STRUCTURAL DETAILS OF LCAT INTERACTION SITE ON HDL REVEALED BY H/D-MS/MS AND MOLECULAR MODELING

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INTRODUCTION: The cardioprotective function of high-density lipoprotein (HDL) is largely attributed to its ability to facilitate reversed cholesterol transport (RCT). Lecithin cholesterol acyl transferase (LCAT) contributes to HDL maturation, an important step in RCT critical for conversion of nascent HDL into a mature spheroidal form.

METHODS: Hydrogen-deuterium exchange tandem mass spectrometry (H/D-MS/MS) experiments have being combined with modeling techniques to produce a three-dimensional all-atom model of nascent HDL, in which two full length apoA1 lipoproteins wrap around 200 molecules of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) and 20 cholesterol molecules. We have developed a theoretical model, based on protein backbone and side chain solvent accessibility, to estimate the H/D exchange probabilities of backbone amide hydrogen atoms from a three dimensional structure of the HDL particle. We have further used these probabilities to calculate theoretical H/D exchange incorporation factors for peptic peptides and compared them with the experimentally measured values.

RESULTS: Hydrogen-deuterium exchange analyses of solvent accessibility within 48 pairs of peptic peptides across the entire sequence of apoA1 (~95% coverage for both lipid free and nascent HDL forms) revealed multiple unique features of both lipid free apoA1 and nascent HDL. First, lipid free apoA1, except for residues 14-18, 90-111 and 148-180, is highly solvent exposed, as demonstrated by a high degree of deuterium incorporation. In contrast, apoA1 within nascent HDL demonstrated numerous regions with marked reductions in deuterium incorporation, consistent with amino acids within these regions being buried within or abutting lipid and/or protein.

DISCUSSION & CONCLUSIONS: We propose that the two chains of apoA1 are in anti-parallel orientation along with a structural conformation that satisfies the experimental hydrogen deuterium exchange, crosslinking and fluorescence resonance energy transfer (FRET) data. Unlike previous models of HDL, we found that the region of amino acid residues 159-170 (the proposed LCAT binding site) is unfolded with the amino acid Tyr_{166} (critical for LCAT interaction) fully exposed to solvent, and its tertiary structure stabilized by a three-way salt-bridge (Arg_{160}-Asp_{168}-His_{162})

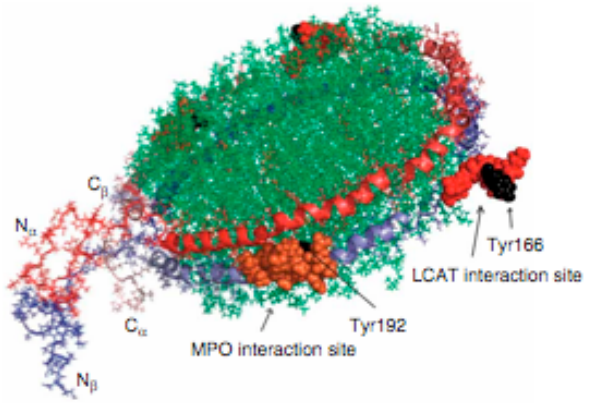

Fig. 1: Computational model of the nascent HDL particle illustrating sites of known protein-protein interactions and site-specific oxidation modifications reported within human atherosclerotic plaque. The LCAT interaction site (residue 159-170, filled red) and MPO binding site (residues 190-203, filled brown) are shown. The sites of the two most abundant site-specific oxidative modifications found in apoA1 recovered from human atherosclerotic lesions (Tyr_{166} and Tyr_{192}) are in black.

REFERENCES: Z. Wu, M. A. Wagner, L. Zheng, S. Liang, J. Parks, G. Vince, J. Smith, J. M. Shy, II, V. Gogonea, S. L. Hazen, *Nat. Struct. Mol. Biol.*, **14**, 861-868 (2007).

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