Probing the Inflammatory Pathways Associated With Increased Levels of Oxidized LDL Through the Structural Analysis of B17

Soumaya Berro, Bassam Hussein, Mohamad Hajj-Hassan, Bassam Hamam, Wiam Ramadan, Hassan M. Khachfe
Lebanese International University
Beirut, Lebanon
{10830001, bassam.hussein, mohamad.hajjhassan, bassam.hamam, wiam.ramadan, hassan.khachfe}@liu.edu.lb

***Abstract*—The structure of the 17% N-terminal domain of apolipoprotein B-100, apo B-17 (or simply B17), was homology-modeled after the structure of the N-terminus of lipovitellin (LV), a protein that shares not only a sequence homology with B17, but also a functional aspect of lipid binding and transport. The model structure was first forced to accommodate the six disulfide bonds found in that region, and then dynamically relaxed to minimize the free energy of the molecule. The content of secondary structural elements in this model structure correlates excellently with the reported data from other biophysical probes. The C-terminus of B17 shows a considerable homology with a conserved region in the constant domain of the T-cell receptor containing several residues that are essential in the interfacial connectivity with the variable domain. This structural insight may be the first potential link between atherogenic LDL and inflammation.**

Keywords–Apolipoprotein; atherosclerosis; homology modeling; LDL; inflammation.

I. INTRODUCTION

Atherosclerosis is a complex disease that has been linked to many risk factors, including hyperlipidemia, dyslipidemia, high blood pressure, and endothelial dysfunction [1]. Oxidative modification to the small Low-density Lipoprotein (LDL) has been dubbed the central event that initiates and propagates coronary artery diseases [2][3], and therefore, LDL is considered a major risk factor for atherosclerosis [4]. It has been also shown that systemic inflammatory mechanisms may underlie the pathogenesis of atherosclerosis [5][6][7]. This is probably why atherosclerosis is called an inflammatory disease [8]. The atherosclerotic process begins when cholesterol-rich LDL particles accumulate in the intima, and then activate the endothelium [8]. Leukocyte adhesion molecules and chemokines help in the recruitment of monocytes and T cells, and thus the inflammatory pathways [8]. However, the specific structural interactions implicated in these mechanisms have not yet been elucidated.

This report introduces a work-in-progress about the relation between inflammation and atherosclerosis, by probing the structural aspects of the apo-B. The next section gives some background information about the topic. It is followed by the experimental design. Subsequently, the results are discussed. Finally, some conclusions are summarized at the end.

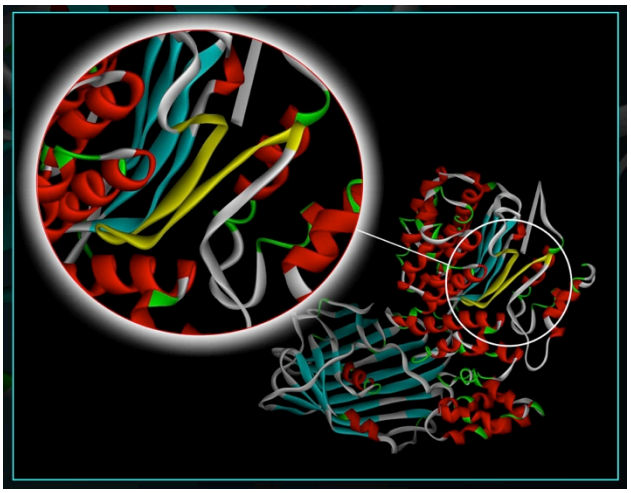

Figure 1. The structure of B17 modeled after lipovitallin, with the TCR homology region (yellow stretch) shown magnified [11].

II. BACKGROUND

Apolipoprotein B-100 (apo B) is the sole protein component of LDL [9], but its large size (4536 a.a.) and the limitation of current experimental techniques require that it be studied in pieces corresponding to its structurally organized domains [10]-13]. Biochemical [13][14], calorimetric [15][16], computational [11][17]-[20], and spectroscopic [21][22] approaches were used to probe the domain arrangement and characterization of the protein, but no molecular structure has ever been assigned to any of the different domains. These techniques, however, helped in the understanding of the overall arrangement of apo B on the LDL particle and the interactions that the various secondary structures have with both the lipid and aqueous phases, and in the ability to genetically engineer protein truncations that correspond to these various domains [14][23]-[25].

In this project, we will use the model structure of the 17% N-terminus of apo B [10] – the first 782 a.a. of apo B (17% of the full-length sequence), a region that is rich in disulfide bonds [26][27], essential for the secretion of the protein from hepatic cells [23] and behaves like an independent globular protein [21] – to further understand

 ISBN: 978-1-61208-359-9

the C-terminus of the protein. This part of B17 shows a considerable homology with a conserved region (25 aa) in the constant domain of the T-cell receptor (TCR) – a protein that is necessarily present during inflammation (Figure 1). It also contains several residues that are essential in the interfacial connectivity with the variable domain, which binds to the different antigens specific to each inflammatory pathway. We will try to establish a potential link between LDL and the inflammatory state correlated with atherosclerosis.

III. EXPERIMENTAL DESIGN

The structure of B17 was modeled using Insight II (Accelrys Inc., Insight Modeling Environment, Release 2000.1, San Diego: Accelrys Inc., 2002), based on the crystal structure of lipovitellin (LV) [11]. The secondary structure of the unstructured region was predicted using the Chou-Fasman Algorithm [28], the PROF methods [29][30] and the Deep View modality [31] (Figure 2). The calculation will be performed using the CHARMm molecular dynamics application [32].

The project will continue to have the following outputs and activities:

- Output 1: Explore the structural characteristics of the potential TCR homology region;
  - Activity 101: Further assessment of the model in various solvated states;
  - Activity 102: In-silico exploration of the ability of the TCR homology region to interact with ligands, by probing the propensities of the different exposed residues;
- Output 2: Study the ability of the region to adopt several conformations based on the lipidation state of the protein or the environmental conditions in the plasma;
  - Activity 201: Simulation of the various conditions to explore the corresponding conformations, by working out the energy minimization of the proposed structure;
  - Activity 202: further refinement of the model based on the environmental interactions and conditions, by introducing parameters pertaining to these conditions into the energy function;
- Output 3: Probe the interactions that might exist between this particular region and inflammation markers, i.e., interleukins and interferon.
  - Activity 301: Possibility of in-vitro interaction between a synthesized peptide corresponding to the TCR homology region with inflammation markers;

IV. RESULTS AND DISCUSSION

Inflammatory mechanisms have been reported to underlie the pathogenesis of atherosclerosis [5][6][7]. The C-terminus of B17 shows a considerable homology to a conserved region in the constant domain of the T-cell receptor – a protein that is necessarily present during inflammation. It also contains several residues that are essential in the interfacial connectivity with the variable domain, which binds the different antigens specific to each inflammatory pathway. The current model clearly shows that this region is fairly exposed and flexible, which – along with the normal protrusion of B17 from the small dLDL particle – may suggest that some kind of interactions may take place between apo B and other cell surface proteins, thus mimicking or competing with the T-cells during atherogeneity. This model, therefore, establishes a potential structural link between LDL and the inflammatory state correlated with atherosclerosis.

Figure 2. The unstructured region of B17 (aa 706-782). The H's below the sequence denote regions predicted to have helical secondary structure.

V. CONCLUSIONS

In this report, a solid link between atherosclerosis and inflammation is established. During atherogenesis, Monocytes recruited upon the activation of the endothelium differentiate into macrophages and upregulate pattern recognition receptors, including scavenger receptors and toll-like receptors. Scavenger receptors are responsible for lipoprotein internalization, and hence, foam-cell formation, one of the major steps in the plaque formation, characteristic of atherosclerosis. On the other hand, toll-like receptors transmit effector signals that lead to the release of cytokines, proteases, and vasoactive molecules. The T cells in these lesions recognize local antigens, and therefore, mount T helper-1 responses characterized by the secretion of pro-inflammatory cytokines, which contribute to local inflammation and the growth of the plaque [8].

While the cascade of events in the above inflammatory response is well organized, the structural link between the various players, in general, and between the oxidized LDL and the inflammation markers, namely the T-cells, has not been observed, let alone elucidated. In the current project / report, an exposed amino acid stretch in the C-terminus of

 ISBN: 978-1-61208-359-9

the first 17% amino-terminal end of apo B-100 was found to adopt the structure of that of the T-cell receptor binding domain. This constitutes the first evidence of a structural link between atherosclerosis-causing LDL and inflammation.

After establishing such a link "*in silico*," we anticipate that *in vitro* experimentations take place, probing the cytokines – and possibly interleukins – involved, then followed by *in vivo* tests to determine the real physiological effects correlated with such a link vis-à-vis the roles of the different involved factors.

REFERENCES

[1] S. Perez, "Thinking intelligently about therapy of atherosclerosis.," American Journal of Therapeutics, vol. 10, 2003, pp. 429-437.

[2] S. Yla-Herttuala, W. Palinski, M. Rosenfeld, S. Parthasarathy, T. Carew, S. Butler, J. Witztum, and D. Steinberg, "Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man," Journal of Clinical Investigation, vol. 84, no. 4, 1989, pp. 1086-1095.

[3] S. Yla-Herttuala, W. Palinski, M. Rosenfeld, D. Steinberg, and J. Witztum, "European heart journal," Lipoproteins in normal and atherosclerotic aorta, vol. (Suppl E), no. 4, 1990, pp. 88-99.

[4] R. A. Archbold and A. Timmis, "Modification of coronary artery disease progression by cholesterol-lowering therapy: the angiographic studies," Current Opinion in Lipidology, vol. 10, 1999, pp. 527-534.

[5] K. Bach-Ngohou, H. Nazih, F. Nazih-Sanderson, Y. Zair, D. L. Carrer, M. Krempf, and J. Bard, "Negative and independent influence of apolipoprotein E on C-reactive protein (CRP) concentration in obese adults. Potential anti-inflammatory role of apoE in vivo," International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, vol. 25, 2001, pp. 1752-1758.

[6] J. Hulthe and B. Fagerberg, "Circulating oxidized LDL is associated with increased levels of cell-adhesion molecules in clinically healthy 58-year old men (AIR study).," Medical Science Monitor, vol. 8, 2002, pp. CRI 48-52.

[7] V. Titov, "The functional role of arterial intima. Endogenous and exogenous pathogens and specificity of atheromatosis as an inflammation," Klinicheskaia Laboratonaia Diagnostika, 2003, pp. 23-24.

[8] G.K. Hansson, A.K. Robertson, and C. Söderberg-Nauclér, "Inflammation and atherosclerosis," Annual Review of Pathology, vol. 1, 2006, pp. 297-329.

[9] R. Mahley and B. Angelin, "Type III hyperlipoproteinemia: recent insights into the genetic defect of familial dysbetalipoproteinemia," Advances in Internal Medicine, vol. 29, 1984, pp. 385-411.

[10] M. A. Mitsche, L. Wang, Z. G. Jiang, C. J. McKnight, and D. M. Small, "Interfacial Properties of a Complex Multi-Domain 490 Amino Acid Peptide Derived from Apolipoprotein B," Langmuir, 2009, pp. 2322-2330.

[11] H. Al-Ali and H. Khachfe, "The N-Terminal Domain of Apolipoprotein B-100: Structural Characterization by Homology Modeling," BMC Biochemistry, 22 July 2007.

[12] C. Claradas, R. Nolte, D. Atkinson, V. Zannis, and C. M. Hadzopoulou-Claradas, "The Complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48 forms," EMBO Journal, vol. 5, 1986, pp. 3495-3507.

[13] C. Y. Yang, Z. Gu, S. A. Weng, T. W. kim, S. H. Chen, H. J. Pownall, P. Sharp, S. W. Liu, W. H. Li, and A. M. Gotto Jr, "Structure of apolipoprotein B-100 of human low density lipoproteins," Arteriosclerosis, vol. 9, 1989, pp. 96-108.

[14] H. M. Khachfe and D. Atkinson, "Expression, purification, and quantification of the 17% N-terminal domain of apolipoprotein b-100," Journal of Cell and Molecular Biology 9(2), 2011, pp. 37-42.

[15] H. M. Khachfe and D. Atkinson, "Confirmation and stability properties of B17-I: analytical investigations using circular dichroism," Eur Biophys J, 25 July 2012.

[16] M. T. Walsh and D. Atkinson, "Calorimetric and spectroscopic investigation of the unfolding of human apolipoprotein B," Journal of lipid research, vol. 31, 1990, pp. 1051-1062.

[17] R. T. Nolte, "Structural Analysis of the human apolipoproteins: an integrated approach utilizing physical and computational methods," In PhD Dissertation, Boston University, Department of Biophysics, 1994.

[18] J. Segrest, D. W. Garber, C. Brouillette, S. Harvey, and G. Anantharamaiah, "The amphipathic alpha helix: a multifunctional structural motif in plasma apolipoproteins," Advances in protein Chemistry, vol. 45, 1994, pp. 303-369.

[19] J. P. Segrest, M. K. Jones, V. K. Mishra, V. Pierotti, S. Young, J. Boren, T. Innerarity, and N. Dashti, "Apolipoprotein B-100: conservation of lipid-associating amphipathic secondary structural motifs in nine species of vertebrates," Journal of lipid research, vol. 39, 1998, pp. 85-102.

[20] J. Segrest, M. Jones, H. De Loof, and N. Dashti, "Structure of apolipoprotein B-100 in low density lipoproteins," Journal of lipid research, vol. 42, 2001, pp. 1346-1367.

[21] H. khachfe and D. Atkinson, "Confirmation and stability properties of B17-II: analytical investigations using differential scanning calorimetry," Eur Biophys J, vol 42, 2013, pp. 309-314.

[22] M. Walsh and D. Atkinson, "Physical properties of apoprotein B in mixed micelles with soduim deoxycholate and in a vesicle with dimyristoyl phosphatidylcholine," Journal of lipid research, vol. 27, 1986, pp. 316-325.

[23] C. Cladaras, M. Hadzopoulou-Cladaras, H. Herscovitz, M. T. Walsh, V. Zannis, and D. Small, "Expression, secretion and lipid-binding characterization of the N-terminal 17% of apolipoprotein B," Proceedings of the National Academy of sciences of the United States of America, vol. 88, 15 October 1991, pp. 7313-7317.

[24] H. Herscovitz, A. kritis, I. Talianidis, E. Zanni, V. Zannis, and D. Small, "Murine mammary-derved cells secrete the N-

 ISBN: 978-1-61208-359-9

terminal 41% of human apolipoprotein B on high density lipoprotein-sized lipoproteins containing a triacylglycerol-rich core," Proceedings of the National Academy of Sciences of the United States of America, vol. 92, 1995, pp. 659-663.

[25] H. M. Khachfe, "Spectroscopic and Calorimetric Studies of the 17% N-terminal domain of apolipoprotein B-100," In PhD Dissertation, Boston University, Department of Biophysics, 2002.

[26] G. S. Shelness and J. T. Thornburg, "Role of Intramolecular disulfide bond formation in the assembly and secretion of apolipoprotein B-100-containing lipoproteins," Journal of lipid research, vol. 37, 1996, pp. 408-419.

[27] C. Y. Yang, T. Kim, S. A. Weng, B. R. Lee, M. Yang, and A. M. Gotto, "Isolation and Characterization of sulfhydryl and disulfide peptides of human apolipoprotein B-100," Proceedings of the National Academy of Sciences of the United States of America, vol. 87, 1990, pp. 5523-5527.

[28] P. Y.Chou and G.D. Fasman, "Prediction of protein conformation," Biochemistry, 13(2), 1974, pp. 222-245.

[29] B. Rost and C. Sander, "PROF," J Mol Biol, 232, 1993, pp. 584-599.

[30] B. Rost, P. Fariselli, and R. Casadio, "PROFhtm," Prot Science, 7, 1996, pp. 1704-1718.

[31] Swiss-PDB viewer [http://www.expasy.org/spdbv]

[32] B.R. Brooks et al., "CHARMm: A program for macromolecular energy, minimization, and Dynamics Calculations," J Comp Chem, 4, 1983, pp. 187-217

 ISBN: 978-1-61208-359-9