MicroRNA-Induced Structural Changes in Reconstituted HDL Probed by Synchrotron SAXS

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Reconstituted high-density lipoprotein (rHDL) nanoparticles, discoidal assemblies of apolipoprotein A-I (apoA-I) and zwitterionic phospholipids, have emerged as promising endogenous-mimetic carriers for nucleic acid therapeutics targeting atherosclerosis. The capacity of rHDL to associate with and deliver microRNAs has been demonstrated in foam cell models of atherosclerotic plaque, with miRNA binding saturating at a 1:1 miRNA:apoA-I molar ratio [1]. Despite this therapeutic potential, the structural consequences of nucleic acid association with the rHDL disc remain poorly characterised at the colloidal level. Molecular dynamics simulations predict that single-stranded miRNA adsorbs at the headgroup region of zwitterionic phospholipid bilayers via hydrogen bonding and electrostatic salt bridges, an interaction mode strongly enhanced by divalent cations such as Ca^{2}+ and Mg^{2}+, which adsorb onto the bilayer surface and electrostatically recruit the negatively charged miRNA backbone [2] [3]. Whether these atomistic predictions translate into measurable structural remodelling of intact rHDL nanodiscs under physiologically relevant ionic conditions is an open question.

Here, we address this gap using synchrotron small-angle X-ray scattering (SAXS) to characterise the structural response of rHDL (apoA-I / DPPC:LysoPC:EC, 75:20:5 mol%) to single-stranded miRNA addition across different concentrations of the divalent salt Ca^{2+} or Mg^{2+}. SAXS patterns of rHDL alone were modelled with a core-shell bicelle elliptical belt model; miRNA alone was described by a flexible cylinder consistent with its hairpin secondary structure; and rHDL–miRNA mixtures were fitted using a combined plugin model in which disc radius and head-region thickness were refined as free parameters. Systematic miRNA addition produced statistically significant increases in both particle core radius and lipid head thickness, with the miRNA-induced signal exceeding concentration-handling noise in all buffer conditions. Crucially, the magnitude of the radius and lipid head thickness increase with divalent cation concentration, consistent with cation-mediated enhancement of miRNA adsorption at the lipid headgroup interface. These results provide direct structural evidence that rHDL nanodiscs are remodelled upon miRNA addition, with changes in particle radius and headgroup layer thickness consistent with miRNA adsorption at the lipid surface, an effect progressively enhanced by increasing divalent cation concentration.

Keywords:
reconstituted HDL, nanodisc, microiRNA, RNA-lipid interaction, divalent cations, SAXS, structural biology

References:

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