Characterization, Cell-Free Synthesis, and Processing of Apolipoprotein A-I of Rat High-Density Lipoproteins†

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ABSTRACT: Rat apolipoprotein A-I (apoA-I) was isolated from delipidated high-density lipoproteins by sequential chromatography on Sephacryl S-200 and Sephadex G-150 columns in guanidine buffer. The purified protein had an apparent M_r of 27 000 and was homogeneous by $NaDodSO_4$ and urea gel electrophoresis. Its amino acid composition was similar to that previously reported by Swaney et al. [Swaney, J. B., Wraithwaite, F., & Eder, H. G. (1977) *Biochemistry 16*, 271–278]. Microsequencing yielded an N-terminal sequence of Asp-Glu-Pro-Pro-Val-(Ser)-Glu-. Rabbit antisera were generated against the purified rat apoA-I and were shown to be monospecific against the protein by immunodiffusion and immunoelectrophoresis. Total poly(A) RNA was isolated from the rat liver by extraction in guanidine hydrochloride buffer and oligo(dT)–cellulose chromatography. In vitro translation of the RNA was performed in both wheat germ and nuclease-treated reticulocyte lysate systems, using [^{35}S]Met as the radioactive amino acid precursor. Immunoreactive ^{35}S-labeled apoA-I synthesized in vitro was precipitated by a rabbit antirat apoA-I serum. It was analyzed on an $NaDodSO_4$–acrylamide slab gel and visualized by fluorography. The in vitro product was found to have an apparent M_r of 28 500, being larger than the authentic plasma protein by approximately 1500 daltons. When translation was performed in the presence of dog pancreatic microsomal membranes, the immunoprecipitable material was cotranslationally cleaved to a product identical in size (M_r 27 000) with plasma apoA-I. Thus, we have synthesized in vitro a putative precursor to rat apoA-I, designated preapoA-I. The preapoA-I has been processed in a cell-free system to its mature plasma counterpart by the addition of exogenous microsomal membranes.

The study of apolipoprotein A-I (apoA-I)[1] in human and animal species has been prompted by the observation that increased levels of plasma high-density lipoproteins (HDL), of which apoA-I is the major protein constituent, are inversely correlated with the incidence of atherosclerosis (Barr et al., 1951; Gofman et al., 1966; Miller & Miller, 1975; Gordon et al., 1977). ApoA-I is important in lipoprotein metabolism. Jackson et al. (1975) demonstrated that apoA-I was a necessary component in mixtures with phospholipid to successfully remove cholesterol from ascites cell membranes. ApoA-I also stimulates the esterification of cholesterol to cholesteryl ester by the enzyme lecithin–cholesterol acyltransferase (LCAT) (Fielding et al., 1972a,b). In the perfused rat liver, Hamilton (1978; Hamilton et al., 1976) proposed that HDL are initially synthesized as discoidal bilayer structures when the action of LCAT is inhibited. Mature HDL are then produced by the action of the enzyme in the presence of apoA-I, most likely supplied from the plasma.

Human apoA-I has been well characterized. The primary amino acid sequence is known (Baker et al., 1974; Brewer et al., 1978), and its ability to spontaneously bind phospholipid and phospholipid–cholesterol complexes has been reported (Jonas et al., 1977; Pownall et al., 1978, 1979). The rat apoprotein, however, is less well characterized.

Rat apoA-I is synthesized in both the liver (Noel & Rubenstein, 1974; Marsh, 1976) and the intestine (Wu & Windmueller, 1979). Stimulation of synthesis in the intestine can be achieved by fat loading (Glickman & Green, 1977).

Although the synthesis and secretion of apoA-I have been studied in whole animals (Wu & Windmueller, 1979) and in the isolated perfused liver (Noel & Rubenstein, 1974; Marsh, 1976) as well as in hepatocytes in culture (Dashti et al., 1980), its biosynthesis at the molecular level remains undescribed. In the following report, we present a detailed characterization of apoA-I isolated from the rat HDL including its N-terminal amino acid sequence. We have also translated in vitro apoA-I mRNA from the rat liver and characterized its product. Furthermore, we demonstrate that the in vitro product is a putative precursor, designated preapoA-I, which can be processed to its plasma counterpart by the inclusion of dog pancreatic microsomal membranes in the translation mixture.

Materials and Methods

Materials

Dithiothreitol was purchased from Sigma; oligo(dT)–cellulose (T3) was obtained from Collaborative Research. Wheat germ was a gift of General Mills. [^{35}S]Methionine (1260 Ci/mmol) was purchased from Amersham. Guanidine hydrochloride (Gdn-HCl) was from Bethesda Research Laboratories. All other chemicals were reagent grade and were obtained from Fisher Scientific Co.

Methods

Animals and Preparation of HDL. Male Sprague-Dawley rats (200–300 g) were obtained from Texas Animal Specialties and were maintained on a Purina chow diet until blood was drawn under ether anesthesia. HDL were isolated from plasma by sequential ultracentrifugation as previously described (Havel et al., 1955). The lipoprotein fractions were washed by overlayering with a solution *d* 1.21 g/mL and

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[1] Abbreviations used: apoA-I, apolipoprotein A-I; HDL, high-density lipoproteins; LCAT, lecithin–cholesterol acyltransferase; RNA, ribonucleic acid; mRNA, messenger ribonucleic acid; poly(A) RNA, polyadenylated RNA; DABITC, 4-(*N*,*N*-dimethylamino)azobenzene 4′-isothiocyanate; PITC, phenyl isothiocyanate; DABTH, 4-(*N*,*N*-dimethylamino)azobenzene 4′-thiohydantoin; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Gdn, guanidine; apoHDL, apolipoproteins in high-density lipoproteins; Tris, tris(hydroxymethyl)-aminomethane; oligo(dT), oligothymidylate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol.

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repeating the centrifugation. The washed lipoprotein solutions were dialyzed exhaustively against 0.9% NaCl and 0.01% EDTA, pH 7.0, and delipidated with ether–ethanol (1:1) solutions.

Isolation of ApoA-I. The delipidated apoHDL proteins were dissolved in 0.2 M Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaN_3, and 3 M Gdn-HCl and applied to a 2.5 × 170 cm column of Sephacryl S-200 equilibrated with the same buffer. Fractions which contained primarily apoA-I were pooled and rechromatographed on a Sephadex G-150 column (2.0 × 170 cm) in 0.05 M Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaN_3, and 3 M Gdn-HCl. Purified apoA-I was analyzed by polyacrylamide gel electrophoresis in $NaDodSO_4$ (Weber & Osborn, 1969) and in urea (Kane, 1973).

Preparation of Antibody. Purified rat apoA-I was emulsified with an equal volume of Freund's complete adjuvant. Two male New Zealand White rabbits were injected with 1 mL of the emulsion containing 100 μg of apoA-I at multiple subcutaneous sites on the back. After the initial immunization, each rabbit received subcutaneous injections of the protein (35 μg each time) every 2 weeks. After 2 months, sera were obtained and tested for anti-apoA-I activity by immunodiffusion and immunoelectrophoresis. An immunoglobulin fraction was prepared by ammonium sulfate precipitation (Chan et al., 1976).

Preparation of Total Liver Cellular mRNA. Total rat liver cellular RNA was extracted by the Gdn-HCl method of Cox (1968), with some modifications. Immediately after excision, livers were homogenized in 6–10 volumes of 20 mM sodium acetate, pH 5.0, containing 7.5 M Gdn-HCl and 1 mM dithiothreitol (DTT). The homogenate was centrifuged at 10000 rpm (11000*g*) for 20 min at 10 °C and the supernatant fraction filtered through a sterile cheesecloth. RNA was precipitated with 0.5 volume of prechilled ethanol at −20 °C for 1 h. The precipitate was collected by centrifugation at 10000 rpm for 10 min and resuspended in 20 mM sodium acetate buffer containing 7.5 M Gdn-HCl, 1 mM DTT, and 20 mM Na_2EDTA. This procedure of ethanol precipitation was repeated an additional 3 times to further purify the RNA. To exchange the sodium salt for the guanidine salt of the RNA, the final pellet was dissolved in a minimum volume of 20 mM Na_2EDTA, pH 7.0, and extracted with an equal volume of chloroform–1-butanol (4:1). This extraction procedure was repeated twice. The aqueous phase was made 0.2 M KCl, and RNA was precipitated with 2 volumes of ethanol at −20 °C. Low molecular weight RNA and DNA were removed by washing the RNA pellet with 3 M sodium acetate, pH 5.0, at 4 °C. The final washed pellet was dissolved in water and precipitated with 2 volumes of ethanol at −20 °C. An RNA fraction enriched in poly(A) was prepared from the total RNA by oligo(dT)–cellulose chromatography by the method of Aviv & Leder (1972).

Cell-Free Translation Systems. Wheat germ extract was prepared by the method of Davies et al. (1977). As in our experience, this preparation proved to be more active than material prepared by the original method of Roberts & Paterson (1973). Nuclease-treated reticulocyte lysate translation system (Pelham & Jackson, 1976) was purchased from Bethesda Research Laboratories.

ApoA-I Protein Synthesis in a Heterologous Cell-Free Protein Synthesizing System. A total reaction mixture of 50 μL contained 20 μL of wheat germ extract and 30 μL of a mixture composed of Hepes (6 nM, pH 7.6), DTT (2 mM), ATP (1 mM), GTP (0.02 mM), creatine phosphate (8 mM), creatine phosphokinase (2 μg), KCl (60 mM), magnesium acetate (0.1 mM), spermidine (0.1 mM), 19 L-amino acids minus Met (each at 2 μM), [^{35}S]methionine (8 μCi), and RNA (2 μg); H_2O was added to adjust the volume to 30 μL. The reaction mixture was incubated at 25 °C for 90 min during which time incorporation of [^{35}S]Met into protein was linear. Translation in the reticulocyte lysate system was performed according to the instructions of the supplier, and incubation was at 25 °C for 90 min. Thirty-microliter assays were used. Aliquots of 5 μL were precipitated in 1 mL of 10% trichloracetic acid, heated to 95 °C for 10 min, cooled in ice, and collected on glass-fiber filters. The remainder of the reaction mixture was used for quantitation of immunoprecipitable apoA-I synthesized in vitro.

Quantitation of Immunoprecipitable ApoA-I Synthesized in Vitro. Inactivated *Staphylococcus aureus* (Cowan I strain) (Pansorbin, Calbiochem) was extensively washed and prepared according to the method of Kessler (1975). Ribosomes were removed from the final translation reaction mixture by centrifugation at 4 °C for 60 min at 105000*g*. The postribosomal supernatant fluid (100 μL) was then incubated with 30 μL of prewashed Pansorbin at room temperature for 5 min. Pansorbin was removed by low-speed centrifugation. Newly synthesized ^{35}S-labeled apoA-I was precipitated in a reaction mixture containing 0.5 μL of rabbit antirat apoA-I IgG and 300 μL of TNENN buffer (50 mM Tris, pH 7.5, 0.15 M NaCl, 5 mM EDTA, 0.02% NaN_3, and 0.5 NP-40, containing 10 mM methionine) per 100 μL of the reaction product. The mixture was incubated at room temperature for 2 h and then at 4 °C overnight. Fifteen microliters of prewashed Pansorbin was added, and the mixture was gently shaken at 4 °C for 40 min. Pansorbin was collected by low-speed centrifugation. It was layered on top of a 1 M sucrose cushion containing 50 mM Tris, pH 7.5, 0.15 M NaCl, 5 mM EDTA, 0.02% NaN_3, 0.5% NP-40, and 10 mM methionine and centrifuged at 2000*g* for 20 min. After washing 3 times with TNENN buffer, immunoprecipitated product was released from the bacteria by incubation in 2.5% $NaDodSO_4$, 10% glycerol, 5% mercaptoethanol, and 65 mM Tris-HCl, pH 8.0, at 95 °C for 5 min. The bacteria were then removed by low-speed centrifugation. An aliquot (5 μL) of supernatant fraction was counted directly by scintillation spectrophotometry. The remainder was analyzed by $NaDodSO_4$ gel electrophoresis.

$NaDodSO_4$–Acrylamide Gel Electrophoresis. Immunoprecipitated proteins were analyzed by 10% or 12% $NaDodSO_4$–polyacrylamide slab gel electrophoresis by the method of Laemmli (1970). Standard proteins as well as purified apoA-I were run on the same gel. Gels were processed for fluorography (Laskey & Mills, 1975) and exposed to X-ray films (Kodak X-Omat). Relative positions of radioactive bands were compared to those of standard proteins.

Amino Acid Analyses. Protein samples were hydrolyzed in 6 N HCl containing 0.1% phenol in sealed, evacuated (<-50-μm) ampules at 110 °C for 18–24 h (Blackurn, 1968). Analyses were performed on a Beckman 119 amino acid analyzer equipped with a Systems AA Autolab data system.

N-Terminal Analysis. N-Terminal sequence analyses of apoA-I was achieved by the microsequence method of Chang et al. (1978) using the 4-(*N*,*N*-dimethylamino)azobenzene 4′-isothiocyanate (DABITC)/phenyl isothiocyanate (PITC) double-coupling technique.

Processing of Putative ApoA-I Precursors. Dog pancreatic microsomal membrane fractions were isolated by the method of Shields & Blobel (1978) as modified from Blobel & Dobberstein (1975). They were added to the translation mixture at a concentration of 8 μL (15 A_{260} units/mL) per 30-μL

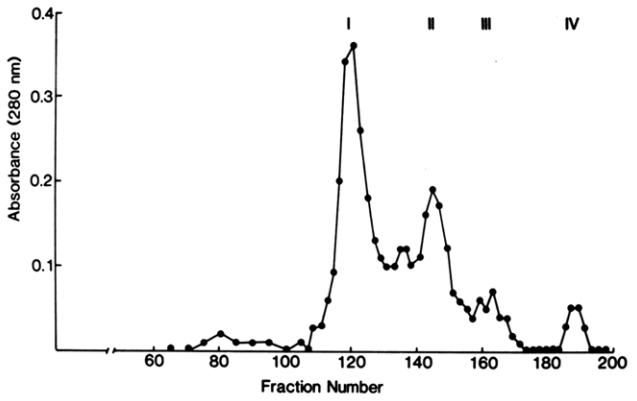

FIGURE 1: Chromatography of delipidated apoHDL on Sephacryl S-200, equilibrated with 0.2 M Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaN_3, and 3 M Gdn-HCl. Thirty milligrams of total delipidated apoHDL were loaded on a 2.5 × 170 cm column at a flow rate of 24 mL/h. Three-milliliter fractions were collected. By $NaDodSO_4$ gel analysis, peak I contained mainly apoA-IV and apoE, both peaks II and III almost exclusively apoA-I with trace amounts of apoE revealed by overloaded $NaDodSO_4$ gels, and peak IV mainly apoA-II and apoC proteins.

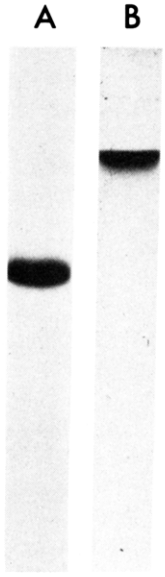

FIGURE 2: (A) 10% acrylamide gel electrophoresis of purified rat apoA-I in 0.1% $NaDodSO_4$ (Weber & Osborn, 1969). (B) 10% acrylamide gel electrophoresis of purified rat apoA-I in 8 M urea at pH 8.8 (Kane, 1973).

reaction prior to the initiation of translation. Immunoprecipitated products were analyzed by $NaDodSO_4$–slab gel electrophoresis and visualized by fluorography as previously described (Laskey & Mills, 1975; Chan et al., 1980a,b).

Results

Isolation and Characterization. HDL were isolated from plasma by sequential ultracentrifugal flotation. The HDL were delipidated, and the lipid-free proteins were fractionated by gel filtration chromatography on Sephacryl S-200 (Figure 1). Those fractions (143–161) which contained almost exclusively apoA-I by $NaDodSO_4$ gel were pooled and reapplied to a Sephadex G-150 column, yielding a single major protein containing peak. Polyacrylamide gel electrophoresis of this material is shown in Figure 2A; only one protein band was detected. This material was used for subsequent studies.

Molecular Weight Analysis. Molecular weight estimates of the purified apoA-I were obtained by $NaDodSO_4$ electrophoresis in 10% acrylamide gels. A linear calibration curve, obtained with the appropriate molecular weight standards,

Table I: Amino Acid Composition of Apolipoprotein A-I from Rat High-Density Lipoproteins

amino acid	apoA-I (mol %)	apoA-I[b] (mol %)
aspartic acid	13.1	13.4
threonine	3.8	4.2
serine	3.0	3.3
glutamic acid	16.5	20.0
proline	1.2	2.4
glycine	6.1	2.4
alanine	6.4	4.8
half-cystine	ND[a]	0.4
valine	5.4	4.6
methionine	3.2	2.8
isoleucine	1.6	1.8
leucine	12.5	12.6
tyrosine	2.3	2.9
phenylalanine	2.9	3.7
lysine	10.3	9.8
histidine	2.8	2.4
arginine	8.9	7.0
tryptophan	ND	1.5

[a] ND, not determined. [b] Swaney et al. (1977).

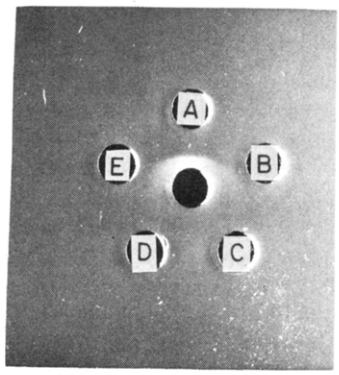

FIGURE 3: Immunodiffusion of apoproteins against rabbit antirat apoA-I. The center well contained 10 μL of the total antiserum. Each of the other wells contained 7μL of the indicated antigen at 1.5 mg/mL. (A) Rat apoA-I; (B) rat apoE; (C) rat apoC; (D) rat albumin; (E) no protein added.

indicated that plasma apoA-I migrated with an apparent M_r of 27 000, in agreement with previously published values (Swaney et al., 1977). The protein also migrated as a single band on urea gels (Figure 2B). Upon isoelectric focusing, this protein showed two major bands at pH 5.9 and 5.8 with a lesser band at pH 5.75 (data not shown).

Amino Acid Analysis. The amino acid composition of the purified apoA-I is shown in Table I. Our data are comparable to those reported by Swaney et al. (1977) for rat apoA-I.

N-Terminal Amino Acid Sequence Analysis. The microsequence methodology of Chang et al. (1978) using DABITC/PITC allowed the determination of seven steps of the N-terminal of rat apoA-I. DABTH derivatives were identified by two-dimensional thin-layer chromatography on polyamide sheets. The inclusion of a synthetic marker, 4-(*N*,*N*-dimethylamino)azobenzene-4′-(thiocarbamoyldiethylamide), facilitated the identification of the DABTH derivatives. Approximately 5 nmol of the apoprotein yielded the N-terminal sequence

$$H_2N\text{-Asp-Glu-Pro-Pro-Val-(Ser)-Glu...}$$

Immunochemical Analysis. On analysis by immunodiffusion, our rabbit antirat apoA-I serum formed a single precipitin line of identity against purified apoA-I and against total rat plasma. It did not cross-react with rat albumin or apoE (Figure 3). The same antiserum also yielded a single pre-

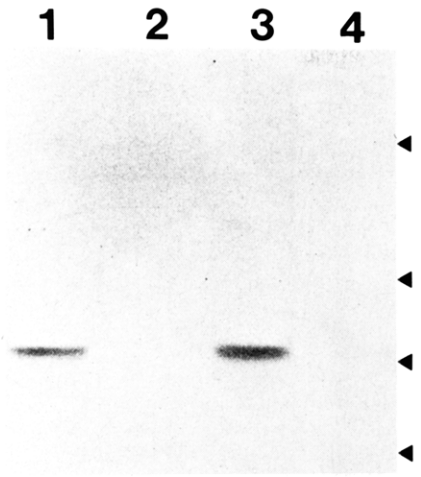

FIGURE 4: $NaDodSO_4$–acrylamide gel electrophoresis and fluorography of immunoprecipitable apoA-I synthesized in vitro. Total mRNA was translated in vitro in the wheat germ system by using [^{35}S]methionine as the radiolabeled amino acid as described under Materials and Methods. The figure shows the products, visualized by fluorography (Laskey & Mills, 1975), separated on a 10% acrylamide gel in 0.1% $NaDodSO_4$ (Laemmli, 1970). (Lane 1) immunoprecipitation using rabbit antirat apoA-I serum; (lane 2) immunoprecipitation in the presence of excess unlabeled apoA-I (50 μg); (lane 3) immunoprecipitation in the presence of an unlabeled unrelated protein (ovalbumin 50 μg); (lane 4) immunoprecipitation using nonimmune serum instead of anti-apoA-I serum. Arrows on the side indicate the position of standard proteins analyzed on the same slab gel. From top to bottom, they are bovine serum albumin (68 000), deoxyribonuclease I (31 000), rat apoA-I, (27 000), and cytochrome *c* (14 300).

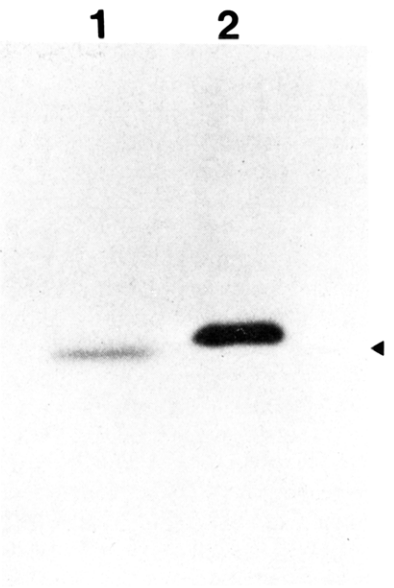

FIGURE 5: In vitro translation of rat apoA-I: effects of inclusion of dog pancreatic microsomal membranes. Translation was carried out in the nuclease-treated reticulocyte lysate system (Pelham & Jackson, 1976) by using [^{35}S]methionine as the radiolabeled amino acid precursor. Translation products were immunoprecipitated, separated on 12% acrylamide slab gel in 0.1% $NaDodSO_4$ (Laemmli, 1970), and visualized by flurography. (Lane 1) Translation performed in the presence of dog pancreatic microsomal membranes (Shields & Blobel, 1978); (lane 2) translation performed without dog pancreatic microsomal membranes. In the presence of exogenous microsomal membranes, there is a 50% inhibition of translational activity as measured by Cl_3AcOH-precipitable radioactivity. The migration position of rat plasma apoA-I is indicated by the arrow.

cipitin arc against apoA-I on immunoelectrophoresis (data not shown). This monospecific antiserum was used for all studies on apoA-I translation.

Translation of Rat Liver mRNA in Vitro. Polyadenylate-enriched RNA was prepared from total liver RNA by oligo-(dT)–cellulose chromatography. After two passages, the amount of poly(A) RNA was approximately 1.5% of the total RNA applied. The mRNA activity of this fraction was tested in the wheat germ system. There was an mRNA-dependent increase in the amount of [^{35}S]methionine incorporated into Cl_3AcOH-precipitable protein. The translation was linear for 90 min.

Characterization of ApoA-I Synthesized in Vitro. Immunoprecipitable apoA-I, synthesized in vitro, was determined to constitute about 1.2% of the newly synthesized protein (data not shown). To ensure the reliability of our antiserum to selectively precipitate only apoA-I under our assay conditions, the immunoprecipitated products were analyzed on 10% polyacrylamide slab gels in the presence of $NaDodSO_4$ (Figure 4). Only a single product was observed by fluorographic analysis of the $NaDodSO_4$ gel. The in vitro product proved to be larger than authentic apoA-I isolated from plasma (Figure 4) having an apparent molecular weight of 28 500. It was designated pre-apoA-I. Specificity of the immunoprecipitated pre-apoA-I synthesized in vitro was confirmed by competition experiments (Figure 4). Addition of unlabeled rat apoA-I to the immunoprecipitation reaction abolished the radioactive band corresponding to the pre-apoA-I. In contrast, the addition of an unrelated protein, ovalbumin, did not result in any detectable inhibition. Finally, when rabbit nonimmune serum was substituted for the anti-apoA-I serum, the pre-apoA-I band was absent.

Cell-Free Translation in the Presence of Dog Pancreatic Microsomal Membranes. Since the immunoprecipitated translation product was larger than authentic plasma apoA-I, we have studied the processing of this product in vitro by the addition of dog pancreatic microsomal membranes. As shown in Figure 5, in the absence of membranes (lane 2), ^{35}S-labeled pre-apoA-I has an apparent molecular weight of 28 500. When membranes were included in the translation mixture (lane 1), there was an approximately 50% inhibition of translation, and the immunoprecipitated ^{35}S-labeled apoA-I synthesized in vitro migrated with an R_f identical with that of authentic plasma apoA-I, corresponding to an apparent M_r of 27 000. Under our conditions, addition of membranes subsequent to the translation reaction was ineffective in processing the product (data not shown). Hence, we have demonstrated the co-translational processing of ^{35}S-labeled preapoA-I into an immunoreactive ^{35}S-labeled apoA-I.

Discussion

The isolation of rat apoA-I from plasma HDL yielded a single molecular weight species of M_r 27 000. This molecular weight is similar to that reported for the apoprotein from human (Baker et al., 1974) and hen (Jackson et al., 1976) as well as pig (Jackson et al., 1973) and dog (Scanu et al., 1975). Unlike human apoA-I, rat apoA-I contains isoleucine (Table I). In this respect, however, it is similar to the chicken protein. The immunological purity of this protein was demonstrated by a single precipitin line of identity both on Ouchterlony plates and by immunoelectrophoresis. The N-terminal analysis of rat apoA-I yielded a single amino acid residue which was aspartic acid. This is in accord with the results of Swaney et al. (1977) who reported Asx by dansylation. Our result unambiguously identifies the N terminal as Asp. No other residue was apparent, confirming the purity of the apoprotein. Aspartic acid has also been reported as the N-terminal amino acid in human, hen, and pig apoA-I. In addition, the N-terminal sequence of rat apoA-I is reminiscent of that of the

human species (H_2N-Asp-Glu-Pro-Pro-Gln-Ser-Pro-; Baker et al., 1974; Brewer et al., 1978). Koga et al. (1969) and later Swaney et al. (1977) demonstrated that rat HDL and apoHDL as well as the isolated apoA-I have circular dichroism characteristics similar to those of the human apoprotein. Isolated rat apoA-I was shown to possess about 60% α helicity (Koga et al., 1969; Swaney et al., 1977) compared to 65% in the human protein (Jackson et al., 1976). This information, taken with our data on the N-terminal amino acid sequence, suggests similar chemical and functional properties between the rat and human apoA-I. With this relatively well-characterized apoprotein, we prepared monospecific antisera to this apoprotein to be used in our studies on the cell-free translation of hepatic mRNA from the rat.

Cell-free translation of rat apoA-I was performed in both the wheat germ (Figure 4) and the nuclease-treated reticulocyte lysate systems (Figure 5). In both systems the immunological identiy of the cell-free product with authentic apoA-I was demonstrated by competition experiments (only the results in the wheat germ system are presented). Furthermore, in both instances, apoA-I was synthesized as a putative precursor, designated pre-apoA-I, which is approximately 1500 daltons greater in apparent molecular weight than the plasma protein.

It is thus apparent that rat apoA-I is initially synthesized as a larger putative precursor. This observation suggests that the biosynthetic pathway of this apolipoprotein must be similar to that of many other secretory proteins (Blobel & Debberstein, 1975; Wickner, 1979; Blobel, 1980) in that the initial product of translation has an additional signal peptide attached to its N terminus. As further evidence for the presence of this peptide, we have carried out the cell-free translation in the presence of dog pancreatic microsomal membranes. Indeed, the presence of the latter results in the processing of pre-apoA-I into a protein identical in size with authentic rat plasma apoA-I. The synthesis of putative apolipoprotein precursors by in vitro translation has been previously demonstrated in avian apoA-I and apoVLDL-II (Chan et al., 1980a,b). This report represents the initial demonstration of such a precursor in a mammalian apolipoprotein. Furthermore, it shows for the first time that the preapolipoprotein is processed in the presence of an exogenous microsomal membrane preparation presumably by the signal peptidase.

At least two mutants in apolipoprotein structure involving apoA-I have been described in man; one of these ($apoA\text{-}I_{milano}$) contains cysteine, an amino acid not found in normal apoA-I (Weisgraber et al., 1980), and the other ($apoA\text{-}I_{marburg}$) is less well characterized (G., Utermann, personal communication). These and potentially other types of mutations may be important instances of alterations in gene products involving apolipoproteins. The demonstration of cell-free translation of a mammalian apolipoprotein represents an initial step toward the elucidation of the primary defect in these disease states as well as other instances of deranged regulation of apolipoprotein synthesis (e.g., hypobeta- and abeta-lipoproteinemia, Tangier disease, and possibly some of the hyperlipoproteinemias). Since rat apoA-I mRNA activity appears to constitute about 1.2% of the total rat hepatic mRNA activity, it should be feasible to purify the gene sequences for this apoprotein by recombinant DNA technology. Our studies on the characterization of the protein and the cell-free translation of its mRNA represent a first step in that direction.

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Expression of Variant Forms of Proopiomelanocortin, the Common Precursor to Corticotropin and β-Lipotropin in the Rat Pars Intermedia†

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ABSTRACT: Proopiomelanocortin, the common glycoprotein precursor to adrenocorticotropin (ACTH) and β-lipotropin (β-LPH), is the most abundant protein synthesized in rat neurointermediate lobes. It represents 30% of the total amount of radioactive proteins obtained after a 1-h pulse incubation with [^{3}H]phenylalanine. Several forms of this protein can be separated by a high-resolution two-dimensional gel electrophoresis technique. The three most abundant species which can be reproducibly characterized by their apparent molecular weights (M_r) and isoelectric points (pI) were called form I (M_r 34 000; pI 8.2), form II (M_r 36 000; pI 8.2), and form III (M_r 35 000; pI 7.3). Additional minor forms, representing together ~30% of the total of forms I, II, and III combined, are also observed. They have very close molecular weights but differ by their isoelectric points. When glycosylation is prevented by tunicamycin, forms I and II are replaced by a new molecule with the same pI of 8.2 but a slightly lower M_r (32 000). This form is referred to as form T_1. Similarly, form III is replaced by form T_2 (M_r 33 000; pI 7.3). Forms T_1 and T_2 are supposed to be nonglycosylated peptides. They were further characterized by microsequencing and peptide mapping. They both have the same N-terminal amino acid sequence with leucine residues in positions 3 and 11, and they both contain identical [^{3}H]phenylalanine-labeled tryptic fragments, two of them corresponding to the sequences 1–8 of ACTH and 61–69 of β-LPH. However, a limited digestion with the *Staphylococcus aureus* (V8 strain) protease generates a collection of peptides different for each form. These results suggest the presence of at least two different gene products corresponding to the major forms of proopiomelanocortin in the rat pars intermedia.

In the rat pars intermedia, β-endorphin and α-melanotropin (α-MSH)[1] are synthesized as part of a large common precursor (Crine et al., 1978, 1979; Mains & Eipper, 1979; Crine et al., 1980) named proopiomelanocortin (Chrétien et al., 1979; Crine et al., 1979). The existence of a similar precursor has also been extensively documented in the mouse pituitary tumor cell line (Roberts et al., 1978; Eipper & Mains, 1978a) and in the anterior lobe of the pituitary (pars distalis) (Taii et al., 1979). The two lobes of the pituitary (pars intermedia and pars distalis) contain, however, very different collections of final maturation products derived from the precursor: adrenocorticortopin (ACTH) and β-lipotropin (β-LPH) are major end products of the maturation process in the anterior lobe while α-MSH and β-endorphin occur in substantial amounts in the pars intermedia [for a recent review, see Krieger et al. (1980)], suggesting that the processing of the common precursor varies according to the tissue. The reason for this in totally unknown. One hypothesis states that there could be tissue-specific differences in the proteolytic enzymes responsible for cleaving the precursor at various sites during the processing. Unfortunately, the nature and specificity of these processing enzymes are still poorly documented and this hypothesis rests on very few experimental observations. Alternatively, since proopiomelanocortin is a glycosylated peptide (Eipper et al., 1976; Roberts et al., 1978), it has been proposed that glycosylation of critically positioned amino acid residues in the polypeptide backbone could direct the cleavage of specific sequences through conformational effects.

Careful analysis of the precursor by sodium dodecyl sulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis revealed that a least two forms of this glycoprotein are synthesized by pars intermedia cells (Loh, 1979; Crine et al., 1979) or AtT-20 tumor cells (Roberts et al., 1978; Eipper & Mains, 1978a). It was proposed that these peptides represent the product of differential glycosylation of a single polypeptide chain (Roberts

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[1] Abbreviations used: α-MSH, α-melanotropin; ACTH, adrenocorticopin, adrenocroticotropic hormone; LPH, lipotropic hormone; $NaDodSO_4$, sodium dodecyl sulfate; Cl_3AcOH, trichloroacetic acid; MEM, minimum essential medium; Me_2SO, dimethyl sulfoxide; LC, liquid chromatography; pI, isoelectric point.

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