Synthesis, transport, and processing of apolipoproteins of high density lipoproteins

Wilhelm Stoffel

Institut für Physiologische Chemie der Universität zu Köln, Joseph-Stelzmann Strasse 52, 5000 Köln 41, Federal Republic of Germany

Abstract Cell biology methods have greatly influenced the elucidation of the biosynthetic pathways of apolipoproteins. In vitro and tissue culture systems allow the study, to a large extent, of the process of synthesis, intracellular processing, secretion, and extracellular processing of the major high density lipoprotein apoproteins apoA-I and A-II and also of a minor component, apoA-IV. Whereas the latter apoprotein is equipped only with a signal sequence, the primary translation products of apoA-I and apoA-II carry N-terminal extensions of prepro-sequence of 24 amino acids for apoA-I and 23 amino acid residues for apoA-II. The pro-form of apoA-I characterized by a hexapeptide extension is completely stable intracellularly and is secreted as such. The pro-form is further processed by a serum protease specific for an unusual -Gln-Gln-Asp-Glu- sequence site. Pro-apoA-II, a pentapeptide sequence, is partially processed intracellularly to its mature form and secreted together with the residual pro-form. The cleavage site of pro-apoA-II is characterized by two basic amino acid residues Arg-Arg, present also in other known pro-proteins. The biological function of the N-terminal pro-sequences and details of their final processing by the serum protease(s) have yet to be established.—**Stoffel, W.** Synthesis, transport, and processing of apolipoproteins of high density lipoproteins. *J. Lipid Res.* 1984. **25:** 1586–1592.

Supplementary key words apoA-I • apoA-II • signal peptide

INTRODUCTION

The major lipid classes of serum such as cholesterol and cholesteryl esters, triglycerides, and phospholipids are transported in four major lipoprotein classes, chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), from and to the liver and the peripheral tissues. The classification of the lipoprotein particles according to density is useful, although it does not reflect the multiple functional aspects adherent to each lipoprotein class, e.g., the transport of triglycerides in chylomicrons and VLDL for energy expenditure, of cholesterol and its esters in LDL and HDL to target cells as plasma membrane constituents, hormone precursors and for their catabolism to bile acids. Also, lipoprotein particles are the substrates of enzymatic reactions such as the degradation of triglycerides, of chylomicrons, and synthesis in the mucosal cells of the intestine. VLDL, secreted by hepatocytes, is affected by lipoprotein lipase, and the LCAT reaction involves HDL. As a consequence lipoproteins are in a highly dynamic state. It has become increasingly apparent that apolipoproteins as protein constituents of the lipoproteins not only function as solubilizers of the water-insoluble lipids, but they also contribute to the size and shape of the lipoprotein particles. Other important functions are relevant to apolipoprotein structure. Among the two major apolipoproteins of HDL, apoA-I (28.3 kDa) and apoA-II (17 kDa), apoA-I is a potent activator of the LCAT enzyme in serum which catalyzes the transesterification of phosphatidylcholine and cholesterol to lysophosphatidylcholine and cholesteryl esters. Similar but less effective LCAT-activating apolipoproteins are apoC-I (6.5 kDa), a major constituent of VLDL, and apolipoprotein C-II (8.8 kDa), which activates lipoprotein lipase. ApoC-III (8.75 kDa) is the main C-apoprotein in VLDL. It is also present in HDL but no particular activating function has been observed. Two apolipoproteins have been recognized as high affinity ligands of specific receptors in plasma membranes which thereby mediate the receptor-dependent uptake of neutral lipids: apoB-100 (400 kDa) present in VLDL and LDL and apoB-48 in chylomicrons. Apolipoprotein E (35 kDa) is present at a 10–20% concentration in VLDL but it is also a minor constituent in all other lipoproteins. ApoE is the apolipoprotein which leads to an avid binding of apoE-containing particles to specific receptors, particularly in the liver plasma membrane. ApoE is responsible for the receptor-mediated lipoprotein remnant catabolism in the liver. It is obvious that these eight main human

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase.

plasma apolipoproteins can carry out their respective functions properly only with their correct protein structure. Analysis of the apolipoproteins on the genomic level, the structural gene and its regulatory units, will shed light on alterations of protein structure. This will become the domain of molecular biology in the lipoprotein field. Important questions regarding the mechanism of apolipoprotein synthesis, co-translational and post-translational processing, secretion and extracellular processing of certain apolipoproteins have been asked and answered to a respectable degree recently due to sophisticated and elegant cell biology methods. The mRNA of the apolipoprotein-secreting tissue is the key with which one may unlock the cDNA clones of the respective apolipoprotein, leading to the gene or a copy of the intracellular and extracellular processes which finally lead to the mature apolipoprotein. With the HDL-apolipoproteins A-I and A-II as examples, it will be demonstrated here how the synthesis of the primary translation products and their further processing and translation have been elucidated by a combination of in vitro and tissue culture methods, leading to the structure of the protein intermediates.

THE PRIMARY TRANSLATION PRODUCT OF RAT AND HUMAN LIVER APOLIPOPROTEIN A-I mRNA AND ITS PROCESSING

Apolipoprotein A-I is the principal apoprotein of serum high density lipoprotein (HDL) and is common to all species from cyclostoma to man (1). ApoA-I is synthesized in the liver (2) and the small intestine (3) and appears in human serum as a 243 amino acid residue polypeptide with an M_r of 28 kDa (4). In its biogenesis this secretory protein requires translocation across the membrane of the endoplasmic reticulum membrane. Despite its propensity for binding lipids, the following experiments proved that its mechanism of translocation does not differ from that of other secretory proteins. Translation of total rat liver poly(A+)RNA was studied in the wheat germ or reticulocyte cell-free translation system containing labeled methionine or other amino acids. The isolation of a primary translation product from the total translation products by immunoprecipitation with rabbit anti-rat apoA-I IgG yielded a primary translation product of apoA-I mRNA (M_r 31 kDa). This was 3 kDa larger than the 28 kDA mature counterpart (5) (**Fig. 1,** lane 1). Purified authentic unlabeled human apoA-I competed with this immunoreactive polypeptide (Fig. 1, lane 2). In the presence of dog pancreatic microsomal vesicles, an additional major band (M_r ca. 28 kDa) arose which also immunoprecipitated with anti-rat apoA-I IgG (Fig. 1, lane 4). Unlabeled authentic

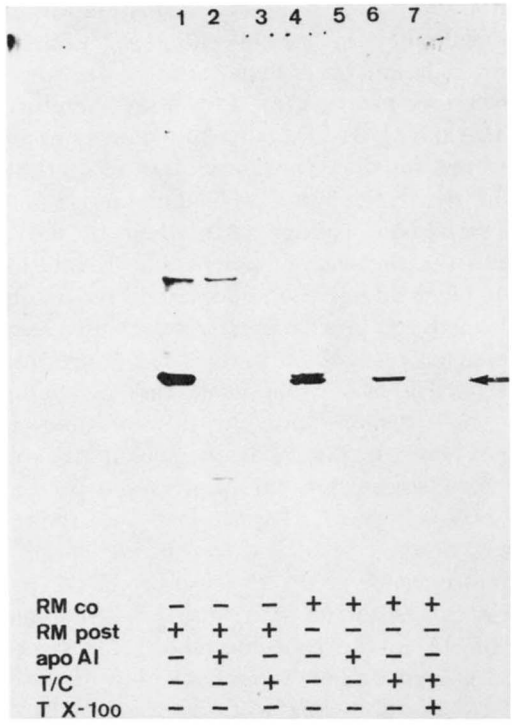

Fig. 1. Translocation in vitro of newly synthesized apolipoprotein A-I. Total rat liver RNA (8 A-260 units/ml) was translated in a wheat germ system (100 μl of translation mixture) in the absence (lanes 1–3) or presence (lanes 4–7) of dog rough microsomes (final concentration 4 A-280 units/ml) (RM co). After a 90-min incubation at 26°C cycloheximide was added to a concentration of 10 μg/ml. Microsomal membranes (RM post) were then added to 4 A-280 units/ml to the tubes which did not contain membrane during translation (lanes 1–3) and the incubation of all tubes was continued for an additional 90 min. Immunoprecipitation using rabbit anti-(apolipoprotein A-I)IgG (30 μg/tube) and protein A-Sepharose (25 μl of packed beads) was performed. The immunoprecipitated products were analyzed by polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate and visualized by fluorography. In lanes 2 and 5 excess native apolipoprotein A-I (20 μg) was added to the translation mix prior to immunoprecipitation. In lanes 3, 6, and 7 trypsin and chymotrypsin (T/C) (0.3 mg/ml each) were added prior to immunoprecipitation and an additional incubation for 60 min at 0°C was performed. In addition to the protease, Triton X-100 (1% final concentration) was added to the sample displayed in lane 7. The arrow indicates the position of authentic apolipoprotein A-I electrophoresed in our gel system (Ref. 5).

apoA-I also immunocompeted with this polypeptide (Fig. 1, lane 5). Of these newly synthesized immunoreactive polypeptides only the lower molecular weight polypeptide was harbored inside the microsomal vesicles. Proteolytic enzymes (trypsin and chymotrypsin) cleaved the 31 kDa primary translation product outside the vesicles (Fig. 1, lane 6). The protection of the lower molecular mass product translocated to the luminal side of the endoplasmic reticulum vesicles depended on the integrity of the vesicle membranes. Non-ionic detergent Triton X-100 abolished the protection (Fig. 1, lane 7). Translation and translocation are strictly coupled: completion of the

translation and post-translational incubation with microsomal membranes yielded only the M_r 31 kDa band, a process which has been demonstrated previously for other secretory proteins (6). This assay therefore suggested that the M_r 31 kDa polypeptide contains a signal sequence that functions in translocation across the membrane of the endoplasmic reticulum and is cleaved during membrane passage. According to the signal hypothesis (7), the nascent polypeptide is translocated to the luminal side of the endoplasmic reticulum in a receptor-mediated process in which the signal sequence and the signal recognition particle (SRP) are involved (8). The SRP isolated from a salt wash of microsomal vesicles (9) is required for the translocation of pre-apolipoprotein A-I, (**Fig. 2**). In the presence of the salt-washed membranes, only the unprocessed pre-form of M_r 31 kDa is formed. (Fig. 1, lane 3). Addition of increasing amounts of SRP led to an increased loss of the signal sequence at the expense of the M_r 31 kDa band (Fig. 2, lanes 4 and 5). Translation of human liver poly(A+)RNA in the reticulocyte cell-free translation system, followed by immunoprecipitation of the translation products with rabbit anti-human apoA-I-IgG and anti-human apoA-II-IgG in the absence and presence of dog pancreas microsomal membranes, yielded very similar results (10). The primary translation product of apoA-I-mRNA was a M_r 31 kDa polypeptide, and that of apoA-II-mRNA a polypeptide of ca. M_r 11 kDa. In the presence of the microsomal membranes both polypeptide chains were processed on translocation with a concomitant reduction of their molecular weights by 2–3 kDa, (**Fig. 3a and b**).

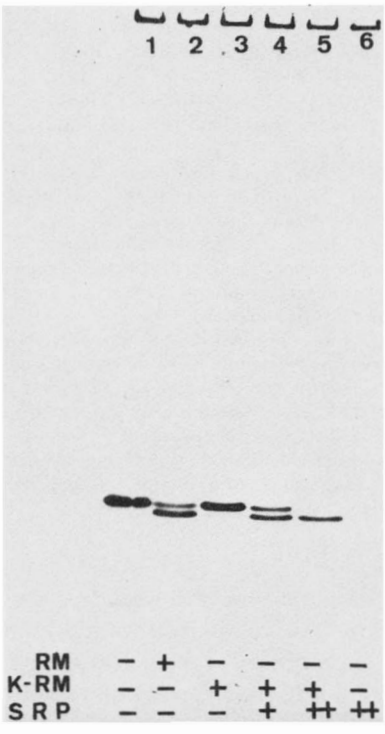

Fig. 2. Translocation of apolipoprotein A-I is dependent on signal recognition protein. Rat liver RNA was translated in the absence (lanes 1 and 6) or the presence of either rough microsomal membranes (lane 2) or salt-extracted rough microsomal membranes (lanes 3–5). Purified signal recognition protein was added to the translation systems at 80 units/ml (lane 4) or 400 units/ml (lanes 5 and 6). The translation products were immunoprecipitated, analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and visualized by fluorography (Ref. 5).

N-TERMINAL SEQUENCES OF PRIMARY AND PROCESSED HUMAN apoA-I AND apoA-II POLYPEPTIDE CHAINS

Proof for the cleavage of an N-terminal signal sequence upon translocation into the lumen of the microsomal vesicles came from partial N-terminal sequence analyses of both the primary translation product and the processed, translocated and membrane-protected polypeptides (5, 10). Cell-free translations were carried out in the presence of [^{35}S]methionine and different [^{3}H]-labeled amino acids. [^{3}H]Leu and [^{3}H]Pro were used for labeling the translation products of rat mRNA, and [^{35}S]Met and [^{3}H]-labeled Val, Asp, Leu, Pro, Ala, Phe, Gln, and Lys were used to label the translation products of human liver mRNA. The results of automated Edman degradations of over 30 cycles of the primary and processed translation products of human apoA-I and apoA-II mRNA not only gave extensive sequence information of the prepro-sequence but also indicated the site of cleavage by the signal peptidase. The difference in molecular weights of the primary and processed translation product of apoA-I mRNA is about 3 kDa, equivalent to 20–30 amino acid residues. In the amino acid sequence of mature apoA-I the first glutamine residue appears at position 5. Labeled glutamine was found in positions 17, 23, 24, and 29 of the primary translation product, aspartic acid at position 25 of the pre-form but at position 7 in the processed polypeptide. Therefore, a signal peptide of 18 amino acid residues was cleaved by the signal peptidase. The labeling experiment with [^{3}H]proline, which was found to be incorporated into positions 9, 10, and 13 of the translocated product, proved further that a hexapeptide extends the N-terminus, since the mature form carries proline in positions 3, 4, and 7. Therefore, the signal sequence is 24 amino acids long and consists of a pre-sequence of 18 residues. This is cleaved upon translocation. An intermediate extended at the N-terminus by a hexapeptide was named the pro-form in analogy to other proteins such as pro-albumin (11). The hexapeptide-extended major apoA-I remains in the lumen of the microsomal vesicles.

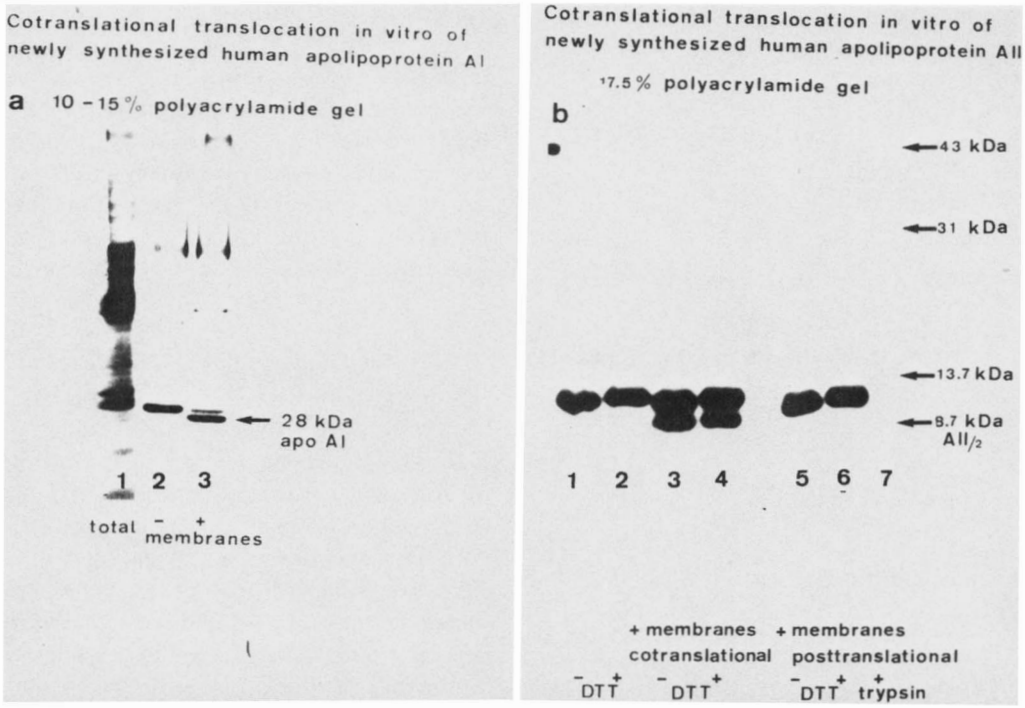

Fig. 3. Translocation in vitro of newly synthesized a) human apolipoprotein A-I. Conditions were those described in the legend of Fig. 1 except that 100 μl of rabbit (anti-human apolipoprotein A-I) IgG and heat-killed, formalin-treated *Staphylococcus aureus* (Cowan strain) were used; 10–15% sodium dodecyl sulfate polyacrylamide gradient gel. Lane 1: total human liver poly(A+)RNA; lane 2: immunoprecipitation products of poly(A+)RNA with rabbit (anti-human apolipoprotein A-I) IgG; lane 3: co-translational cleavage of product of lane 2 by dog pancreas microsomal membranes. b) Human apolipoprotein A-II analyzed in 17.5% polyacrylamide gel. Lane 1: immunoprecipitated primary translation product untreated [−DTT (dithiothreitol)]; lane 2: reductively carboxymethylated (+DTT); lane 3: co-translational cleavage of product in lane 1 (−DTT); lane 4: co-translational cleavage of product in lane 1 (+DTT); lane 5: post-translational addition of membranes (−DTT); lane 6: same as 5 (+DTT); lane 7: trypsin treatment of primary translation product of lane 1 (Ref. 10).

In similar experiments, human apoA-II mRNA was translated in the absence and presence of microsomal membranes and a prepro-apoA-II of 100 amino acids was isolated. The prepro-sequence consisted of 23 amino acids, 18 of which resemble the signal sequence and 5 the pro-form. Both forms were isolated from the in vitro incubation and partially sequenced by automated Edman degradation. These experiments revise our previous proposal of a hexapeptide as present in the pro-form (10). The conclusion that the signal peptidase releases an 18 amino acid residue pre-sequence is based on labeling experiments with [^{35}S]methionine, [^{3}H]alanine, and [^{3}H]lysine. In the pre-form, methionine takes position 1, cysteine positions 14 and 29, and alanine positions 5, 6, 19, and 25. In the pro-form, alanine is in positions 2 and 8, [^{3}H]lysine was incorporated into positions 2 and 26 of prepro-apoA-II and was located in position 8 of pro-apoA-II, while in the mature sequence the first lysine residue takes position 3. In agreement with Gordon et al. (12), therefore, the prepro-sequence is 18 amino acid residues long and the pro-sequence has 5 residues. These data are in agreement with a recent study using RNA isolated from either a human hepatocellular carcinoma cell line (Hep G 2) or intestinal mucosal cells (12). A pre-sequence of 18 amino acids and a pro-segment of 5 residues in apoA-II was deduced. The partial N-terminal sequence studies of the pre- and pro-segments, using many but not all possible amino acids as labeled precursors, are summarized in **Fig. 4** (10, 12). The sequence was completed by recent data derived from the DNA-sequences of the human apoA-I gene (13–15). A comparison of the sequences of the 24 amino acid amino-extensions of human and rat apoA-I clearly indicates a 72% homology in the pre-segment and a 50% homology in the pro-segment. There is no significant homology between the prepro-sequences of human apoA-I and apoA-II. The charged amino acid side chains of the pro-forms lend to human pro-apoA-I a more basic nature and to the rat pro-apoA-I a more acidic nature. Whereas pro-apoA-I of rat and man terminate at a gln-gln sequence, pro-apoA-II ends on two arginine residues. Due to the

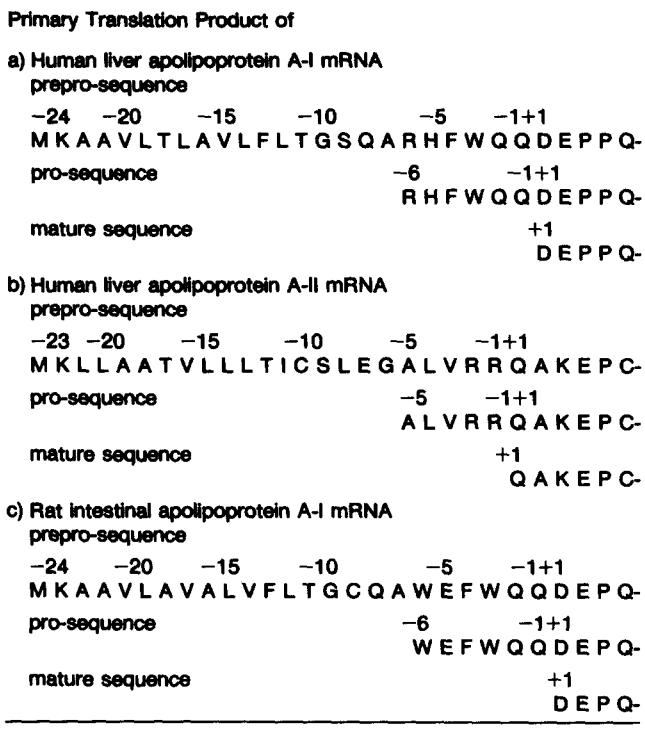

Fig. 4. Comparison of N-terminal sequences of prepro-forms of rat and human apoA-I and apoA-II obtained as primary translation products of rat and human liver apoA-I and apoA-II mRNA, respectively, (10, 12, 19) and completed by data from rat and human apoA-I and apoA-II cDNA clones (13–16).

arginine and histidine residues, the pro-form is more basic than the mature form and exhibits a different migration in isoelectric focusing. The apoA-I polymorphism in isoelectric focusing has led to the apoA-I isoform nomenclature (16). The pro-apoA-I is isoform apoA-I-2, and the mature form is apoA-I-4 according to this nomenclature. The aforementioned studies have now given a molecular explanation for this phenomenon. Automated Edman degradation of pro-apoA-I itself, isolated from human plasma in small amounts, also confirms the pro-sequence of apoA-I (17, 18).

TRANSLATION AND PROCESSING OF RAT INTESTINAL APOLIPOPROTEIN A-I mRNA

Independently and parallel to our studies on the biosynthesis of apoA-I in rat liver (10), Gordon et al. (19) studied the translation and the co-translational cleavage of the in vitro product by ascites cell membrane preparations. The system of these authors, utilizing intestinal apoA-I mRNA and a heterologous processing system, though different from ours led to identical results. They referred to the unusual -gln-gln terminal sequence of the pro-form which is different from previously known C-termini of pro-proteins (20). Their sequence data of the prepro-segments is incorporated in Fig. 4. Cell-free synthesis of a putative precursor to rat apoA-I, which was designated pre-apoA-I and processed in a cell-free system to its mature plasma counterpart, was described earlier by Lin-Su et al. (21). Since no sequence data of the translation product and the processed precursor were obtained, the details of the biosynthesis of apoA-I were not present in that study.

SECRETION OF APOLIPOPROTEIN A-I and A-II

The cell-free system permits a study of the biosynthesis of the primary translation step and the subsequent translocation process into the lumen of the endoplasmic reticulum, including the processing of the products and their preparation for secretion. The secretion process of apoA-I and apoA-II, however, requires the use of cellular systems. The isolated rat hepatocyte suspension culture (22) and a cell line derived from human hepatocellular carcinoma cell lines Hep G 2 (23) were used for these studies (22–24). We demonstrated (22) that monolayer cultures of rat hepatocytes and cell suspensions prepared by recycling liver perfusion with collagenase and hyaluronidase (25) both efficiently synthesize apoA-I. The product isolated from the post-mitochondrial fraction of hepatocyte homogenate is pro-apolipoprotein A-I. The pro-form is secreted into the serum-free medium of the cell suspension. It proved to be identical with the intracellular pro-form, when the two products were prelabeled with [^{3}H]phenylalanine and [^{3}H]valine and sequenced. The radiolabeled thiazolinones of Phe appeared in cycles 3 and 19 and of Val in cycles 16, 22, and 24 of the automated Edman degradation. The pro-form separates discretely in isoelectric focusing from the mature form of rat apoA-I (22), due to the surplus negative charge of Asp in the pro-hexapeptide sequence which is Trp-Asp-Phe-Trp-Gln-Gln. In Hep G 2 cells, when apoA-I was immunoprecipitated from the cell lysate (100,000 *g* supernatant) and the medium was analyzed by N-terminal sequence analysis, similar results were obtained (26, 27). Since the human pro-hexapeptide contains the two basic amino acid side chains of arginine and histidine in its sequence Arg-His-Phe-Trp-Gln-Gln, the observation that basic isoforms of apoA-I are converted to more acidic isoforms in tissue and organ cultures (28–30) found its explanation on a structural level. Analogous to the co-translational processing of human prepro-apolipoprotein A-II to pro-apoA-II, Hep G 2 cells contain intracellularly stable pro-apoA-II and secrete about half of the apoA-II without the cleavage of the pro-sequence (31). Therefore, the two principal

HDL-apolipoproteins A-I and A-II are processed from their prepro-forms to their pro-forms for secretion. Apolipoprotein A-IV, a 46 kDa polypeptide primarily synthesized in the intestine, is also partially associated with rat HDL and with triglyceride-rich lipoproteins; human HDL has less apoA-IV (30, 31). In vitro synthesis, together with biosynthetic studies in Hep G 2 cells, revealed that apolipoprotein A-IV is synthesized with a 20 amino acid long N-terminal signal peptide which is entirely cleaved on translocation through the microsomal membrane (32).

The final processing step, a conversion of pro-apoA-I to the mature form, occurs extracellularly in the plasma by a serum proteinase. This has been demonstrated in rat hepatocyte suspension culture (33) and in Hep G 2 cells (34). The radiolabeled pro-apoA-I secreted by rat hepatocytes is associated with lipoprotein particles of density 1.16–1.20 g/ml. These nascent labeled apolipoprotein A-I-containing particles were isolated and purified by affinity chromatography by adsorption to anti-apoA-I–Sepharose CL-4B (10). The proteinase which cleaves the hexapeptide pro-sequence of apoA-I was found in the greater than 1.21 g/ml density fraction (lipoprotein-deficient serum) of rat serum (33). The serum proteinase that converts pro-apoA-I to mature apoA-I shows very slow kinetics. In order to further characterize this enzyme, we attempted to apply a synthetic substrate (35). The radiolabeled pentadecapeptide [^{3}H]Arg-His-Phe-Trp-Gln-Gln-Asp-Glu-Pro-Pro-Gln-Ser-Pro-Asp-Asp was linked by its C-terminus to the hydrophobic poly-dimethylaminoacryl resin (36) by solid phase synthesis. In this sequence the cleavage site Gln-Gln-Asp-Glu is located in the center. Proteases such as trypsin, thermolysin, and chymotrypsin have free access to this cleavage site. No hydrolysis of the pro-sequence by the converting plasma protease was observed even after prolonged incubation. Thus we deduce from our data that the enzyme of the final processing step requires more than only the primary amino acid sequence with its specific cleavage site.

Much remains to be learned about the biochemical reasons for the pro-sequences of apoA-I and apoA-II and how this may relate to their associations with lipids and with other apoproteins in lipoproteins. We also need to learn more about the nature of the enzyme reaction involved in the pro-peptide cleavage, especially the precise structure required as a substrate.

REFERENCES

1. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21:** 789–853.
2. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18:** 465–473.
3. Green, R. H. R., A. R. Tall, and R. M. Glickman. 1978. Rat intestine secretes discoid high density lipoproteins. *J. Clin. Invest.* **61:** 528–534.
4. Brewer, H. B., R. Fairwell, A. LaRue, R. Ronan, R. Hanser, and T. J. Bonzert. 1978. The amino acid sequence of human apoA-I, an apolipoprotein isolated from high density lipoproteins. *Biochem. Biophys. Res. Commun.* **80:** 623–630.
5. Stoffel, W., G. Blobel, and P. Walter. 1981. Synthesis in vitro and translocation of apolipoprotein A-I across microsomal vesicles. *Eur. J. Biochem.* **128:** 519–522.
6. Blobel, G., and B. Dobberstein, 1975. Transfer of proteins across membranes. II. Reconstitution of functional rough membranes from heterologous components. *J. Cell Biol.* **67:** 852–862.
7. Blobel, G. 1980. Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. USA* **77:** 1496–1500.
8. Walter, P., and G. Blobel. 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature.* **299:** 691–698.
9. Warren, G., and B. Dobberstein. 1978. Protein transfer across microsomal membranes reassembled from separated membrane components. *Nature.* **273:** 569–571.
10. Stoffel, W., E. Krueger, and R. Deutzmann. 1983. Cell free translation of human liver apolipoprotein A-I and A-II mRNA. Processing of primary translation products. *Hoppe-Seyler's Z. Physiol. Chem.* **364:** 227–237.
11. Strauss, A. W., C. A. Bennett, A. H. Donohue, J. A. Rodkey, I. Boime, and A. W. Alberts. 1978. Conversion of rat pre-proalbumin to proalbumin in vitro by ascites membranes. *J. Biol. Chem.* **253:** 6270–6274.
12. Gordon, J. I., K. A. Budelier, H. F. Sims, C. Edelstein, A. M. Scanu, and A. W. Strauss. 1983. Biosynthesis of human preproapolipoprotein A-II. *J. Biol. Chem.* **258:** 14054–14059.
13. Shoulders, C., A. R. Kornblihtt, B. S. Munro, and F. E. Baralle. 1983. Gene structure of human apolipoprotein A-I. *Nucleic Acids Res.* **11:** 2827–2837.
14. Karathanasis, S. K., V. I. Zannis, and J. Breslow. 1983. Isolation and characterization of the human apolipoprotein A-I gene. *Proc. Natl. Acad. Sci. USA.* **80:** 6147–6151.
15. Cheung, P., and L. Chan. 1983. Nucleotide sequence of cloned cDNA of human apolipoprotein A-I. *Nucleic Acids Res.* **11:** 3703–3715.
16. Law, S. W., and H. B. Brewer, Jr. 1984. Nucleotide sequence and encoded amino acids of human apolipoprotein A-I mRNA. *Proc. Natl. Acad. Sci. USA.* **81:** 66–70.
17. Zannis, V. I., J. L. Breslow, and A. J. Katz. 1980. Isoproteins of human apolipoproteins A-I demonstrated in plasma and intestinal organ culture. *J. Biol. Chem.* **255:** 8612–8617.
18. Brewer, H. B., Jr., T. Fairwell, L. Kay, M. Meng, R. Ronan, S. Law, and A. L. Light. 1983. Human plasma proapoA-I: isolation and aminoterminal sequence. *Biochem. Biophys. Res. Commun.* **113:** 626–632.
19. Gordon, J. I., D. P. Smith, R. Andy, D. H. Alpers, G. Schonfeld, and A. W. Strauss. 1982. The primary translation product of rat intestinal apolipoprotein A-I mRNA is an unusual pre-proprotein. *J. Biol. Chem.* **257:** 971–978.
20. Steiner, D. F., P. S. Quinn, S. J. Chan, J. Marsh, and

H. S. Tager. 1980. Processing mechanisms in the biosynthesis of proteins. *Ann. NY Acad. Sci.* **343:** 1–16.
21. Lin-Su, M. H., Y. C. Lin-Lee, W. A. Bradley, and L. Chan. 1981. Characterization, cell-free synthesis and processing of apolipoprotein A-I of rat high density lipoproteins. *Biochemistry.* **20:** 2470–2475.
22. Stoffel, W., C. Bode, and K. Knyrim. 1983. Serum apolipoprotein A-I synthesis in rat hepatocytes and its secretion as proform. *Hoppe-Seyler's Z. Physiol. Chem.* **364:** 439–445.
23. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209:** 497–499.
24. Bojar, H., K. Balzer, K. Reiners, M. Basler, W. Reipen, and W. Staib. 1975. Isolierung intakter Leberparenchymzellen durch eine modifizierte enzymatische Methode. *Z. Klin. Chem. Klin. Biochem.* **13:** 25–30.
25. Gordon, J. I., H. F. Sims, S. R. Lentz, C. Edelstein, A. M. Scanu, and A. W. Strauss. 1983. Proteolytic processing of human prepro-apolipoprotein A-I. *J. Biol. Chem.* **258:** 4037–4044.
26. Zannis, V. I., S. K. Karathanasis, H. T. Keutmann, G. Goldberger, and J. L. Breslow. 1983. Intracellular and extracellular processing of human apolipoprotein A-I: secreted apolipoprotein A-I isoprotein 2 is a propeptide. *Proc. Natl. Acad. Sci. USA.* **80:** 2574–2578.
27. Zannis, V. I., J. L. Breslow, T. R. San Giacomo, D. P. Aden, and B. B. Knowles. 1981. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* **20:** 7089–7096.
28. Zannis, V. I., D. Kurnit, and J. L. Breslow. 1982. Hepatic apoA-I and apoE and intestinal apoA-I are synthesized in precursor isoprotein forms by organ cultures of human fetal tissues. *J. Biol. Chem.* **257:** 536–544.
29. Gordon, J. I., K. A. Budelier, H. F. Sims, C. Edelstein, A. M. Scanu, and A. W. Strauss. 1983. Biosynthesis of human preproapolipoprotein A-II. *J. Biol. Chem.* **258:** 14054–14059.
30. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the $d < 1.006$ lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
31. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins: Studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
32. Gordon, J. I., C. L. Bisgaier, H. F. Sims, O. P. Sachdev, R. M. Glickman, and A. W. Strauss. 1984. Biosynthesis of human preapolipoprotein A-IV. *J. Biol. Chem.* **259:** 468–474.
33. Stoffel, W., K. Knyrim, and C. Bode. 1983. A serum proteinase converts proapolipoprotein A-I secreted by rat hepatocytes to the mature apolipoprotein. *Hoppe-Seyler's Z. Physiol. Chem.* **364:** 1635–1640.
34. Edelstein, C., J. I. Gordon, K. Toscas, H. F. Sims, A. W. Strauss, and A. M. Scanu. 1983. In vitro conversion of proapoprotein A-I to apoprotein A-I. *J. Biol. Chem.* **258:** 11430–11433.
35. Stoffel, W., and B. Niedel. 1984. The proteolytic cleavage of the pro-apoA-I-sequence require specific conformation. *Hoppe-Seyler's Z. Physiol. Chem.* In press.
36. Shepard, R. C. 1973. *In* Peptides 1971. H. Nesvada, editor. North-Holland Publishers, Amsterdam. 111–125.