ABSTRACT

Angiopoietins-Like protein (ANGPTLs) are a group of eight secretory mammalian proteins which share structural similarity with the members of the angiopoietin (ANG) protein family. The physiological role of ANGPTL protein family is not well established and only few of them have been functionally characterized. Among these, ANGPTL3, ANGPLT4 and ANGPTL8 are known to play important roles in triacylglycerol metabolism. Lipoprotein lipase (LPL) is key regulatory enzyme known in triacylglycerol metabolism pathways. Structural analysis of human LPL enzyme suggested that it has two functional domains, N-terminal active site domain (LPL^{NTD}) and C-terminal substrate binding domain (LPL^{CTD}). Mechanisms involved in regulating triacylglycerol metabolism are currently under investigation to find solutions to treat metabolic syndrome. Role of ANGPTL4 in triacylglycerol metabolism is well established as it interacts with LPL and inhibit its function by dissociating its oligomers into inactive monomers. Later on, ANGPTL3 and ANGPTL8 have also been reported to have a role in lipid metabolism. Multiple sequence alignment suggests that they all have a common SE1 domain implicated in LPL inhibition. Though their role for LPL binding and direct inhibition is not yet clear but *in vitro* and *in vivo* studies confirmed that they have ability to inhibit LPL however, not as efficient as ANGPTL4.

Available literature suggests that ANGPTLs may use a different mechanism to inhibit LPL, so we hypothesized that ccdANGPTL3, ccdANGPTL4, and ANGPTL8 target different domains of LPL for its inhibition. To test this hypothesis, we performed pull-down assays and analytical size exclusion chromatography experiments which suggested that CCD domain of ANGPTL4 binds LPL N-terminal domain (LPL^{NTD}) while both ANGPTL3 and ANGPTL8 binds LPL C-terminal domain (LPL^{CTD}).

We also performed quantitative biochemical approach to study mechanistic differences in LPL inhibition and found that like ANGPTL4, both ANGPTL8 and ANGPTL3

have LPL inhibitory effect. The important differences in their mode of action is, ANGPTL4 could almost completely inhibit LPL activity even in the nanomolar concentration range whereas, both ANGPTL3 and ANGPTL8 were not able to completely suppress LPL activity even at micro-molar range of concentrations. These findings combined with previous studies suggested that both ANGPTL3 and ANGPTL8 targets LPL^{CTD} and inhibit LPL in substrate dependent manner while on the other hand ANGPTL4 block LPL^{NTD} and inhibit LPL in the substrate independent manner.

According to a recent study, though ANGPTL3 and ANGPTL8 are week inhibitors, but they have a tendency to form a complex which have more potential against LPL activity. We further confirmed ANGPTL3 and ANGPTL8 interaction using pull-down assays and size exclusion chromatography experiments. The data suggests that these two proteins do not interact when in folded state however, they form a complex when partially folded states are mixed and allowed to refold. We also incubated ANGPTL3 and ANGPTL8 in different molar ratios with LPL and observed an additive effect on LPL inhibition.

Fatty acids are known to modulate ANGPTL4 structural features upon binding and block the LPL inhibition effect by destabilization oligomeric structure of the latter. In this study, using ITC and thermoflour assays, we demonstrate that AGNPTL8 preferentially binds saturated fatty acids. Our thermal melting studies suggested that fatty acid binding destabilizes ANGPTL8. Therefore binding of fatty acids with ANGPTL8 may possibly modulate its LPL inhibition effect as reported for ANGPTL4. Based on these studies we have been able to proposed models highlighting roles of ANGPTL3, ANGPTL4 and ANGPTL8 in regulation of triacylglycerol metabolism.