Design of pH-responsive lipid-based nanocarriers for antimicrobial peptide delivery

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The delivery of antimicrobial peptides (AMPs) suffering from chemical instability in biological environment is one of the major research challenges in pharmaceutical field. In the present study, we design a structurally tunable pH-responsive lipid-based delivery system with the promise of delivery of AMPs while protecting them from degradation. These nanocarriers are prepared through the self-assembly of 1,2-dioleoyl-3-dimethylammonium-propane (DODAP) with the human cathelicidin LL-37 in excess water. Advanced experimental methods including Small-angle X-ray scattering, cryogenic transmission electron microscopy, and dynamic light scattering were used to characterize firstly the DODAP self-assemblies in absence and presence of a second stabilizer upon pH modification, and secondly the DODAP/LL-37 self-assemblies and their structural transitions in response to pH and composition changes. Investigations of DODAP self-assemblies in presence of a second stabilizer reveal further transitions from inverse bicontinuous cubic phase (*Im3m*) coexisting with vesicles via *Im3m* coexisting with inverse hexagonal phase (H_2), and H_2 phase to normal emulsion upon an increase of pH in the pH-range varying from 3 to 8. The further observed differences in the internal nanostructure of DODAP stabilized particles compared to the free-stabilizer DODAP system demonstrate that charged DODAP acted as a first stabilizer of DODAP particles in absence of a second stabilizer. Encapsulation of LL-37 into DODAP nanostructures are successfully observed across all pH range. Extensive two-phase regimes are detected upon gradual pH and composition changes. At pH higher than 5, increasing the concentration of LL-37 from up to 30 wt% relative to DODAP led to the gradual formation of small nanostructures coexisting with the larger DODAP emulsion droplets. At pH lower than 5 , LL-37 actively participate to the packing of DODAP. Increasing the LL-37 content up to 30 wt% in DODAP dispersion in this pH range lead to an increase in the fraction of vesicles that coexist with non-lamellar structures such as *Im3m*, H_2 and sponge like phases. These detailed understanding into the formation of this DODAP/LL-37 nanocarriers and their pH and composition tunable structural features may contribute to the rational design of pH-triggered antimicrobial systems for the delivery of AMPs.

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