; even at 100% DSPE-PEG, oblate ellipsoidal micelles are formed. As more and more EYPC is incorporated in the micelles, the micelles elongate, and rodlike micelles with an elliptical cross section form. In the region where the growth takes place, the DSPE-PEG2000:EYPC micelles have larger aggregation numbers than the DSPE-PEG5000 micelles for identical mixing ratios. As the EYPC content increases, these micelles elongate, and at a DSPE-PEG:EYPC ratio of 30:70, rigid, rodlike micelles longer than 1000 Å are formed. By mixing DSPE-PEG and EYPC, considerable latitude in controlling the particle shape is obtained, and we have been able to demonstrate that the aggregation numbers and observed transition from spheroid to rodlike shape follows the predictions of a simple theory using the mean curvature of the surfactants. This may be useful for controlling micelle shape in controlled drug delivery applications.

Protein:Surfactant Complexes

SAXS data were taken from proteins and surfactants, both alone and in different mixing ratios. The comparison of the scattering curves allowed us to determine if the protein and surfactants were interacting, and, if so, to determine the stoichiometry of the interaction and the saturation level. We found that SDS forms complexes with all of our proteins. This is seen, for example, by analyzing the forward scattering extracted from the SAXS data, where the dependence of $I(0)$ with the mixing ratio (Fig. 3) follows a model calculation for complex formation to a molar ratio of about 250 (SDS to protein), after which $I(0)$ shows that complexes and pure micelles coexist. However, polysorbate interaction is minimal. The latter conclusion comes from the observation that the SAXS data for the complexes at all mixing ratios are the

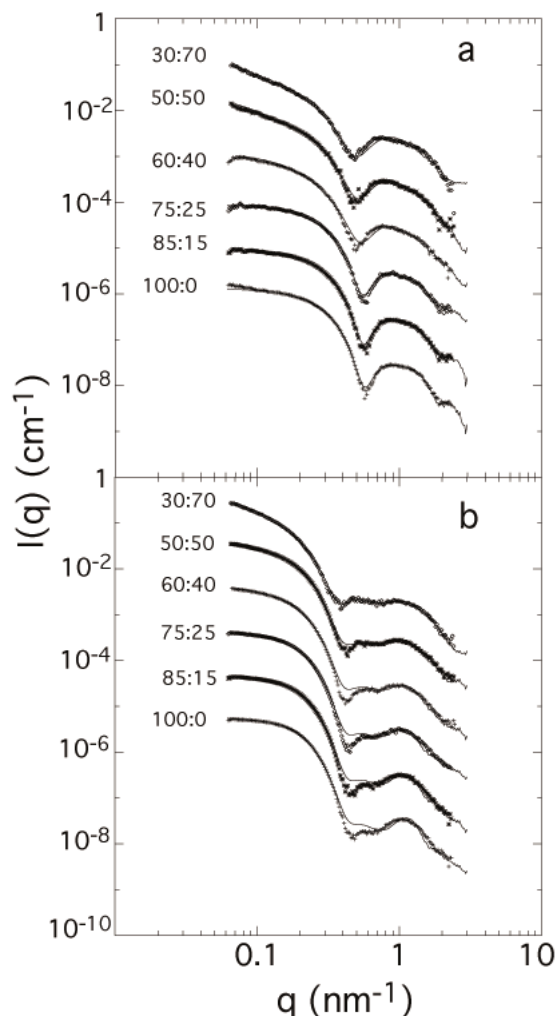


FIG. 1. SAXS data from the mixed micelle systems. Top graph a shows DSPE-PEG2000:EYPC. Bottom graph b shows DSPE-PEG5000:EYPC. Points indicate data. Lines indicate model fits. The DSPE-PEG2000:EYPC molar mixing ratios are indicated on the graphs. The data are plotted on an absolute scale. The data are rescaled by 10^n ; n runs from -5 to 0, starting from the lowermost curve.

sums of the SAXS data from the individual components (Fig. 4). Both results were anticipated from other analytical methods described in the literature. In contrast, PEGylated phospholipid-protein interaction is complex and concentration dependent. One of our proteins becomes saturated with PEGylated phospholipid at phospholipid-to-protein molar ratios of $\sim 40:1$ and $>75:1$ for our model enzyme inhibitor. The complex size increases until saturation is achieved, suggesting that the proteins are coated by phospholipid. Complex sizes above

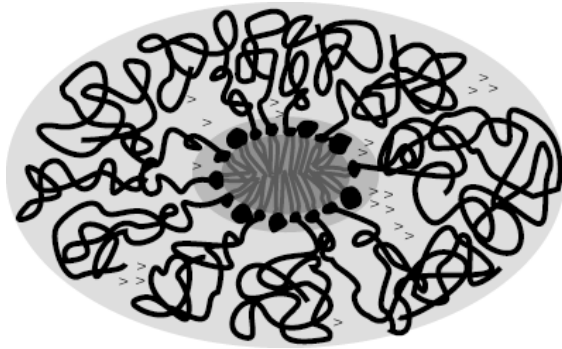


FIG. 2. Model for the mixed DSPE-PEG:PC micelles.

saturation approach the size of micelles. (Unlike standard phospholipids, PEGylated phospholipids form micelles in aqueous solutions.) In conclusion, our SAXS data have enabled an increased understanding of our complex systems.

Acknowledgments

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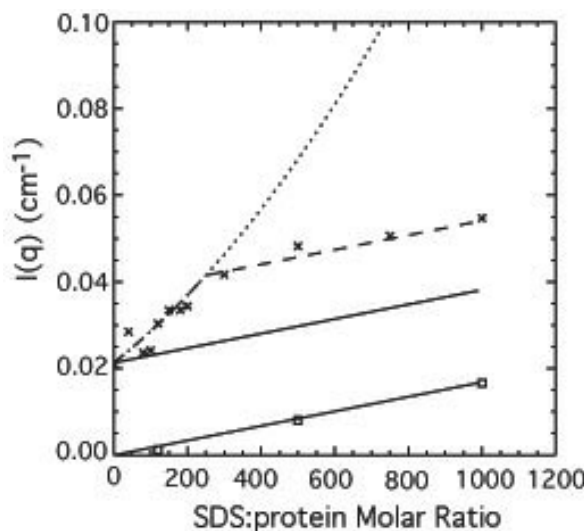


FIG. 3. Model calculations and experimental data: $I(q)$ as a function of the SDS-to-protein mixing ratio. Solid line = no interaction. Dotted line = interaction and full complex formation. Dashed line = interaction, saturation level for complex formation, equilibrium micelle model (calculation for $N_{smax} = 140$). Dotted/dashed line = pure SDS micelles with a mean aggregation number of 57.2. Squares = experimental data for pure DSPE-PEG5000 micelles. X's = experimental data for DSPE-PEG5000:MPO mixtures.

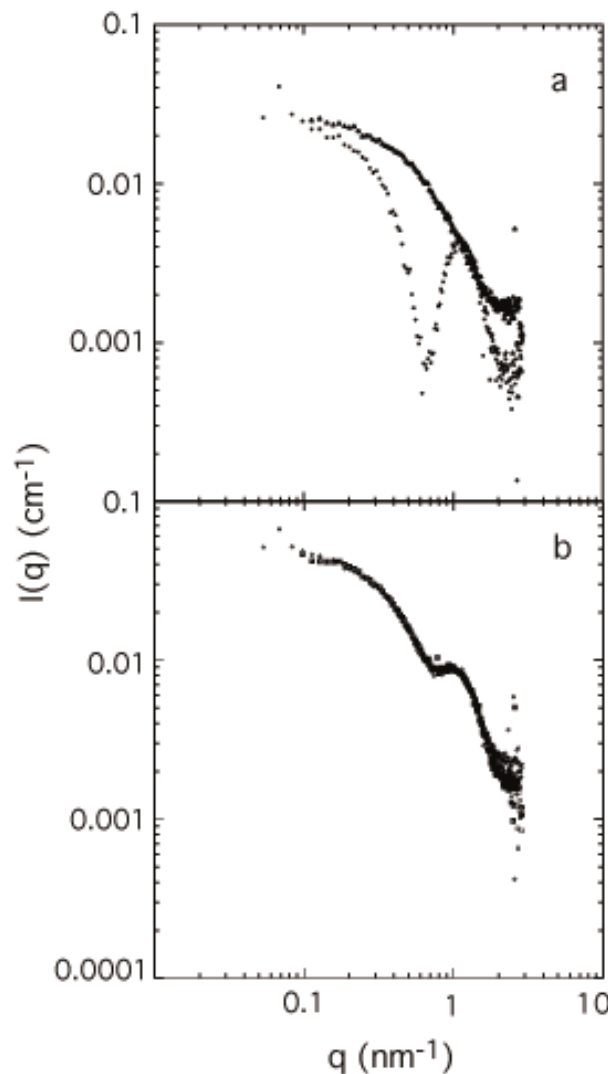


FIG. 4. SAXS from polysorbate-protein systems. Top graph a: Triangles = protein. Plus signs = polysorbate. Bottom graph b: Squares = mixed polysorbate-protein data. The molar ratio of polysorbate to protein is 86.4.

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