

RESEARCH INTERESTS

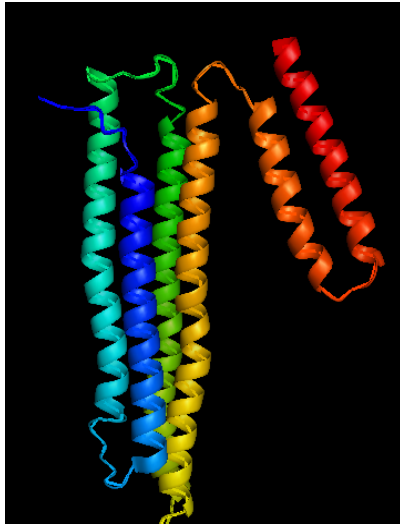
Cardiovascular disease (CVD) is the number one cause of death in American men and women. One of the major factors in heart disease is blood cholesterol, which is carried by lipoproteins. One of the most commonly used risk factors for heart disease is the serum level of HDL (high density lipoprotein) and LDL (low density lipoprotein). High HDL and low LDL levels are correlated with reduced risk for heart disease. HDL lowers the risk for CVD because it elicits the removal of cholesterol from cells in peripheral tissues, a process called reverse cholesterol transport.¹ This process is facilitated by the major protein in HDL, apolipoprotein A-I (apo A-I). In my laboratory, we are interested in the structure-function relationships of apolipoprotein A-I (apo A-I). In addition to its lipid-binding functions in HDL formation and cholesterol transport, apo A-I has also been shown to possess a number of other properties. For example, it plays a role in reducing the inflammation associated with the development of CVD.² As apo A-I circulates throughout the serum (either in a lipid-free or one of many lipid-bound forms), it also interacts with a number of cellular membrane proteins and other proteins in serum. Although the interest in apo A-I is very high and it has been studied for many years, the specific mechanisms by which apo A-I carries out its multiple functions and its interaction with other proteins are still rather poorly understood.

A hallmark feature of all apolipoproteins is the amphipathic helix. This motif allows the protein to bind to lipid surfaces.³ Because the protein must bind to lipid surfaces as part of its function, apo A-I in the absence of lipid is not like typical globular proteins. It is more flexible and has a lower stability than most globular proteins.^{4,5} In spite of this, it has a characteristic three dimensional shape as demonstrated by a recent crystal structure of the full-length protein⁶ (Figure 1A). The lipid-free protein has a helix bundle composing the amino-terminal two-thirds of the protein. The crystal structure also shows a carboxy-terminal helical hairpin. Since the crystal structure is at least 25% more helical than apo A-I in solution,^{7,8} crystallization of the protein induces a significant amount of helix. A variety of measures suggest that the carboxy-terminus is less helical in solution than in the crystal structure.⁹⁻¹² Furthermore, the lipid-free protein in solution possesses about 10% β -sheet, most likely occurring in both the amino- and carboxy-termini.^{11,13,14}

A computer model of the lipid-bound structure of apo A-I,¹⁵ developed by Jere Segrest's group at University of Alabama, Birmingham is shown in Figure 1B. This

model has been largely verified by a number of experimental methods.¹⁶⁻¹⁹ The amphipathic helices bind to the surface of the disc so that the helix axis is perpendicular to the acyl chains of the lipid. Since lipid-bound apo A-I particles come in a variety of shapes and sizes, apo A-I must undergo conformational changes to accommodate these differences.

A.



B.

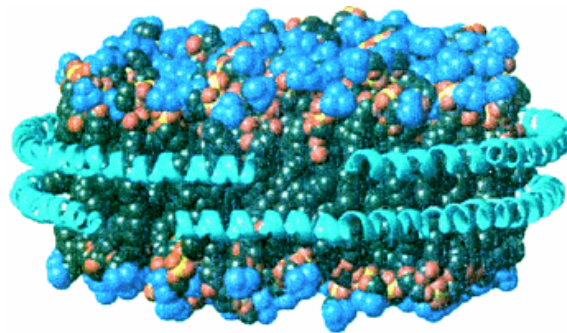


Figure 1. **A.** Crystal structure⁶ of lipid-free apo A-I showing the amino-terminal four-helix bundle and the carboxy-terminal helical hairpin; the carboxy-terminus in solution probably has less helix than found in the crystal structure. **B.** Computer model¹⁵ of lipid-bound apo A-I on synthetic HDL discs. Note the arrangement of helices relative to the lipid acyl chains.

Projects in the lab

There are two main projects involving apo A-I structure in my lab.

Conservation of structural elements.

Although human apo A-I has been heavily studied for over 30 years, very little is known about apo A-I from other organisms. We have been exploring the structural properties of apo A-I from species either closely or distantly related to homo sapiens in order to understand what features of the protein are conserved. For example, we are exploring the role of the amino-terminus in maintaining the helix bundle structure of the protein in the absence of lipid through comparison of

amino-terminal deletion mutants prepared from human and zebrafish apo A-I. Several studies have shown that the lack of residues 1-43 in human apo A-I results in opening of the helix bundle and self-association of apo A-I molecules into the ring structure characteristic of lipid-bound protein, even though lipid is absent.^{20,21} We are examining this and smaller deletions in both human and zebrafish apo A-I to determine precisely which residues are required to maintain the structure of the lipid-free state. In other words, we are looking for the residues that act as a conformational switch between lipid-free (helix bundle) and lipid-bound (ring) protein structures. We are also examining the properties of dog and salmon apo A-I isolated from plasma. The salmon apo A-I project is part of a larger project involving the development of lipoproteins as biological markers for monitoring the health of salmon populations on the American River.

Amyloidogenic apo A-I

We are currently collaborating with the laboratory of Dr. John Voss at UC Davis to understand what mechanisms are responsible for the formation of amyloids by certain apo A-I mutants.²² A variety of amyloid diseases are known, including Alzheimer's and the spongiform encephalopathies. Apo A-I also forms amyloids, with deposits being found in kidney, heart, skin and elsewhere in the body, depending on the mutation. There is almost nothing known about the structures of amyloidogenic apo A-I variants or the mechanisms of amyloid formation. We have been using limited proteolysis in conjunction with a number of methods employed by the Voss laboratory (in particular, site-directed spin-label EPR spectroscopy) to explore the structures of amyloidogenic apo A-I variants.

Methodologies

Students working in my lab gain experience in the following:

- Cloning
- Bacterial cell growth and expression of recombinant proteins
- Protein purification, including a variety of affinity and non-affinity chromatographies
- Structural analysis employing a variety of spectroscopies as well as limited proteolysis. Through a collaboration recently established through my sabbatical in the laboratory of Dr. David Wilson at UC Davis, we also now have the opportunity to try to grow crystals of the proteins we produce.
- Lipid-binding analyses

These techniques provide very good experience for students planning to attend PhD programs or for students desiring jobs in the biotechnology industry.

Student participation

A large number of Sac State students have worked on apo A-I in my laboratory. Many of these students have gone on to PhD programs, jobs in industry, or medical school. Students interested in participating in this research should contact me in person (check my current office hours). I welcome master's students as well as undergraduates at all levels. I also work with students from both the Chemistry and Biological Sciences departments. Since the training students receive in my lab requires a lot of my time, I also require a one-year commitment so that sufficient progress can be made for a research presentation at a regional or national meeting.

References

1. Glomset, J.A. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9**: 155-167, 1968.
2. Nicholls, S.J., et al., Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normo-cholesterolemic rabbits. *Circulation* **111**(12): p. 1543-503, 2005.
3. Segrest, J.P., Jackson, R.L., Morrisett, J.D., Gotto, Jr, A.M. A molecular theory of lipid-protein interactions in the plasma lipoproteins. *FEBS Lett.* **38**: 247-258, 1974.
4. Davidson, W.S., Silva, R.A. Apolipoprotein structural organization in high density lipoproteins: belts, bundles, hinges and hairpins. *Curr Opin Lipidol.* **16**, 295-300. 2005.
5. Edelstein, C., Scanu, M. Effect of guanidine hydrochloride on the hydrodynamic and thermodynamic properties of human apolipoprotein A-I in solution. *J Biol Chem.* **255**, 5747-54, 1980.
6. Ajees, A.A., Anantharamaiah, G.M., Mishra, V.K., Hussain, M.M., Murthy, H.M. Crystal structure of human apolipoprotein A-I: insights into its protective effect against cardiovascular diseases. *Proc Natl Acad Sci U S A*, . **103**, 2126-31, 2006.
7. Rogers, D.P., Roberts, L.M., Lebowitz, J., Engler, J.A., Brouillette, C.G. Structural analysis of apolipoprotein A-I: effects of amino- and carboxy-terminal deletions on the lipid-free structure. *Biochemistry* **37**, 945-55, 1998.
8. Roberts, L.M., Ray, M.J., Shih, T.W., Hayden, E., Reader, M.M., Brouillette, C.G., Structural analysis of apolipoprotein A-I: limited proteolysis of methionine- reduced and oxidized lipid-free and lipid-bound human apo A-I. *Biochemistry* **36**, 7615-7624, 1997.
9. Fang, Y., Gursky, O., Atkinson, D. Lipid-binding studies of human apolipoprotein A-I and its terminally truncated mutants. *Biochemistry* **42**, 13260-13269, 2003.

10. Gorshkova, I., Liu, T., Kan, H.Y., Chroni, A., Zannis, V.I., Atkinson, D. Structure and stability of apolipoprotein a-I in solution and in discoidal high-density lipoprotein probed by double charge ablation and deletion mutation. *Biochemistry* **45**, 1242-54, 2006.
11. Oda, M.N., Hargreaves, P.L., Beckstead, J.A., Redmond, K.A., van Antwerpen, R., Ryan, R.O. The C-terminal domain of apolipoprotein A-I contains a lipid-sensitive conformational trigger. *Nat Struct Biol.* **10**, 455-60, 2003.
12. Silva, R.A., Hilliard, G.M., Fang, J., Macha, S., Davidson, W.S. A three-dimensional molecular model of lipid-free apolipoprotein A-I determined by cross-linking/mass spectrometry and sequence threading. *Biochemistry* **44**, 2759-69, 2005.
13. Nolte, R.T., Atkinson, D. Conformational analysis of apolipoprotein A-I and E-3 based on primary sequence and circular dichroism. *Biophys. J.* **63**, 1221-1239, 1992.
14. Lagerstedt, J.O., Budamagunta, N.S., Oda, M.N., Voss, J.C., EPR spectroscopy of site-directed spin labels reveals the structural heterogeneity in the N-terminal domain of apo-AI in solution. *J Biol Chem*, 2007.
15. Segrest, J.P., Jones, M.K., Klom, A.E., Sheldahl, C. J., Hellinger, M, Deloof, H, Harvey, S.C. A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein. *J. Biol. Chem.* **274**, 31755-31758, 1999.
16. Koppaka, V., Silvestro, L., Engler, J.A., Brouillette, C.G., Axelsen, P.H., The Structure of Human apolipoprotein A-I. Evidence for the "belt" model. *J. Biol. Chem.* **274**, 14541-14544, 1999.
17. Maiorano, J.N., Davidson, W.S. The orientation of helix 4 in apolipoprotein A-I-containing reconstituted high density lipoproteins. *J. Biol. Chem.* **275**, 17374-17380, 2000.
18. Li, H.H., Lyles, D.S., Thomas, M.J., Pan, W., Sorci-Thomas, M.G. Structural determination of lipid-bound apo A-I using fluorescence resonance energy transfer. *J.Biol. Chem.* **275**, 37048-37054, 2000.
19. Panagotopoulos, S.E., Horace, E.M., Maiorano, J.N., Davidson, W.S., Apolipoprotein A-I adopts a belt-like orientation in reconstituted high density lipoproteins. *J. Biol. Chem.* **276**, 42965-42970, 2001.
20. Rogers, D.P., Brouillette, C.G., Engler, J.A., Tendian, S.W., Roberts, L.M., Mishra, V.K., Anantharamaiah, G.M., Lund-Katz, S., Phillips, M.C., Ray, M.J. Truncation of the amino-terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. *Biochemistry* **36**, 288-300, 1997.
21. Borhani, D.W., Rogers, D.P., Engler, J.A., Brouillette, C.G. Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. *Proc. Natl. Acad. Sci., USA* **94**, 12291-12296, 1997.
22. Joy T, Wang J, Hahn A, Hegele RA APOA1 related amyloidosis: a case report and literature review. *Clin Biochem* **36**: 641-645, 2003.