Human Placental Tissue Expresses a Novel 22.7 kDa Apolipoprotein A-I-like Protein[†]

B. Richardson,*[,‡] M. N. Palgunachari,[§] G. M. Anantharamaiah,[§] R. G. Richards,[‡] N. Azrolan,[∥] D. Wiginton,[⊥] and S. Handwerger[‡]

Divisions of Endocrinology and Developmental Biology, Children's Hospital Medical Center, and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, Atherosclerosis Research Unit, Department of Medicine, University of Alabama, Birmingham, Alabama 35294, and Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York, New York 10021

Received November 10, 1995; Revised Manuscript Received February 29, 1996[⊗]

ABSTRACT: Since apolipoprotein A-I (apo A-I) and HDL stimulate the expression of the placental hormone human placental lactogen (hPL), experiments were performed to determine whether the human placenta synthesizes apo A-I. Western blot analysis of a partially purified extract of human term placenta with an antiserum to human apo A-I yielded an immunoreactive band with an apparent mass of approximately 23.5 kDa, which is smaller than human plasma apo A-I (28 kDa). HPLC chromatography of the partially purified placental extract on a preparative reverse-phase C-18 column yielded two fractions that reacted to the apo A-I antiserum. The mass of both fractions by mass spectral analysis was 22 721 daltons, and N-terminal amino acid sequences were identical to the first four amino acids of apo A-I (Asp, Glu, Pro, Pro). The apo A-I-like protein was not a proteolytic product of apo A-I since Northern analysis of placental RNA with a 641 bp apo A-I cDNA fragment encoding most of the 5′ region of the apo A-I mRNA detected a single band of 850 nt, which is smaller than the size of apo A-I mRNA (1100 nt). Placental mRNA, however, did not hybridize with a 3′ apo A-I riboprobe, indicating that the 3′ region of the apo A-I-like mRNA is different from that of apo A-I mRNA. Differences in the mRNAs were confirmed by S1 nuclease analysis of placental RNA with a cDNA probe that included the 3′ end of the apo A-I cDNA and by RT-PCR analysis with a series of oligonucleotide primers that span the entire cDNA for apo A-I. Since there is only a single apo A-I gene in the human genome, these findings strongly suggest that human placental tissue expresses a novel 22.7 kDa apo A-I-like protein (ALP) that results from alternative splicing of the apo A-I primary transcript.

Apolipoprotein A-I (apo A-I) is the major apolipoprotein constituent of high-density lipoproteins (HDL), comprising about 95% of the total protein content of the HDL particle (Schonfeld & Pfleger, 1974). The major sites of apo A-I synthesis are the liver and intestine (Wu & Windmuller, 1979). Apo A-I mRNA has also been detected in other tissues, including testes (Lenich et al., 1988) and brain (Weiler-Guttler et al., 1990). However, an exhaustive search for apo A-I mRNA in all human tissues has not been performed.

A possible role for the placenta as a site of apo A-I synthesis is suggested by the isolation of a novel apo A-I-containing lipoprotein particle in human placental extracts that differs from HDL in size and apolipoprotein content (Park et al., 1988). The novel particle floats at a density similar to VLDL (<1.006 mg/mL) and, like VLDL, contains apo B-100. However, the presence of an apo A-I-containing particle in the human placenta is not proof that the placenta synthesizes apo A-I since apo A-I might originate from another source.

In the present study, we have examined whether the human placenta expresses apo A-I. While we were unable to detect full-length apo A-I, we purified an apo A-I-like protein (ALP) from extracts of human placenta that is smaller in molecular weight than plasma apo A-I. Northern blot hybridization, S1 nuclease analysis, and RT-PCR amplification of placental RNA suggest that ALP is synthesized in the placenta and arises from alternative splicing of the apo A-I primary transcript.

EXPERIMENTAL PROCEDURES

Purification and Chemical Characterization of ALP

Purification Scheme. Informed consent to use term placentas for this study was approved by the Human Investigation Committees of the Children's Hospital Medical Center and the University of Cincinnati College of Medicine. The placental tissue, which was obtained from women with normal pregnancies, was cut into small pieces of approximately 0.5 cm^3. The placental pieces were then placed in 4 L of cold saline, stirred at 4 °C for 3−4 h, collected on a 150 μm Nitex filter (Tetco, Briarcliff Manor, NY), and rinsed once more with cold saline to remove unbound HDL and free apo A-I from blood. The placental pieces were homogenized in phosphate-buffered saline (PBS) containing 1 mM EDTA and 1 mM phenylmethanesulfonyl fluoride (PMSF) using a Waring blender with a minisample container. The homogenate was then centrifuged at 6750*g* for 1 h at 4

[†] Supported by NIH Grants HD-15201, HD-07447, and PO1 HL 34343.

[‡] Division of Endocrinology, Children's Hospital Medical Center.
[§] University of Alabama.
[∥] The Rockefeller University.
[⊥] Division of Developmental Biology, Children's Hospital Medical Center.

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1996.

S0006-2960(95)02695-X CCC: $12.00

°C, and the supernatant was concentrated to a volume of approximately 100 mL by osmotic diffusion across a 3500 MW cutoff dialysis tubing (Spectrum Medical Industries, Los Angeles, CA) using 15−20 kDa poly(ethylene glycol) (PEG) (Sigma Chemical Co., St. Louis, MO).

The concentrated soluble extract was then fractionated by sodium bromide flotation at a density of 1.215 g/mL (Schumaker & Puppione, 1986). After centrifugation, the supernatant was fractionated into top quarter, middle half, bottom quarter, and pellet fractions. The top and bottom fractions and the pellet were discarded, and the middle fraction (which contained apo A-I immunoactivity by Western blot analysis) was dialyzed against three changes of PBS containing 1 mM EDTA and 0.1 mM PMSF. The proteins were then concentrated to approximately 50 mL by osmotic diffusion with poly(ethylene glycol), dialyzed again against PBS, and lyophilized for long-term storage and transport. The crude mixture was then dissolved in 6 M guanidine hydrochlorides dialyzed against water for 24 h using a 12 000−14 000 MW cutoff dialysis membrane (Spectrum Medical Industries), and subjected to further purification on a C-18 reverse-phase glass column (20 mm inner diameter × 30 cm) using a Waters 600E HPLC system with a 10−80% acetonitrile gradient containing 0.1% trifluoroacetic acid. The presence of apo A-I-related protein(s) in the column fractions was detected by immunoblot analysis as described below. One of the fractions from the preparative column that reacted to an antiserum to apo A-I was subsequently subjected to analytical HPLC (Beckman Gold System 200; Beckman Instruments, Fullerton, CA) using a C-18 reverse-phase column (VYDAC, 4.6 mm inner diameter × 25 cm) with the same gradient conditions.

Mass Spectral and Amino Acid Sequence Analyses. The molecular weights of the two apo A-I immunoreactive fractions were determined by electrospray ionization mass spectrometry on a PE Apt III biomolecular mass analyzer (Sciex Instruments, Toronto, Canada). N-Terminal amino acid sequence analysis was performed using a Model PI 2090E amino acid analyzer (Proton Instruments, Inc., Tarzana, CA).

Western Blot Analyses. Western blot analysis was performed on the partially purified placental extract prior to HPLC chromatography. The soluble proteins (400 μg) were separated on a 12% SDS−polyacrylamide gel (Laemmli), transferred to Immobilon (Milipore, Bedford, MA), and blocked with 5% instant milk. The blots were incubated overnight at room temperature with a 1:1000 dilution of goat anti-apo A-I serum (IncStar, Stillwater, MN). The goat antibody was then detected using an ECL detection system (Amersham, Arlington Heights, IL) with a 1:5000 dilution of donkey anti-goat horseradish peroxidase-conjugated IgG (Chemicon, Temecula, CA). Purified apo A-I, prepared by the method of Anantharamaiah and Garber (1995), was used as a control. Analysis of the fractions eluting from the preparative and analytical C-18 columns was performed by dot blot analysis using a second polyclonal antibody specific for human apo A-I (Advanced Chem Tech, Louisville, KY).

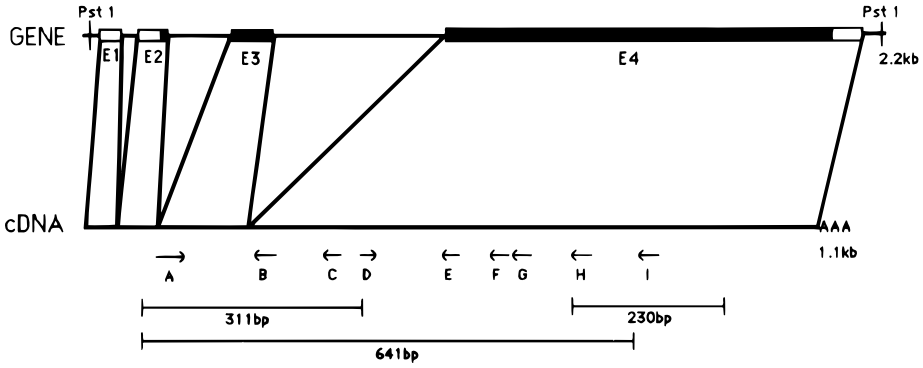
Molecular Characterization of ALP

RNA Isolation and Northern Blot Analysis. The human hepatoma cell line HepG2 was obtained from the American Type Culture Collection (Rockville, MD) and was grown in Eagles MEM supplemented with 2 mM glutamine and 10% heat-inactivated fetal bovine serum. The hepatoma cells were maintained at 37 °C in a humidified atmosphere of 95% air/5% CO_2. The cells were grown to 90% confluency in 100 cm^2 plastic tissue culture plates, and three plates were used for RNA isolation. For the placenta studies, placental tissue was washed 3 times with cold PBS. Total cellular RNA from the HepG2 cells and placental tissue were isolated by the single-step guanidinium thiocyanate−phenol−chloroform extraction method of Chomczynski and Sacchi (1987). The RNA preparations were stored at −70 °C. Human liver RNA was a gift from Dr. David Witte, Department of Pathology, Children's Hospital Medical Center, Cincinnati, OH. Human uterine decidual RNA was extracted from tissue peeled from the chorion membranes at the time of harvesting the placenta tissue.

For Northern analysis, 2 μg of human liver RNA and 20 μg of placental RNA were electrophoresed in formaldehyde-denaturing gels (Sambrook et al., 1989). The liver was used since this tissue expresses relatively large amounts of apo A-I. The electrophoresed RNA was transferred to nylon membranes (Genescreen, NEN, Boston, MA) via capillary blotting, covalently linked to the membranes using a Stratagene (Stratagene, La Jolla, CA) Model 1800 UV Crosslinker (according to the manufacturer's instructions), and then utilized immediately for hybridization to ^{32}P-labeled probes. One probe was a 641 bp *Pst*I/*Stu*I fragment that includes nt 110−750 of the apo A-I mRNA (Figure 1) (Law and Brewer, 1985). The other probe was a riboprobe synthesized from a 231 bp fragment of apo A-I cDNA comprising the 3′ end of the cDNA (nt 636−866). The fragment was isolated by *Bss*HII/*Pst*I digestion of apo A-I cDNA clone pAI-113 (Breslow et al., 1982) and ligated into a pGem 1 vector. The pGem 1 vector with the cDNA insert was then linearized by restriction enzyme digestion 5′ to the insert, and a ^{32}P-UTP-labeled riboprobe corresponding to the antisense DNA strand was synthesized using T7 RNA polymerase (Sambrook et al., 1989).

Hybridization of the 641 bp cDNA was performed at 37 °C for 18 h in the presence of 1.25 M sodium chloride, 0.75 M sodium citrate, 0.1% SDS, 5× Denhardt's solution, 50% formamide, and 10^6 dpm/mL probe (specific activity > 10^8 dpm/μg). Filters were washed at 65 °C in 0.1× SSC containing 0.2% SDS. The washed filters were exposed to Kodak XAR film at −70 °C with intensifying screens. Sizes of mRNA signals were determined by direct comparison to RNA standards electrophoresed on the same gel. RNA (20 μg) from human uterine decidual tissue was used as a negative control. Hybridization with the apo A-I 3′ riboprobe was performed under more stringent conditions. Hybridization was performed at 65 °C in 0.65 M sodium chloride, 50 mM sodium phosphate, pH 7.4, 50 mM EDTA, 0.2% Blotto, and 0.5% SDS in the absence of formamide. The membrane was washed at 65 °C in 0.1× SSC and 0.1% SDS.

S1 Nuclease Protection Assay. The 641 bp *Pst*I/*Stu*I fragment (nt 110−750) was ligated into the pBS SK+ and KS+ vectors (Stratagene). Single-stranded phagemid DNA was isolated from each clone to give antisense and sense strand apo A-I insert DNA. The S1 nuclease protection assay was performed by the method described by Adrian et al. (1984). HepG2 (1 μg) and placental (5 μg) poly(A^+) RNA, isolated by the method of Sambrook (1989), were hybridized to 50 ng of antisense or sense single-stranded apo A-I cDNA.



OLIGO	NUCLEOTIDES	AMINO ACID	SEQUENCE
A	127-160 S	8-19	5'-TGGGATCGAGTGAAGGACCTGGCCACTGTGTAC-3'
B	299-324 AS	65-73	5'-CCCAGAATTCCTGGGTCACAGGGCC-3'
C	393-413 AS	97-101	5'-TCGTCCAGGTAGGGCTGCAC-3'
D	418-438 S	105-111	5'-CAGAAGAAGTGGCAGGAGGA-3'
E	516-536 AS	138-144	5'-AGTGGGCTCAGCTTCTCTTG-3'
F	551-571 AS	148-155	5'-ATGGGCGCGCGCGCGGTCGC-3'
G	572-592 AS	156-164	5'-TGCGTGCGCAGCGCGTCCAC-3'
H	638-658 AS	178-184	5'-GTTCTCCTTGAGAGCCTCAA-3'
I	778-798 AS	225-231	5'-CTCAGGAAGCTGACCTTGAA-3'

FIGURE 1: Diagram of cDNA probes and oligonucleotide primers used in this study. The nucleotides are given for the cDNA (S = sense strand, AS = antisense strand) as reported in Law and Brewer (1985). The 641 bp and 230 bp lines represent the cDNAs used in the Northern blots. The 641 bp cDNA was also used in the S1 nuclease experiment. The 311 bp cDNA was used to probe the S1 nuclease assay. The amino acids are given for the mature hepatic apo A-I protein. The seventh base in primer B was changed to an A to create an *Eco*RI site.

The digested products were size-fractionated on a formaldehyde (6%)/agarose (1%) gel. The nucleic acids were then transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH), and the membrane was probed with a 311 bp apo A-I cDNA fragment (nt 110−420) that was labeled with ^{32}P to a specific activity 10^8 dpm/μg by the random primer method.

Oligonucleotide Primers. Two sense oligonucleotide primers corresponding to nt 127−160 and 418−438 of the apo A-I cDNA (primers A and D) and seven antisense primers corresponding to nt 299−324 (primer B), 393−413 (primer C), 516−536 (primer E), 551−571 (primer F), 572−592 (primer G), 638−658 (primer H), and 778−798 (primer I) of the apo A-I cDNA were synthesized Law & Brewer, 1985). The sequence of primer B had an A substituted for G at position 318 to create an *Eco*RI site. The sequence and location of these primers relative to apo A-I cDNA and the genomic structure are shown in Figure 1.

PCR Analysis and DNA Sequencing. Poly(A$^+$) RNA was obtained using an oligo(dT)−cellulose column as previously described; 1 μg of HepG2 or 5 μg of placental poly(A$^+$) RNA was reverse-transcribed with oligo(dT) for 60 min at 42 °C in 1 mM dNTPs, 10 units of RNase inhibitor (BRL, Gaithersburg, MD), 2 mM DTT, 1× RT buffer (BRL), 5 μg of oligo(dT), and 5 units of AMV-RT (BRL) in a total volume of 60 μL. For PCR amplification, 2 μL of placental and 1 μL of HepG2 RT products were amplified with 50 pmol of 5′ and 3′ primers, 1.5 mM magnesium chloride, 1× PCR buffer (Cetus, Norwalk, CT), 0.2 mM dNTPs, and 2.5 units of Taq polymerase (Cetus) in a total volume of 50 μL. The samples were heated to 94 °C for 5 min, and then subjected to 35 cycles of amplification at 94 °C for 1 min, 30 s, 65 °C for 2 min, 72 °C for 3 min, and finally at 72 °C for 10 min. Eight microliters of each PCR product was electrophoresed on a 2% agarose/TAE gel, and the bands were visualized with ethidium bromide staining.

Sequence analysis of the 198 bp (nt 127−324 of apo A-I cDNA) and the 411 bp (nt 127−536) PCR products was done by ligating the products into the PCR II plasmid in the TA cloning kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions. Double-stranded DNA purified from each clone was used for dideoxy chain termination sequencing in each direction using Sequenase (United States Biochemical, Cleveland, OH) according to the manufacturer's instructions.

RESULTS

Chemical Characterization of ALP. Western blot analysis of a partially purified placental extract with a polyclonal antibody to apo A-I demonstrated an immunoreactive band with an apparent mass of 23.5 kDa. The mass of human plasma apo A-I under identical conditions was 28 kDa (Figure 2). Following further purification of the placental extract by reverse-phase chromatography on a C-18 column using a 10−80% acetonitrile gradient with 0.1% TFA, two immunoreactive fractions (fractions 4 and 10) were detected by Western dot blot analysis (Figure 3). The apparent mass of fraction 4 by SDS−PAGE was 23−25 kDa, and the apparent mass of fraction 10 was approximately 68 kDa. Mass spectral analysis revealed that each fraction had a mass of 22 721 daltons, and the amino acid sequence of the first four amino acids of each fraction was identical with those of human apo A-I (Asp, Glu, Pro, and Pro). Taken together,

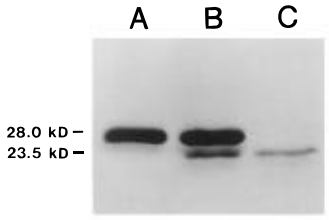


FIGURE 2: Western blot analysis of proteins from a partially purified human placental extract and purified apo A-I using an antiserum to apo A-I. Lane A is control apo A-I (50 ng), lane C is placental extract, and lane B is a mixture of apo A-I and placental extract. The placental extract had been subjected to sodium bromide flotation (density = 1.215 g/mL) as described under Experimental Procedures. The proteins were separated on a 12% SDS−polyacrylamide gel, transferred to Immobilon, and incubated with goat anti-apo A-I serum. The goat antibody was detected by an ECL detection system with horseradish peroxidase-conjugated second antibody. ALP and apo A-I migrated with apparent molecular masses of approximately 23.5 and 28.0 kDa, respectively.

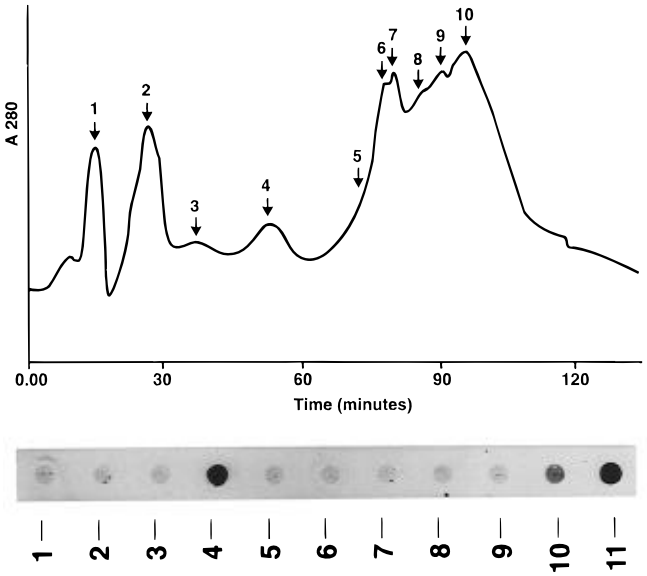


FIGURE 3: Profile of placental extract proteins recovered from the middle fraction of a 1.215 g/mL sodium bromide flotation and separated by HPLC on a preparative C-18 reverse-phase column. The column (30 cm × 20 mm) was eluted with a gradient of 10−80% acetonitrile with 0.1% trifluoroacetic acid, and 1.0 mL fractions were collected. Ten peak fractions were analyzed for apo A-I immunoreactivity by Western dot blot using a polyclonal antibody specific for apo A-I (panel B). Only two of the fractions (4 and 10) were positive for apo A-I immunoreactivity. Fraction 11 is a purified apo A-I positive control.

these findings strongly suggested that the 68 kDa fraction was an aggregate of the 23 kDa fraction. Subsequent analytical HPLC of the 23 kDa material on a C-18 reverse-phase column with a 10−80% acetonitrile gradient with 0.1% trifluoroacetic acid (flow rate 1.0 mL/min) indicated that the placental protein eluted much later than human apo A-I (29.06 min compared to 22.32 min), indicating that ALP is more hydrophobic than apo A-I (Figure 4).

Molecular Characterization of Placental RNA. Hybridization of placental RNA with a 641 bp apo A-I cDNA fragment (Figure 5) detected a hybridization band of approximately 850 nt (lane B). In contrast, hybridization of total RNA from human liver resulted in a single band of 1100 bp (lane D), a size that corresponds to that reported for human apo A-I mRNA (Cheung & Chan, 1983). Hybridization of a mixture of placental and liver mRNAs resulted in two bands of 850 and 1100 bp (lane C). Even though 10 times more placental

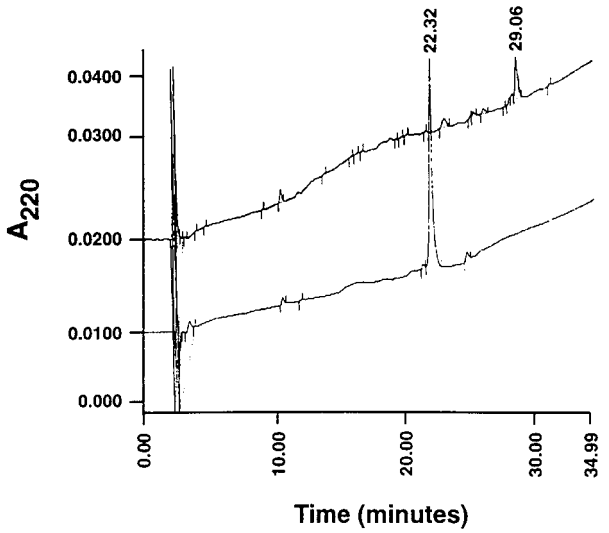


FIGURE 4: Comparison of HPLC retention times for ALP and apo A-I on a C-18 reverse-phase column. The column (VYDAC, 4.5 mm × 25 cm) was eluted with a gradient of 10−80% acetonitrile with 0.1% trifluoroacetic acid at a flow rate of 1.0 mL/min. The retention times of ALP and apo A-I were 29.06 and 22.32 min, respectively.

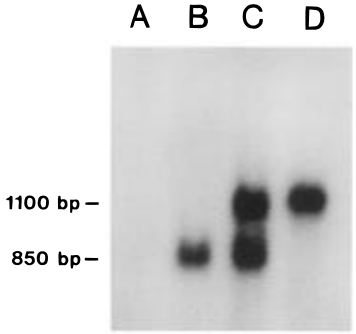


FIGURE 5: Northern blot hybridization of decidua, placenta, and liver RNAs: Hybridization with a 641 bp apo A-I cDNA (nt 110−750). Lane A, decidua (20 μg); lane B, placenta (20 μg); lane C, placenta (20 μg) and liver (2 μg); and lane D, liver (2 μg). The decidua RNA was used as a negative control.

RNA was loaded on the gel than liver RNA (20 μg vs 2 μg of total RNA), the intensity of the placental band was still less than that of the liver band, suggesting a low abundance of the placental ALP RNA.

When placental and liver RNA were probed with an apo A-I 3′ riboprobe under stringent conditions, no signal was observed for the placenta while the liver RNA gave a strong signal (data not shown). This is in contrast to the Northern blot in Figure 5 which showed a strong signal in both samples when a 5′ probe was used, suggesting that the placental and liver RNAs differ at the 3′ end.

S1 nuclease protection assay performed on placental and HepG2 mRNAs using a *Pst*I−*Stu*I cDNA (nt 110−750) fragment of apo A-I cDNA confirmed striking differences between the two mRNAs (Figure 6). S1 nuclease digestion of placenta mRNA revealed a single product of approximately 460 bp. In contrast, S1 nuclease digestion of HepG2 mRNA revealed two products of approximately 641 and 460 bp. The larger product of 641 bp is the expected size for apo A-I, while the smaller product of approximately 460 bp in the placenta is consistent with the shorter apo A-I-like mRNA detected by Northern blot hybridization. The presence of a 460 bp fragment in HepG2 mRNA suggests that

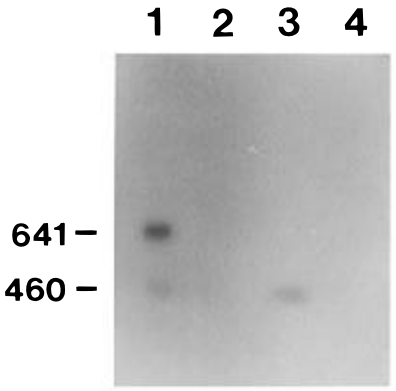


FIGURE 6: Autoradiogram of S1 nuclease analysis of placenta and HepG2 RNA. A 641 bp cDNA fragment (nt 110−750) was subcloned into pBS SK+ and KS+ plasmids. Single-stranded phagemid DNA was hybridized to RNA. Unhybridized RNA and single-stranded DNA were digested with S1 nuclease. The samples were electrophoresed on a 6% formaldehyde/1% agarose gel. Products were probed with a 311 bp *Pst*I/*Sau*I cDNA (nt 110−420) labeled with ^{32}P. Lane 1, HepG2 RNA with antisense ssDNA−pBS KS+; lane 2, HepG2 RNA with sense ssDNA−pBS SK+; lane 3, placental RNA with antisense ssDNA; lane 4, placenta RNA with sense ssDNA.

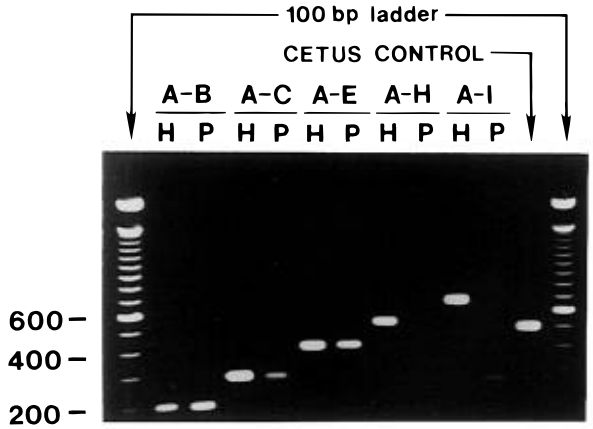


FIGURE 7: Ethidium bromide stain of RT-PCR analysis. RNA was reverse-transcribed with oligo(dT) and amplified with the primers indicated. Letters above the lines (A, B, C, E, H, and I) are primers used for RT-PCR as described in Figure 1. Letters below the lines are samples used as templates; H is Hep G2 RNA; P is placenta RNA. Cetus control is a 500 bp Perkin-Elmer Cetus control amplified product. The faint 200 bp band seen in the P(A-I) lane is due to the homology of oligo I to the 380−410 region of the cDNA.

HepG2 cells also express an alternatively spliced apo A-I-like mRNA that is undetectable by Northern blot analysis using 2 μg of total RNA (see Figure 5).

RT-PCR Amplification of Placental RNA and Sequencing of the PCR Products. To define more clearly the differences in the placental and liver apo A-I RNAs, a series of PCR analyses was performed on reverse-transcribed products of mRNA from human placental tissue and HepG2 cells with oligonucleotide primers corresponding to eight different regions of the apo A-I cDNA. PCR analysis of HepG2 RNA with sense primer A and antisense primers B, C, E, H, or I (see Figure 1) gave products with the expected sizes of 198, 288, 411, 531, and 672 bp, respectively (Figure 7).

PCR amplification of reverse-transcribed placental RNA with sense primer A and antisense primers B, C, or E also yielded products with sizes of 198, 288, and 411 bp. However, no PCR products were obtained when RT-PCR

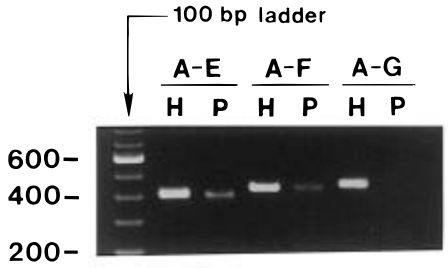


FIGURE 8: Ethidium bromide stain of RT-PCR analysis adjacent to the putative splice site at nt 570−571. Letters above the lines (A, E, F, and G) are primers described in Figure 1. Letters below the lines are samples used as templates; H is HepG2 RNA; P is placenta RNA.

amplification of placental mRNA was performed using sense primer A and antisense primers H or I, even though the PCR amplifications were performed in the same experiment, under identical conditions, as the PCR amplifications of the HepG2 RNA (Figure 7). In addition, no products were obtained when sense primer D at positions 418−438 was paired with primers E, H, or I (data not shown). DNA sequencing of the 198 and 411 bp placental RT-PCR products indicated 100% homology to segments of the apo A-I cDNA, corresponding to nt 127−324 and 127−536 of apo A-I cDNA as reported by Law and Brewer (1985).

Analysis of the apo A-I cDNA sequence reveals a single putative splice site between primers E and H that is consistent with the 460 bp placental mRNA fragment detected in the S1 nuclease protection assay. The site is at nucleotides 569−576 of the cDNA. To examine the possibility that alternative splicing of apo A-I pre-mRNA occurs at this site of the apo A-I cDNA, two antisense PCR primers (F and G) that surround this site were synthesized. Primer F corresponds to apo A-I cDNA sequences that are 20 bp immediately upstream (5′) of nt 570, and primer G corresponds to sequences 20 bp immediately downstream (3′) of nt 570. As shown in Figure 8, RT-PCR amplification of HepG2 RNA with primer A and either primer F or primer G yielded products with the expected sizes of 443 and 463 bp. In contrast, RT-PCR amplification of placenta mRNA with primers A and F gave a product of 443 bp, but amplification with primers A and G resulted in no product.

DISCUSSION

The results of this study indicate that human term placenta expresses an apo A-I-like protein, ALP. Western blot analysis of placental extracts using an antiserum to apo A-I indicated that ALP has a molecular mass of approximately 23.5 kDa, which is smaller than that of apo A-I (28 kDa). Mass spectral analysis of the placental extract purified by reverse-phase HPLC revealed a mass of 22 721 daltons. Amino acid sequence analysis of the amino-terminal four amino acids of ALP indicated a sequence identical to that of apo A-I. Although ALP has striking similarities to apo A-I, ALP does not appear to be a degradation product of apo A-I since Northern blot analyses of placental and HepG2 RNAs with an apo A-I cDNA probe indicate that the cDNA probe hybridizes to an mRNA in the placenta that is approximately 250 nucleotides smaller than apo A-I mRNA. Placenta mRNA hybridized to apoA-I cDNA probes that encompassed the 5′ end of the gene. However, a riboprobe corresponding to the 3′ end of the apo A-I cDNA did not

Table 1: Exon/Intron Junction Sequences in the Human Apolipoprotein A-I Gene[a]

exon 1/intron A	GAG/GTGCGT
exon 2/intron B	CGG/GTAGGT
exon 3/intron C	AAA/GTAAGG
exon 4/intron D (putative)	CAT/GTGGAC
consensus	$NA^{64}G^{73}/G^{100}T^{100}A^{62}A^{68}G^{84}T^{63}$

[a] The splice junction of apo A-1-like protein (ALP) is shown. Exons 1−3 and introns A−C are those of the hepatic apo A-1 gene. Intron D, proposed in this paper, interrupts exon 4 at position 570 of the cDNA. A consensus sequence is given for mammalian genes (Breathnach et al., 1978; Sharpe, 1981). The superscript numbers for the consensus sequence show the number of times, reported as a percentage (Sharpe, 1981), that this base appears at this position of a junction. The sequence of exon 5 in ALP has yet to be determined.

hybridize to placental RNA under conditions of stringency that were optimal for hybridization to apoA-I mRNA, indicating that the 3′ sequence of apo A-I-like mRNA is markedly different from the published apo A-I sequence (Law & Brewer, 1985).

A further difference in the two mRNAs was shown by a S1 nuclease protection assay in which a 641 bp apo A-I cDNA fragment extending from nt 110−751 of the full-length mRNA detected a digestion product of 460 nt. RT-PCR analysis of the placental RNA with primers that extend into the 3′ end of the molecule also shows a difference in the two mRNAs. RT-PCR analysis of placental mRNA with sense and antisense primers that are located before nt 570 shows identical products to hepatic apo A-I. However, when a sense primer located before nt 570 is paired with an antisense primer located after nt 570, no products are obtained in the placental mRNA. Since there is only a single copy of the apo A-I gene in the human genome and the sequence of the RT-PCR products revealed 100% homology to hepatic apo A-I, these data strongly suggest the existence of an alternatively spliced apo A-I-related mRNA species in the placenta that codes for a protein that differs from apo A-I at the carboxyl terminus. Previous studies have shown a consensus sequence of AGGTAAGT for a donor splice site in mammalian genes, with a requirement for the first GT dinucleotide (Breathnach et al., 1978). Analysis of the apo A-I cDNA sequence reveals only a single putative splice site at nucleotides 569−576 (ATGTGGAC) that is consistent with the 460 bp placental mRNA fragment detected in the S1 nuclease protection assay (Table 1) and confirmed by RT-PCR analysis (Figures 7 and 8). A donor site at this location would give a fourth intron to the gene encoding ALP.

At present, the biological role of the placental apo A-I-like protein is unknown. Studies from our laboratory strongly suggest a novel role for HDL and apo A-I in the regulation of placental lactogen (hPL) expression during pregnancy (Handwerger et al., 1987, 1995). In these investigations, HDL and apo A-I at physiological concentrations were shown to stimulate the synthesis and release of hPL from human trophoblast cells and placental explants. The stimulation of hPL release by HDL was not due to the lipid constituents of HDL but rather to the apolipoprotein constituents. It is possible that the apo A-I-like protein may also be involved in the regulation of hPL expression by an autocrine/paracrine mechanism. It is also possible that the apo A-I-like variant may be a constituent of the novel VLDL-like particle isolated from human placental extracts (Park et al., 1988).

In summary, the results of this study indicate that the human term placenta expresses a 22.7 kDa apo A-I-like protein, ALP, that appears to arise from an alternative splice of the apo A-I primary transcript. The amino-terminal end of ALP appears to be identical or nearly identical to apo A-I, while the carboxyl-terminal ends of the two molecules are different. The complete sequence and the physiological role(s) of this novel apo A-I-like protein, ALP, remain to be elucidated.

ACKNOWLEDGMENT

We thank Darrell Capel, Cherie Kessler, and Anthony Meyer for technical assistance, Drs. Jeffrey Whitsett, Jeffrey Robbins, James Greenberg, Richard Akeson, Barbara Burkhardt, and Philip Zeitler for valuable advice, and Vicki Livengood for secretarial assistance.

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BI9526950