commentary

Maturation of apolipoprotein A-I: unrecognized health benefit or a forgotten rudiment?[1]

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Apolipoprotein A-I (apoA-I) is the principal protein of high density lipoprotein and is most likely responsible for many, if not all, of HDL's antiatherogenic properties. In humans, apoA-I is synthesized in hepatocytes and enterocytes as a prepro-protein. The 18 amino acid pre-peptide is cleaved prior to secretion and pro-apoA-I is secreted and processed into mature protein in blood or lymph; the understanding of how and why this happens is surprisingly poor. It was originally found that a metalloprotease is responsible for the cleavage of the six amino acid pro-peptide (1) and the cleavage site on apoA-I has been characterized (2). More recently, the cleavage enzyme was identified as bone morphogenic protein-1 (BMP-1) (3). In this issue of the *Journal of Lipid Research*, Zhu et al. (4) further unravel a complex mechanism of apoA-I maturation and also provide clues as to why the pro-peptide needs to be cleaved.

In a series of carefully crafted biochemical experiments, Zhu et al. (4) demonstrated that, in addition to BMP-1, there is another player in the process of apoA-I maturation, procollagen C-proteinase enhancer-2 (PCPE2). PCPE2 is not a protease, but was found to have a homology with cubulin, a protein once considered an HDL receptor and capable of binding apoA-I, suggesting that PCPE2 may bind apoA-I as well. Yes, PCPE2 was able to bind apoA-I, but no, it couldn't cleave the pro-peptide out of it. Instead, it did what it does best, greatly enhancing the cleavage of apoA-I by BMP-1.

Zhu et al. (4) then conducted an elegant set of experiments to establish the molecular mechanisms of interaction of apoA-I, BMP-1, and PCPE2. They demonstrated that BMP-1 binds to pro-apoA-I first, followed by PCPE2. Formation of the ternary complex in this order stabilizes the complex and doubles the rate of pro-peptide cleavage. However, PCPE2 is also capable of binding apoA-I in the absence of BMP-1. When PCPE2 binds pro-apoA-I before BMP-1, it hinders binding of BMP-1 and cleavage of pro-peptide; thus, it is a negative regulator. It is unclear under what circumstances PCPE2 switches from positive to negative regulator. It is also unclear whether BMP-1 and PCPE2 dissociate from apoA-I after the pro-peptide is cleaved. PCPE2 was found in mature HDL particles, indicating that either it remains associated with apoA-I after its maturation or maturation occurs in HDL particles. This also raises an intriguing possibility that PCPE2 has other functions and that its binding to pro-apoA-I is required to promote incorporation of PCPE2 into the HDL particle rather than cleave the pro-peptide.

An unanswered question, however, is why. Why does the pro-peptide have to be cleaved? Is there a biological purpose in this? Generally, maturation of proteins is a feature of secreted proteins allowing for safe delivery of inactive protein out of cells to their extracellular destination where they are unblocked, becoming functional where and when their action is required. A classic example is a protease that would do harm if it became active prematurely. One function of apoA-I that we are sure of is that of binding lipids. It is reasonable to assume that it would be unfortunate if apoA-I binds to lipids while still inside the cell. This may impair secretion of apoA-I and there is evidence suggesting that secretion of mature apoA-I is less effective. When expressed as a heterologous protein, up to 70% of mature apoA-I was retained inside the cells (5) compared with 90% of pro-protein secreted even when the pro-peptide was artificially positioned in the middle of the molecule (6). Further, there is emerging evidence that lipidation of apoA-I during formation of HDL must follow a strict sequence of operations sequentially performed by various ABC transporters that are functioning on the outer surface of the cells (7). Nonspecific lipidation of apoA-I (as happens, e.g, in Tangier disease patients) does not result in formation of "proper" HDL. The possibility that pro-apoA-I has impaired capacity to bind lipids was investigated by Chau et al. (3) and our group (8). Both groups demonstrated that the efflux of cholesterol and phospholipids to pro-apoA-I is about half that of mature apoA-I in a head-to-head comparison.

Whereas the above considerations provide some indirect clues to why the pro-peptide might be needed, they say little about why it has to be cleaved. One possibility mentioned above is that pro-apoA-I has reduced capacity to

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support cholesterol efflux and may not form proper HDL. Kono et al. (9) have demonstrated that the N-terminal domain in apoA-I is capable of dissociation from apoA-I/phospholipid complexes when the surface concentration of apoA-I increases, a property that may be important in HDL remodeling and potentially affected by pro-peptide. However, the differences in cholesterol efflux between pro- and mature forms of apoA-I hardly constitute a complete block of lipid binding. Pro-apoA-I is still capable of binding lipids and supporting cholesterol efflux and, when even minimally lipidated, all the differences between the mature and pro-form disappear (8). There is no evidence that any other reverse cholesterol transport-related property of HDL is directly affected by the pro-peptide. Could it be that cleavage of pro-peptide of apoA-I is a rudimentary process retained needlessly through evolution because it does no harm? Mammalian apoA-I evolved from apolipoprotein E after the divergence of the tetrapod and teleost (10). Its pro-peptide has an unusual structure and the cleavage site may be an evolutionary by-product without a biological function. Zhu et al. (4) approached this issue from a different perspective; if maturation of apoA-I is a prerequisite for HDL formation, there must be a connection between polymorphism in the PCPE2 gene and HDL plasma levels. Upon investigation of three populations, only a tentative connection was found between PCPE2 polymorphism and HDL levels. It is important to recognize that polymorphism of very few genes shows a consistent association with HDL levels in genetic studies, with *CETP*, *LIPC*. and *ABCA1* having the best credentials. A weak genetic association between HDL level and PCPE2 does not rule out that apoA-I maturation contributes to HDL level, but neither does it provide a confirmation.

The antiatherogenic capacity of HDL, however, is not fully determined by its level, nor is reverse cholesterol transport the only function of HDL. Even if pro-peptide cleavage is not required for HDL formation and cholesterol efflux, it may be required for other functions of HDL, such as antiinflammatory, antioxidation, and antithrombotic properties. Not much is known about mechanisms of HDL involvement in these processes, but it is conceivable that protein-protein interactions requiring specific amino acid sequences leading to signal transduction cascades are involved. Vaughan et al. (11) have recently demonstrated activation of Janus kinase 2 resulting in interaction of apoA-I with ABCA1. The N-terminus is the most conserved region of apoA-I (12); is that because the region is involved in an important function? Would pro-peptide interfere with this function? Would pro-peptide affect properties of apoA-I and HDL other than binding of lipids? If so, how important is this level of regulation of HDL function in determining its antiatherogenic capacity? The findings of Zhu et al. (4) illustrate the complexity of the mechanism of apoA-I maturation; now we need to find a reason.

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