Interaction of Lipid-Conjugated Poly(ethylene glycol) Micelles with Bovine Serum Albumin

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Statement of Purpose: Micelles formed from the amphiphilic molecule, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG(2000)) have shown promise as targeting, therapeutic, and diagnostic nanoparticles. Two saturated 18-carbon alkyl tails provide a strong driving force for self-assembly in aqueous environments while hydrophilic PEG forces curvature at the core-corona interface. Spheroidal micelles are formed of approximately 10-20 nm in diameter with aggregation numbers near 90. Biofunctionality is added to the micelles through the end-functionalization of the PEG chain and subsequent conjugation of peptides, fluorophores, antibodies, or other relevant molecules. This system provides a path to arbitrarily complex mixed micelles in which functional molecules can be separately conjugated to DSPE-PEG(2000) and mixed together in precise ratios before micelle formation. With their small size, ease of synthesis, and high density of biomolecules, these micelles offer new opportunities for the design of nanoparticles that interact with biological systems.

One barrier to the *in vivo* use of these micelles is the potential for micelle breakup by proteins in the blood. Micelle degradation would eliminate any functional advantage of a mixed micelle by separating the individual components (i.e. targeting and therapeutic peptides). In particular, it has been suggested that DSPE-PEG(2000) micelles complex with bovine serum albumin (BSA) such that micelles are absent even in the presence of moderate amounts of BSA. (Castelletto V. Biomacromolecules. 2007;8:2244-2249.) At odds with this result is the observation that mixed micelles, with separate targeting and fluorescent components, reach *in vivo* targets with their fluorescence in tact. (Karmali PP. Nanomedicine. 2008;*In press, available online*.) This work examines this discrepancy by quantifying both the equilibrium partitioning of DSPE-PEG(2000) between the micellar state and the BSA-bound state as well as the kinetic behavior of the system. We believe that although micelles are not thermodynamically stable at certain concentrations of BSA, micelle breakup happens slowly, providing time for *in vivo* targeting and drug delivery.

Methods: We track the kinetics and steady state behavior of the micelle / BSA system using the self-quenching behavior of fluorescein molecules separated by nano-scale distances. Micelles formed from fluorescein-labeled DSPE-PEG(2000) are highly quenched due to high density of fluorophores on the micelle surface. As monomers move from the micelle to complex with individual BSA proteins, self-quenching decreases and increased fluorescence is observed. We vary the ratio of BSA to DSPE-PEG(2000) in order to determine the equilibrium partitioning coefficients for the system. Kinetic behavior is studied for these systems and compared to the mixing rate between populations of labeled and unlabeled micelles to determine the mechanism of micelle breakup in the presence of BSA.

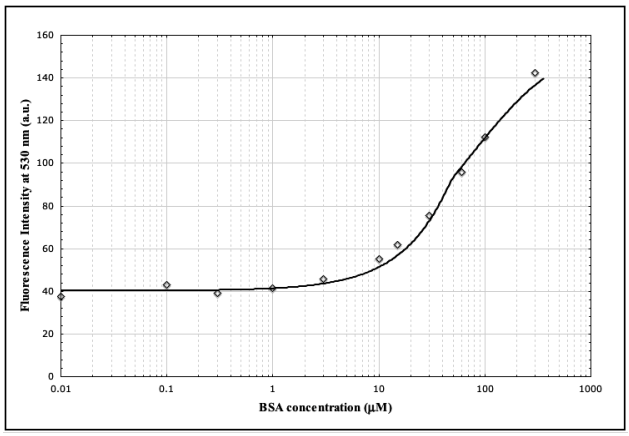

Figure 1. Fluorescence intensity (◇) is shown as a function of BSA concentration for 30 μM DSPE-PEG(2000). Data modeling (solid line) yields equilibrium constants.

Results: Our results show that the critical micelle concentration (CMC) of DSPE-PEG(2000) micelles is dependent on BSA concentration. Three DSPE-PEG(2000) monomers are required for every five BSA proteins in order to maintain a DSPE-PEG(2000) population above the CMC that is observed in the absence of BSA. While affinity of DSPE-PEG(2000) for BSA is not particularly strong, it is possible to eliminate micelles at a given BSA concentration. Kinetic data show that the change from micelles to BSA-bound monomers occurs over a period of hours at 25ºC, and over several minutes at 37ºC. At neither temperature do the monomers associate with BSA faster than they mix with themselves. This result depicts BSA as a passive sink for monomers rather than an active catalyst for micelle breakup.

Conclusions: DSPE-PEG(2000) micelles coexist with BSA when BSA concentration is comparable to that of DSPE-PEG(2000). This result contradicts previous work that implied strong association of DSPE-PEG(2000) with BSA as studied by scattering techniques that looked at size distributions between particles of comparable size. In contrast, our technique is highly sensitive to the presence of micelles as fluorescence is strongly dependent on the fraction of monomers in the micellar versus BSA-bound states. Kinetic data shows that micelles at 37ºC have several minutes to function *in vivo* before being separated by BSA, a result that may explain the successful use of mixed targeted micelles *in vivo*. In order to further develop lipid-PEG micelles for *in vivo* use, we plan to vary both the size of the lipid and PEG portions of the amphiphile in order to favor the formation of micelles over BSA-lipid complexes.