Proteolytic events affecting plasma apolipoproteins at the co- and post-translational levels and after maturation

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The application[1] of the techniques of molecular and cell biology to the study of plasma lipoproteins is rapidly yielding a better understanding of the biosynthetic steps involving the plasma apolipoproteins and of the attending intra- and extra-cellular proteolytic events leading to the mature products. Additional studies have indicated that some of the mature apolipoproteins may themselves be subject to proteolytic cleavage with the generation of discrete fragments amenable to structural and functional studies. Many of the data obtained thus far have originated from studies in organ or cell cultures; thus, in most cases, their physiological meaning remains to be determined although it has become apparent that proteolytic events play a role in the intra- and extra-cellular remodeling of plasma apolipoproteins. In this review we have provided a summary of the current advances made in this field and whenever applicable we have identified the potential physiological importance of these observations.

A. PROTEOLYTIC ENZYMES INVOLVED IN THE CO- AND POST-TRANSLATIONAL PROCESSING OF PLASMA APOLIPOPROTEINS

ApoA-I

The early experiments by Lin-Su et al. (1) and by Stoffel, Blobel, and Walter (2) in the rat and the studies by Zannis et al. using organ cultures of human adult (3) and fetal intestine (4), liver (4), and hepato-cellular carcinoma cell lines Hep G2 and Hep 3B (5), led to the conclusion that apolipoprotein A-I (apoA-I) is synthesized with a signal sequence of 18 amino acid residues and secreted in a precursor form which is further modified post-translationally into the mature product found in the circulation. The subsequent studies of Gordon et al. (6, 7) clarified that the primary translation product of rat and human apoA-I mRNA is a preproprotein which is processed co- and post-translationally to the mature form (see ref. 8 and **Fig. 1**). These studies now corroborated by cDNA sequence data (9–12) have shown that the amino terminal extension of human or rat apoA-I is 24 amino acids long, containing an 18 amino acid presegment and a hexapeptide prosegment. The prepeptide, also referred to as a signal peptide, is removed during the translocation of the nascent peptide chain across the lipid bilayer of the rough endoplasmic reticulum and appears to be similar in size, hydrophobicity, and primary structure to other prepeptides (13). Little information is available on the nature of the signal peptidase acting on apoA-I and other prepeptides. This may be due to the poor solubility of this enzyme in aqueous media and/or the lack of a suitable assay for it. However, since the peptidase present in canine pancreatic microsomes correctly cleaves prepeptides from various animal species (13), this may be taken to suggest that a single enzyme is involved. A characteristic of this signal peptidase is that it is an integral membrane protein which requires detergent for its unmasking. When the hydrophobicity of the central region of the signal sequence is decreased, e.g., by replacing leucine with β-hydroxyleucine, translocation and processing of the growing polypeptide chain is inhibited (14). Based on this evidence and on the sequence and secondary structure of the signal peptides cleaved, it is believed that the enzyme may exhibit specificity for the conformational state of the substrate.

The cleavage of the presegment of preproapoA-I

Abbreviations: HDL, high density lipoproteins, d 1.063–1.21 g/ml; LDL, low density lipoproteins, d 1.006–1.063 g/ml; VLDL, very low density lipoproteins, d < 1.006 g/ml; HTG, hypertriglyceridemia; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; PMN, polymorphonuclear cells; PMSF, phenylmethylsulfonylfluoride; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; DMPC, dimyristoylphosphatidylcholine; AA, tissue amyloid A protein; SAA, serum component related to amyloid A protein.

[1] The literature search for this review was completed on July 31, 1984.

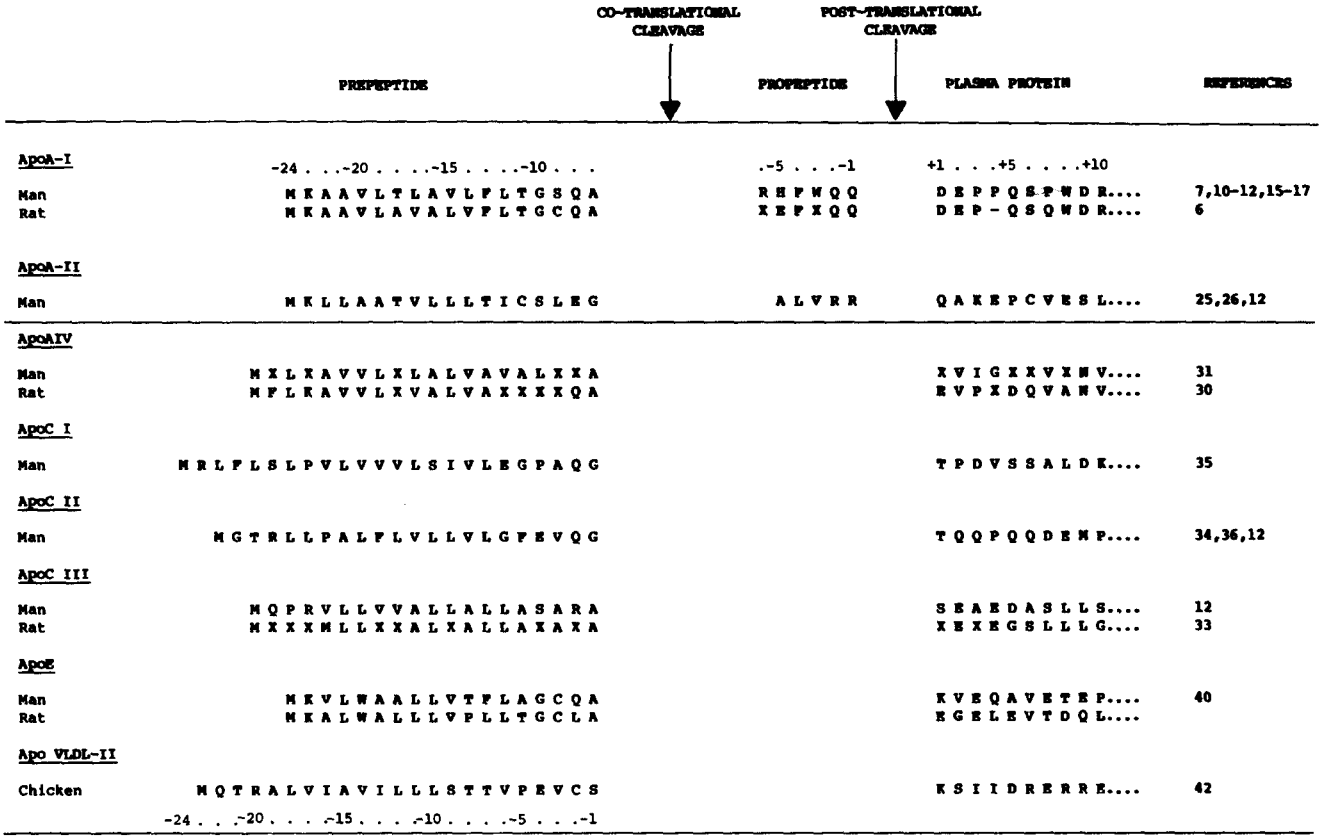

Fig. 1. Amino acid sequences of the pre- and propeptides of the various plasma apolipoproteins. The arrows indicate the sites of co- and post-translational cleavage. Only the first ten amino acids are presented for the mature plasma protein. (X) indicates an unidentified amino acid. (——) indicates a deletion applied to obtain alignment of the sequences of man and rat. The single letter notation is used for the amino acids and is defined as: D, aspartic; N, asparagine; T, threonine; S, serine; P, proline; E, glutamic; Q, glutamine; G, glycine; A, alanine; V, valine; C, cysteine; M, methionine; I, isoleucine; L, leucine; Y, tyrosine; F, phenylalanine; K, lysine; H, histidine; R, arginine; W, tryptophan.

generates a proprotein which differs from its mature form by the presence of a hexapeptide amino terminal extension which both in the rat and human subjects contains a pair of glutamines at the carboxyl terminal (6–8, 15–19). The studies by Gordon et al. (6, 7) have shown that proapoA-I is secreted uncleaved from HepG2 and intestinal cells and undergoes proteolytic processing to the mature product extracellularly at an unusual gln-asp cleavage site (**Table 1**). The original work conducted in the authors' laboratory (18) and later corroborated by other studies (19, 20) has shown that the enzyme responsible for this cleavage is present in the circulation, is metal-dependent, is insensitive to phenylmethylsulfonylfluoride (PMSF) and other serine-protease inhibitors, and in the normolipidemic state is mainly associated with high density lipoproteins (HDL). The extracellular processing that this enzyme effects in vitro is on the order of hours (18, 19) perhaps due to suboptimal assay conditions and/or enzyme lability. This would be consistent with the observation that in vivo the conversion of human proapoA-I to its mature form occurs comparatively more rapidly than in vitro (21). The rate of proapoA-I to apoA-I conversion may be one of the determinants in maintaining steady state levels of proapoA-I in the plasma. In man, the proportion of proapoA-I in plasma is less than 2% (22) of the total apoA-I mass; in the rat, the proportion is about 29% (19). However, these differences may also reflect the dissimilar half-lives of apoA-I in man, 5.04 days (23) and the rat, 10 hours (24).

ApoA-II

ApoA-II, like apoA-I, is synthesized as a preproapolipoprotein (25). The signal peptide exhibits a typical 18 amino acid sequence with glycine at the carboxyl terminal position (12, 26; see Fig. 1) and is cleaved intracellularly by a signal peptidase to generate a monomeric proprotein (25, 27), which after secretion from the cell contains a pentapeptide prosegment with a pair of arginines at the carboxyl end (Fig. 1). The data by Stoffel, Kruger, and Deutzmann (15) have instead indicated that proapoA-II contains a hexapeptide prosegment with a glutamine in the carboxyl terminal position. However, recent cDNA sequence data (12, 26) support

TABLE 1. Sites of cleavage in the proteolytic processing of apolipoproteins

Apoliprotein	Bond Cleaved	Site of Cleavage: Intracellular	Site of Cleavage: Extracellular	Product	References
PreproapoA-I					
Man	ala-arg	Intestine		proapoA-I	7, 10–12, 15, 16
Rat	ala-X[a]		Liver		6
ProapoA-I					
Man	gln-asp		Plasma	apoA-I	7, 10–12, 15–17
Rat	gln-asp		Plasma	apoA-I	6, 19
PreproapoA-II					
Man	gly-ala		Liver Intestine	proapoA-II	12, 25, 26
ProapoA-II					
Man	arg-gln		Plasma	apoA-II	12, 25–27
PreapoA-IV					
Man	ala-X	Intestine		apoA-IV	31
Rat	ala-glu	Intestine		apoA-IV	30
PreapoC-I					
man	gly-thr	Liver		apoC-I	35
PreapoC-II					
Man	gly-thr	Liver		apoC-II	12, 34, 36
PreapoC-III					
Man	ala-Ser	Liver		apoC-III	12
Rat	ala-X	Liver and Intestine		apoC-III	33
PreapoE					
Man	ala-Lys	Liver		apoE	40
Rat	ala-glu	Liver		apoE	39
Proapo VLDL-II					
Chicken	ser-lys	Liver		apo VLDL-II	42

[a] X denotes an unidentified amino acid.

the findings by Gordon et al. (25, 27). The penultimate arg-arg residues appear to be typical of other propeptide sequences although there is a striking difference in the site of cleavage between the processing of proapoA-II and that of other propeptides. ProapoA-II is cleaved extracellularly at the arg-gln bond (Table 1); in contrast, most prohormones and proalbumin (28) undergo intracellular cleavage after the paired carboxyl terminal basic residues. In the Hep G2 cells, the extracellular processing of the apoA-II propeptide was initially reported to be only 45% efficient (25). However, recent in vitro pulse chase experiments in the same cell system indicate that the proteolytic cleavage of the pentapeptide extension occurs essentially all extracellularly (27). The enzyme involved in this process has been identified tentatively as a thiol protease which is secreted into the extracellular medium (27). ProapoA-II has not been observed in plasma HDL (27), although a considerable polymorphism of this apolipoprotein has been reported (29). Similarly, no report has appeared on the presence of proapoA-II converting enzyme in plasma. Thus, the in vivo compartment where proapoA-II is converted to mature apoA-II remains to be established.

ApoA-IV

Proteolytic processing of human and rat intestinal apoA-IV proceeds co-translationally with the release of the mature product (30, 31). The primary translation product is preapoA-IV which contains a 20 amino acid presegment. Both the rat and human presegments are cleaved intracellularly by a signal peptidase. The sequence of this signal peptide resembles other known signal peptides (Fig. 1) except that, in the rat, the initial methionine is followed by phenylalanine, an aromatic residue not previously reported at this site (28). For the human counterpart, the amino acid residue for this position is still undetermined. ApoA-IV does not have a propeptide segment, thus it differs from apoA-I and apoA-II.

ApoB

Information on the biosynthesis and proteolytic processing of apoB is still scanty. Recently, Olofsson et al.

(32) isolated a protein from human liver believed to represent the primary translation product of apoB. In vitro synthesis using RNA from human liver produced a polypeptide with an estimated molecular weight of 80,000, immunologically related to a 75kDa protein isolated from low density lipoproteins (LDL). These interesting studies await further developments.

ApoC

The results reported thus far show that the C peptides are synthesized with signal peptide presegments which are co-translationally cleaved by a signal peptidase to yield the mature product (12, 33–36). The signal peptide sequences of the C peptides represented in Fig. 1 resemble those observed in the presegments of other apolipoproteins and secretory proteins in general. One exception is the penultimate arginine residue of the signal peptide of apoC-III. A positively charged amino acid in this position is uncommon but has been reported for the pre-IgG light chain as well as for the bovine preproparathyroid hormone (37). The amino terminal sequence of mature apoC-II has a close resemblance to the propeptide sequence of proapoA-I. In particular, residues 5,6, and 7 of apoC-II are identical to residues −2,−1, and +1 of proapoA-I (Fig. 1). This similarity suggests that apoC-II has a propeptide sequence which may be slowly processed by the same or a similar metal-dependent endopeptidase which acts on proapoA-I. However, an apoC-II form lacking the first six amino terminal residues has not been documented. Nevertheless, it would be of interest to determine whether the protease which cleaves proapoA-I acts against mature apoC-II.

ApoE

The synthesis and processing of apoE has been studied in the rat (38, 39) and human liver (40). The primary translation product of apoE contains an 18 amino acid residue presegment which is cleaved co-translationally by a signal peptidase. The protein is then glycosylated, secreted, and subsequently desialylated in plasma (40). The homology between the human apoE and apoA-I signal peptide sequences (Fig. 1) appears to be more significant than that among other presequences.

Chicken apo very low density lipoproteins (apoVLDL)-II

ApoVLDL-II, a major apolipoprotein in avian VLDL (41), is synthesized in the cockerel liver with a signal peptide (23 amino acids) extension that is co-translationally cleaved (42). This is another example of a lipid binding protein whose presegment sequence is very similar to other apolipoproteins as well as to other secretory proteins.

B. PROTEOLYSIS OF MATURE APOLIPOPROTEINS

ApoA-I

In addition to the proteolytic events attending the maturation of apoA-I from its primary product of synthesis, reports of additional proteolytic modifications of the mature apolipoproteins in vitro have appeared (see **Table 2**). Lijnen and Collen (43) have studied the proteolysis of human apoA-I by plasmin. Cleavage of apoA-I resulted in the initial release of peptides of M_r = 11,000–14,000 followed by smaller ones. Moreover, apoA-I was found to competitively inhibit the hydrolysis of D-val-leu-lys-4-nitroanilide by plasmin, with a K_i of 5 μM. Interestingly, the apoA-I substrate utilized in these studies was obtained nonconventionally as a side product of α_2-antiplasmin isolation and had the same amino terminal sequence and a similar amino acid composition as apoA-I. The peptides generated from the cleavage by plasmin were not chemically identified nor was the enzyme directly tested for its affinity for HDL and capacity to hydrolyze HDL. In a recent study relating to plasmin and its affinity for lipoproteins, Gilmore and Moroz (44) have shown that approximately 20% of the normal human plasma samples analyzed contained a unique subclass of VLDL which co-chromatographed

TABLE 2. Proteolytic modifications of mature apolipoproteins

Apolipoprotein	M_r ($\times 10^{-3}$)	Protease	Bond Cleaved	Size of Fragments Produced (kDa)	References
A-I	28.0	Plasmin	unknown	11–14	43
A-II	17.4	PMN elastase	val^{18}–thr^{19}	7, 11	48
B	550.0	Kallikrein	unknown	410, 145	54
		Thrombin	unknown	385, 170	
E	34.0	Thrombin	arg^{191}–ala^{192}	12, 22	55, 56
SAA	11.5	Unknown; found in serum or recalcified plasma	unknown	3–5	60, 61
		Monocyte elastase	unknown	7–9	64–66
		PMN elastase	unknown	unknown	67

with plasminogen; however, the actual significance of this observation remains to be determined.

ApoA-II

HDL-associated apoA-II has been recently shown to undergo hydrolysis by proteases derived from human polymorphonuclear cells (PMN) (45–48), and human skin fibroblasts (49). In both instances, apoA-I in HDL was unaffected by exposure to these cells. However, when lipid-free apoHDL was tested in a similar manner, both apoA-I and apoA-II underwent extensive proteolytic degradation. Recently, Polacek et al. (47) have shown that HDL promotes the release of proteolytic activity from PMN and that the amount of enzyme secreted is dependent on the concentration of HDL in the medium. Once released from the cell, the enzyme becomes associated with HDL at physiological ionic strength but can be dissociated from the lipoprotein by either ultracentrifugation or chromatography at d 1.21 g/ml. Additional studies have shown that the PMN enzyme responsible for cleavage of apoA-II is of the elastase family (48). When HDL_3 is incubated with the enzyme, in the first phase of the enzymatic reaction, 11kDa and 7kDa fragments are generated as a consequence of the cleavage of the val^{18}–thr^{19} bond in one of the two apoA-II chains (see **Fig. 2**). By prolonging the incubation, the val^{18}–thr^{19} of the second chain is hydrolyzed leading to a total conversion of the 11 kDa components to fragments in the 7kDa and 4kDa range (48). Although the physiological significance of these in vitro studies remains to be determined, they suggest that proteases with an affinity for an amphiphilic surface may be associated with lipoproteins. Indeed, Jacob et al. (50) have reported that an elastase-like activity which hydrolyzes succinyl-$(ala)_3$-4-nitroanilide is associated with HDL in human serum. However, contrary to PMN elastase, the elastase-like enzyme was unaffected by serine protease inhibitors and was metal ion-dependent. Maeda, Kobori, and Uzawa (51) have recently observed two distinct proteolytic activities associated with human HDL. One of these activities was similar to that described by Jacob et al. (50). More work is needed before the actual role of these activities in the structure and function of HDL is established.

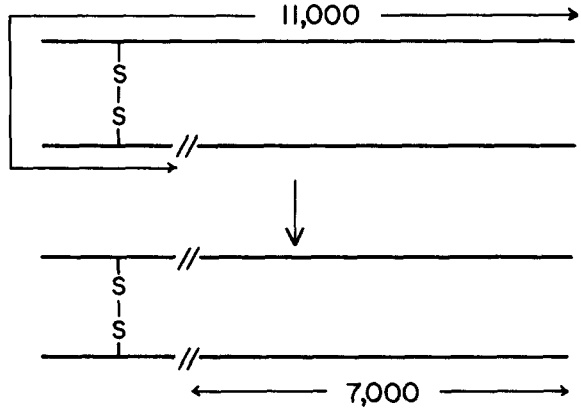

Fig. 2. Schematic diagram of the cleavage of mature apoA-II by PMN elastase. ApoA-II is represented in its disulfide-linked 2-chain form. Each identical chain has a molecular weight of approximately 8,700. The (//) represents the cleavage site at val^{18}–thr^{19}. The generation of the 7kDa and 11kDa peptides (upper panel) results from the cleavage occurring at only one chain. The cleavage at both chains (lower panel) produces two peptides of 7kDa each and one of 4kDa.

ApoB

The tendency of apoB-containing lipoproteins to undergo proteolysis has long been recognized (52, 53), but the interest in the subject has been heightened by the recent discovery of multiple molecular forms of this apolipoprotein, i.e. B-100 (M_r = 549,000), B-74 (M_r = 407,000), B-48 (M_r = 264,000), and B-26 (M_r = 144,500). Although a precursor-product relationship among these various forms has been suggested, no conclusive evidence for this concept has been obtained and a definitive conclusion may have to await further developments in the study of the primary structure of apoB. Cardin et al. (54) have recently observed that besides apoB-100, which is the major protein of human plasma LDL, some preparations contain the minor components apoB-74 and apoB-26. When apoB-100 in LDL was digested in vitro with tissue and plasma kallikrein or with thrombin and the resulting peptide fragments were analyzed by gel electrophoresis, those generated by kallikreins migrated in the position of apoB-74 and apoB-26, whereas those generated by thrombin had molecular weights of 385,000 and 170,000 unlike any molecular form of apoB so far reported (54).

ApoE

Bradley et al. (55) have shown that incubation of purified human apoE with thrombin generates fragments of 12,000 (E-12) and 22,000 (E-22) due to the cleavage of the arg^{191}–ala^{192} bond. The E-22 fragment had the same amino-terminal as intact apoE, whereas E-12 had the identical amino-terminal of a 12,000 dalton fragment isolated from the plasma VLDL of patients with hypertriglyceridemia (HTG). Although the presence of thrombin in the HTG-VLDL was not established, the preparations did contain proteolytic activity against the substrate [^{14}C]methylated-methemoglobin in the presence of the non-ionic detergent, Kyro EOB. The activity was inhibited by PMSF and was not detected in LDL or HDL by the same assay. The presence of this endogenous proteolytic activity in isolated HTG-VLDL prevented this lipoprotein from suppressing 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) in human skin fibroblasts. Moreover, Gianturco et al. (56) have shown that thrombin treatment of HTG-

VLDL at 37°C for 2 hr abolishes or severely reduces its ability to compete with the specific uptake and degradation of ^{125}I-labeled LDL or to suppress HMG-CoA reductase. Contrary to HTG-VLDL, thrombin treatment of LDL had no effect on receptor-mediated suppression of HMG-CoA reductase. The addition of intact apoE to thrombin-treated HTG-VLDL restored the suppressor capacity, whereas the addition of E-22, which is normally lost during ultracentrifugation of the modified lipoprotein, was 50% less effective than intact apoE. The addition of E-12 failed to restore this biological activity. Recently, Innerarity et al. (57) utilized thrombin treatment of apoE2 as an approach to define the molecular basis of the deficiency in receptor binding (1% of normal apoE3) of this apoE variant in which arg^{158} is replaced by cys. The amino terminal 22kDa apoE2 fragment–dimyristoylphosphatidylcholine (DMPC) complex displayed a 12-fold higher binding activity than that exhibited by the apoE2-DMPC complex and was equivalent to that of cysteamine-modified apoE2-DMPC. Moreover, the cysteamine-modified 22kDa apoE2 fragment–DMPC complex attained the level of activity of normal apoE3-DMPC. These studies suggest that the thrombin-mediated cleavage of apoE2 induced a conformational change in the apoE2 receptor binding domain and that this binding was prevented by the carboxyl-terminal third of the molecule under conditions in which a positive charge (arg) at residue 158 was absent.

Serum amyloid A (SAA)

Another example of proteolysis relating to plasma lipoproteins is that affecting SAA. The SAA protein is an acute phase reactant of M_r = 11,500 that is carried in plasma in association with HDL (58, 59). It is generally believed that SAA is the precursor of the tissue amyloid A (AA) protein (M_r = 8,500) present in secondary amyloidosis, although there is no direct evidence for a precursor-product relationship. Proteolysis of SAA to intermediates of similar size as tissue AA by several serum proteases has been reported by Skogen and Natvig (60). However, these findings appear to disagree with those reported recently by Bausserman and Herbert (61). The latter authors found that most of the SAA is initially cleaved by an unidentified serum protease, to peptides of M_r = 3,000–5,000 and eventually to smaller peptides. Those studies also suggested that the degradation of SAA does not directly involve enzymes of either the clotting, kinin, or fibrinolytic systems. It is possible that the unknown protease is activated by one of the clotting factors or calcium ions since serum or recalcified ethylenediaminetetraacetic acid (EDTA) plasma which had been allowed to clot were both capable of degrading SAA, whereas EDTA plasma alone exhibited no proteolytic activity (61). An activity which degrades tissue AA fibrils has been reported in serum (62); but a recent study by Skinner et al. (63) suggests that such a proteolytic activity is already bound to the AA type amyloid fibrils when used as a substrate in the absence of serum. This activity had the properties of PMN elastase inasmuch as it was inhibited by an elastase inhibitor, succinyl-ala-ala-pro-val-chloromethylketone, as well as by an antibody specific for PMN elastase. In addition to serum proteases, human blood monocytes have been reported to have on their surface an elastase-like protease which cleaves SAA to products in the same size range as AA (64–66). SAA is also degraded by zymosan-activated PMN or by the supernatants from the activated cells (67). As with the monocytes, an elastase-like enzymes was considered responsible for the degradation of SAA.

C. CONSIDERATIONS ON THE SIGNIFICANCE OF PROTEOLYSIS IN THE PROCESSING OF APOLIPOPROTEINS BEFORE AND AFTER MATURATION

All of the apolipoproteins studied thus far conform to the signal peptide hypothesis proposed by Blobel and Dobberstein (68) in that they contain a prepeptide which is co-translationally cleaved during translocation of the primary translation product across the rough endoplasmic reticulum. The primary sequences of these prepeptides are grossly similar to each other though not identical and more information is needed on the specificities and structural relationships of the signal peptidase(s) involved in the presegment removal. In the case of apoE, apoA-IV, and the C-peptides, the intracellular co-translational processing leads to the formation of the mature polypeptides which upon glycosylation, whenever applicable, are ready for export. On the other hand, at the time of secretion, apoA-I and apoA-II still contain a prosegment which is cleaved during and/or after secretion. The presence of a gln-gln sequence at the carboxyl end of the propeptide of proapoA-I may be responsible for the lack of intracellular cleavage, since this process is believed to require the presence of dibasic residues, i.e., arg-arg at the prosegment carboxyl-terminus as exemplified by proinsulin and proalbumin. This view is supported by the case of albumin Christ church, a variant in which the carboxyl terminal residue, arginine, of the hexapeptide prosegment is replaced by glutamine. As a result of such a substitution, the prosegment instead of undergoing the usual intracellular cleavage is secreted covalently bound to mature albumin (69). However, in the case of proapoA-II, the prosegment cleavage occurs extracellularly, even if an arg-arg is present at the carboxyl end. Thus, alternative explana-

tions for the extracellular cleavage must be provided. These may have to do with conformational requirements of the converting enzymes and/or perhaps with a need for an initial integration step of proapoA-I and proapoA-II into a lipoprotein particle. The prosegments in apoA-I and apoA-II may be viewed as necessary for the targeting of the mature polypeptide to specific sites on the plasma membrane and, in this context, each prosegment may assume the role which in glycoproteins is exhibited by carbohydrates. The enzymes that are responsible for the cleavage of the prosegment of apoA-I and apoA-II appear to exhibit different properties and are likely to have different regulatory and substrate requirements. The observation that the enzyme affecting the conversion of proapoA-I into the mature protein is metal-dependent, and is associated with lipoproteins such as HDL, VLDL and chylomicrons, points to a possible requirement for a lipoprotein surface for optimum activity. Both proapoA-I and proapoA-II are capable of integrating into the HDL particles and, in the case of proapoA-I, a capacity for activating lecithin: cholesterol acyltransferase comparable to mature apoA-I has been reported (70). The fate of the prosegment after release from each proprotein is also unknown, and the possibility that this prosegment plays a regulatory role in apolipoprotein synthesis should not be overlooked. An example is provided by the hexapeptide prosegment of proalbumin. The addition of a synthetic prosegment to the medium of isolated hepatocytes in culture or to cell-free lysates of reticulocytes or wheat germ has been reported to inhibit the synthesis of albumin, suggesting that this prosegment exhibits a feedback inhibition (71).

As we look at the information gathered on the enzymes acting on the mature apolipoproteins in plasma, the number of questions that arise is not less challenging than those posed by the processing enzymes. Of particular interest is the in vitro observation that an elastase derived from circulating PMN cleaves apoA-II at a single bond site. Presently we know little on the function(s) of apoA-II and we may speculate that such a cleavage may be important for the functional expression of this apolipoprotein; for instance, its uptake by cells. Should the elastase-mediated cleavage of apoA-II control the rate of the removal of this protein from plasma, an increment in the activity of this enzyme might explain why species such as the dog or the cow have no detectable amounts of apoA-II in the plasma. Studies are needed to assess whether the liver and other organs of these animals can synthesize apoA-II. It would also be significant to establish whether the two fragments of apoA-II, which are produced in vitro by the action of the PMN elastase, are present in the circulation.

The importance of proteolysis in apolipoprotein function is well exemplified by the specificity of action of thrombin on the apoE of HTG-VLDL and the attending loss of biological activity by this apolipoprotein, as well as by the observation that, after thrombin action, the apoE2 isoform increases its binding capacity for the apoB,E receptor. In this regard, it would be important to establish whether specific proteolytic cleavages of either LDL or Lp(a) of the type elicited by the action of kallikrein may alter the affinity of either of these two lipoprotein complexes for the apoB,E receptor and their uptake and degradation by cells.

In the case of apoSAA, the role played by proteolytic enzymes is still uncertain. In a recent study, Parks and Rudel (72) and Bausserman et al. (73) reported that the association of apoSAA with the HDL particles significantly changes the rate of disappearance of these particles from the circulation. Both reports suggest that SAA-(HDL) is catabolized faster than apoA-I(HDL), although the mechanism of SAA removal is unclear. As Bausserman et al. (73) point out, SAA may be dissociated from HDL before clearance from plasma or contained in particles with a catabolic rate different from that of the majority of HDL. In vitro apoSAA is susceptible to proteolysis, but how proteolytic fragmentation influences the behavior in vivo of this apolipoprotein remains to be established.

The presence of proteolytic enzymes associated with plasma lipoproteins has been recognized for many years. However, only recently we have become aware that some of these enzymes might be involved in the structural remodeling of apolipoproteins and the metabolism of the lipoprotein particles they are associated with. Because of the number of proteolytic enzymes present in the plasma, it should be the goal of future experiments to identify those with a specificity of action on apolipoproteins. The recent studies demonstrating that lipoproteins can effect the efflux of proteolytic enzymes from PMN isolated from human blood raise the possibility that this process may represent a general mechanism by which cellular enzymes enter the circulation. This notion raises considerations of practical importance with regard to the isolation of plasma lipoproteins. The studies by Polacek et al. (47) have shown that when the elastase released from PMN becomes associated with HDL it retains its lipoprotein association in media of low ionic strength but not in high salts. It follows that when column chromatography is used in the isolation of plasma lipoproteins in the presence of buffers of a low ionic strength, these lipoprotein preparations may be more readily contaminated by proteolytic enzymes than those obtained by the more conventional ultracentrifugal procedures which are conducted in high salt media.

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