Plasma lipid transport in the hedgehog: partial characterization of structure and function of apolipoprotein A-I

Doris A. Sparrow,* P. Michel Laplaud,[1,†] Michel Saboureau, Guoying Zhou,†† Peter J. Dolphin,†† Antonio M. Gotto, Jr.,* and James T. Sparrow***

Department of Medicine,* Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030; Unité 321 INSERM,† Pavillon Benjamin Delessert, Hôpital de la Pitié, 83 boulevard de l'Hôpital, 75651 Paris Cedex 13, France; Centre d'Etudes Biologiques de Chizé,** Centre National de la Recherche Scientifique, 79360 Beauvoir-sur-Niort, France; and Department of Biochemistry,†† Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Abstract Apart from exhibiting the presence of lipoprotein [a] in its plasma, another interest of the European hedgehog in lipoprotein research lies in the quantitative prominence of a complex spectrum of high density lipoproteins (HDL) and very high density lipoproteins (VHDL) as cholesterol transporters in plasma (Laplaud, P. M. et al. 1989. *Biochim. Biophys. Acta.* **1005:** 143–156). We, therefore, initiated studies in the field of reverse cholesterol transport in the hedgehog. As a first step, we characterized apolipoprotein A-I (apoA-I), the main protein component of hedgehog HDL and VHDL. Proteolytic cleavage of apoA-I (M_r approx. 27 kDa) using two different enzymes resulted in two sets of peptides that were subsequently purified by high performance liquid chromatography, and that allowed us determination of the complete protein sequence. Hedgehog apoA-I thus consists of 241 amino acid residues and exhibits an overall 58% homology to its human counterpart, i.e., the lowest value observed to date among mammalian species. However, it retained the general organization common to all known apoA-Is, i.e., a series of amphipathic helical segments punctuated by proline residues. Circular dichroism experiments indicated a helical content of approx. 45%, increasing to approx. 58% in the presence of lecithin unilamellar liposomes. Apart from other differences, amino acid composition analysis shows that hedgehog apoA-I contains four isoleucine residues, while this amino acid is totally absent from the corresponding protein in higher mammals. Polyclonal antibodies raised against hedgehog apoA-I failed to detect any cross-reactivity between the animal and human proteins, although comparative prediction of the respective antigenic structures using the Hopp-Woods algorithm indicated that several potentially antigenic sites may occur in similar regions of the protein. Finally, hedgehog apoA-I was shown to be able to activate lecithin:cholesterol acyl transferase, although it was 4 to 5 times less efficient in this respect than the human protein.—**Sparrow, D. A., P. M. Laplaud, M. Saboureau, G. Zhou, P. J. Dolphin, A. M. Gotto, Jr., and J. T. Sparrow.** Plasma lipid transport in the hedgehog: partial characterization of structure and function of apolipoprotein A-I. *J. Lipid Res.* 1995. **36:** 485–495.

Supplementary key words preparative electrophoresis • amino acid sequence • circular dichroism • lecithin:cholesterol acyl transferase • high density lipoproteins • very high density lipoproteins • reverse cholesterol transport • phylogeny

The European hedgehog is a species of considerable interest in lipoprotein research. Indeed, we have demonstrated (1) that this hibernator is among the few species whose plasma contains lipoprotein [a] (Lp[a]), a lipoprotein particle with highly probable implication in both atherogenesis and thrombosis (2–4). In addition, we have demonstrated (5) that the plasma lipoprotein spectrum of the hedgehog included another unusual feature, i.e., the occurrence of considerable amounts of VHDL, defined as particles with hydrated density 1.162–1.259 g/ml. These latter lipoproteins appeared heterogeneous and subject to season-dependent changes in both their distribution and chemical composition (6). During summer, which is the period of the year when the hedgehog is constituting fat stores before hibernation, VHDL appeared responsible for the transport of as much as 25% of total plasma cholesterol, while this value decreased to about 10% by the end of winter. For its part, the spectrum of HDL-like lipoproteins displayed a variety of particles with considerable size heterogeneity according to density (6) and exhibited in SDS gels the presence of a protein with apoA-I-like M_r as their prominent apolipoprotein component. This spectrum extended down to hydrated densities as low as 1.030 g/ml, approximately, during the fall, but only to about 1.080 g/ml in late spring, and preliminary results obtained in this laboratory revealed that such seasonal changes were equally present with

Abbreviations: HDL, high density lipoproteins, density as defined; VHDL, very high density lipoproteins, density as defined; apo, apolipoprotein; EDTA, ethylene diamine tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; LCAT, lecithin:cholesterol acyl transferase; DMPC, dimyristoyl phosphatidylcholine; HPLC, high performance liquid chromatography; CD, circular dichroism.

[1]To whom correspondence should be addressed.

regard to lipid composition (Laplaud, P. M., unpublished results). Considering the fact that the hedgehog exhibits seasonal variations of large amplitude of many biological parameters (see ref. 6 for a review), and especially of thyroid and male sex hormones (7), it appears that this species is of obvious interest for studies on the hormonal regulation of the metabolism of HDL and VHDL subfractions.

Such experiments are especially relevant with respect to reverse cholesterol transport (8, 9) and the characterization of particles specifically responsible for the so-called "protective role" of HDL against ischemic cardiovascular disease. Indeed, studies in this field have shown the physiological importance of various apoA-I-containing HDL subfractions, including some with electrophoretic pre-beta mobility and/or whose density corresponds at least partly to the VHDL range (10–12). These results have prompted us to initiate experimental work directed towards hedgehog HDL and VHDL structural and metabolic analysis. As a first step, we report in the present manuscript on the structural and functional characterization of the main apolipoprotein present in these particles, apoA-I.

MATERIALS AND METHODS

Animals and diets

Male adult hedgehogs were caught in the fields surrounding the Centre d'Etudes Biologiques de Chizé, located in western central France. They were kept individually in 6 m^2 parks under natural conditions of light, temperature, and rainfall. The animals were fed daily with a mixture of crushed chicken meat and commercial food for dogs (Canina Duquesne-Purina) containing the following proportions by weight of the major constituents: protein, 20%; animal fat, 6%; carbohydrate, 5%; vitamin A, 15,000 I.U./kg; vitamin D_3, 1,500 I.U./kg. Water was provided ad libitum.

Blood specimens

All the experiments reported in the present study were performed on blood samples obtained between April and mid-September from active animals. In each case, blood was taken from hedgehogs that had been fasted overnight for approximately 18 h. The animals were slightly anesthetized with fluothane (ICI Pharma) in oxygen, and blood was withdrawn by heart puncture.

Blood was collected on EDTA (final concentration 1 mM). Plasma was then separated by low-speed centrifugation at 4°C, and sodium azide (0.01%) and the proteolytic inhibitor PMSF (final concentration 1 mM) were added immediately. In every series of experiments, all manipulations leading to the purification of apoA-I were performed as quickly as possible.

Purification of hedgehog apoA-I

Hedgehog HDL (d 1.100–1.210 g/ml) were obtained by sequential ultracentrifugal flotation according to established procedures (13), in an MSE Prepspin 50 ultracentrifuge (MSE Scientific Instruments, Crawley, UK), using an MSE 8 × 14 ml aluminum fixed-angle rotor. Lipoproteins were subsequently dialyzed (1), delipidated with ethanol–diethyl ether 3:1 (v/v) (14), and the apolipoprotein residue was dried under nitrogen.

Apolipoprotein A-I was isolated using preparative electrophoresis according to the methodology of Stephens (15). For this purpose apolipoproteins were dissolved to a concentration of 3 mg/ml in a 0.05 M phosphate buffer (pH 8.2), containing 4% SDS; a 100 μl aliquot was dansylated according to the technique of Talbot and Yphantis (16). The dansylated aliquot was mixed with the bulk of the sample, and the mixture was heated at 90°C for 3 min. The apolipoprotein solution was then dialyzed in Spectrapor tubing (exclusion limit 3,500) against 2.5 mM Tris-glycine containing 0.1% (w/v) SDS, pH 8.3 at room temperature for 3 × 12 h. After addition of 50 μl of bromophenol blue as a tracking dye, aliquots containing 1 mg protein were placed on polyacrylamide gels (7.5% monomer; 1.5 cm in diameter and 10 cm long) containing 1% SDS. Electrophoresis was then carried out at 300 V (about 7 mA/gel) and 15°C; migration was monitored by UV illumination in the dark. Gels were subsequently removed from the glass tubes and bands corresponding to apoA-I were sliced out. Slices were stacked in a similar glass tube which was then tied to a dialysis bag of Spectrapor tubing (exclusion limit 3,500). Electrodialysis was carried out in the same electrophoresis chamber as above for approx. 3–4 h at 150 V and 15°C, using a buffer solution of 2.5 mM Tris-glycine containing 0.1% SDS. On completion, the fluorescent content of the dialysis bag was recovered and exhaustively dialyzed against a solution of 5 mM NH_4HCO_3. The protein solution was then lyophilized.

Analytical electrophoresis

The molecular weight of hedgehog apolipoprotein A-I was estimated using gradient gel SDS-polyacrylamide electrophoresis on Excel Gel SDS, 8–18% monomer concentration (Pharmacia, Uppsala, Sweden). Gel calibration was performed using Low Molecular Weight protein calibration kit (Pharmacia).

Immunological studies

Double immunodiffusion was performed on glass slides according to Ouchterlony (17) in 1.2% agarose in a 0.05 M veronal buffer at pH 7.2. After 24 h at 37°C, slides were washed for 48 h in saline, dried in air, and then stained for protein with Amido black. Human apoA-I used in these studies was from Sigma (St. Louis, MO) and sheep monospecific antiserum to human apoA-I was

from Immuno AG (Vienna, Austria). A monospecific antiserum to purified hedgehog apoA-I was raised in rabbits and its specificity was verified by immunoelectrophoresis.

Sequence analysis

Enzymatic hydrolysis with arginylendopeptidase. One milligram of hedgehog apoA-I was dissolved in 200 μl 50 mM Tris-HCl buffer, pH 8.0, in a microfuge tube and 20 units arginylendopeptidase (Takara Biochemical Inc., Berkeley, CA) was added. The digestion was performed at 37°C for 18 h, and the reaction was terminated by the addition of 1 ml 0.1% TFA in 6 M guanidine hydrochloride. The hydrolysate was subjected to HPLC fractionation as described below.

Enzymatic hydrolysis with endoproteinase Asp-N. One milligram hedgehog apoA-I in 200 μl 0.1 M Tris-HCl buffer, pH 8.0, was added to a microfuge tube containing 2 μg endoproteinase Asp-N (sequencing grade, Boehringer Mannheim, Indianapolis, IN) (substrate:enzyme ratio = 250:1). The mixture was incubated at 37°C overnight (18 h); the hydrolysate was then subjected to HPLC fractionation after the addition of 1 ml 0.1% TFA, 6 M guanidine hydrochloride.

Peptide purification by HPLC. The peptide mixture was purified on a Spectra Physics 8000B HPLC with a Vydac 214TP1010 column (1.0 × 25 cm) (The Separations Group, Hesperia, CA) and a linear gradient of 0.1% TFA and 2-propanol (Burdick and Jackson, Muskegon, MI) from 0 to 70% over 1 h at a flow rate of 5 ml per min. The column temperature was set to 30°C and the eluted peptides were detected at 220 nm. The eluent under each peak was collected manually and prepared for gas phase sequence analysis by evaporation in a Speed-Vac.

Automatic sequence analysis. The peptides isolated by HPLC were dissolved in TFA and transferred to a Biobrene-treated glass filter. Automated sequence analysis was performed with an Applied Biosystems Model 477 Gas Phase Sequencer connected to a 120 PTH amino acid analyzer using the programs supplied with the instrument (Applied Biosystems, Inc., Foster City, CA).

Analysis of sequence data. Sequence alignment of the different apoA-Is compared was performed using the method of Altschul and Erickson (18). The evolutionary tree for apoA-I was constructed using calculations described by Klotz and Blanken (19).

Circular dichroism (CD)

The circular dichroism spectra were recorded on a Jasco 500 Spectropolarimeter equipped with an IBM compatible computer for data collection and manipulation. The protein (0.28 mg) was dissolved in 1 ml 0.01 M potassium phosphate, 0.1 M KF, pH 7.4, and the spectrum was recorded from 260 to 185 nm using a 0.05 cm circular cell. An aliquot of unilamellar DMPC liposomes was then added to give a lipid to protein molar ratio of 200:1. The mixture was incubated at the transition temperature overnight and the CD spectrum was recorded as above. The CD spectra were analyzed for secondary structure using the program of Chang, Wu, and Yang (20).

Activation of lecithin:cholesterol acyl transferase

Proteoliposomes were prepared by the cholate dialysis method essentially as reported by Chen and Albers (21). The molar ratios were egg lecithin:cholesterol:apoA-I, 250:15:0.8, and the radiolabel was [^{3}H]cholesterol. For comparative purposes, a similar series of experiments was conducted using human apoA-I purified from a pool of human sera by preparative isoelectric focusing as described by Jauhiainen and Dolphin (22). LCAT was purified as reported (22).

Each incubation contained: *a)* 125 μl 2% fatty acid-free BSA in assay buffer; *b)* 100 μl of substrate (experiments with proteoliposomes containing hedgehog apoA-I: 366933 cpm = 24462 cpm/nmol cholesterol; experiments with proteoliposomes containing human apoA-I: 229112 cpm = 15274 cpm/nmol cholesterol); *c)* assay buffer (10 mM Tris-HCl, pH 7.4, containing 150 mM NaCl to a final incubation volume of 500 μl). The incubation mixtures were pre-incubated at 37°C with gentle shaking for 20 min. Twenty-five μl 2-mercaptoethanol and 1.5 μg pure human LCAT were then added and the mixture was incubated at 37°C for the indicated period of time. The reaction was stopped by addition of chloroform–methanol 2:1 and the lipids were extracted (Folch extraction). Carrier cholesteryl ester was added and the lipids were separated by TLC (Silica Gel G in n-heptane–isopropyl ether–glacial acetic acid–methanol 60:40:4:2). Spots were identified with iodine vapor; the cholesteryl esters were scraped off and counted in a Beckman LS-7000 liquid scintillation counter. Cholesteryl ester formation was calculated from the known specific activity of the cholesterol in the substrate.

Protein determination

Lipoprotein protein and purified apolipoproteins were quantitated by the procedure of Lowry et al. (23) using bovine serum albumin (Sigma) as the working standard.

RESULTS

Molecular weight determination

Purified hedgehog apoA-I consistently migrated as a single band with M_r 27,350 ± 458 (n = 9) upon SDS-polyacrylamide gradient gel electrophoresis (data not shown).

Immunological reactivity

Immunodiffusion of a monospecific antiserum to hedgehog apoA-I produced a single, intensely stained precipitin line with the corresponding protein; a similar

reactivity was observed with hedgehog whole plasma. By contrast, no precipitation line could be detected between antiserum to hedgehog apoA-I and human apoA-I, or antiserum to human apoA-I and hedgehog apoA-I or whole plasma (data not shown).

Sequence determination

Hedgehog apoA-I was cleaved using arginylendopeptidase and endoproteinase Asp-N. After amino terminal analysis of each fraction, pure peptides were subjected to automated sequence analysis. Contaminated peptides were further purified by HPLC separation on a Vydac 218TP104 column with a gradient of 0.1% TFA and acetonitrile.

The complete amino acid sequence of hedgehog apoA-I is shown in **Fig. 1.** The amino-terminus of the protein was confirmed by the automatic gas phase sequencer for 49 cycles. The sequenced peptides were aligned on the basis of their homology with bovine apoA-I as reported previously (24). The primary structure of hedgehog apoA-I thus consists of 241 amino acid residues, taking into account the two deletions occurring at positions corresponding, respectively, to Pro_3 and Glu_{183} in the 243 residue-containing human apoA-I.

Circular dichroism

The CD spectrum of hedgehog apoA-I in the absence of phospholipid had a mean residue ellipticity of −14,700 at several different concentrations. Computer analysis of the CD spectrum indicated an helical content of approx. 45%; β-sheet, 20%; β-turn, 5%; and random coil, 30%. After overnight incubation with DMPC unilamellar liposome at a molar ratio of 200:1, the ellipticity decreased to −21,840 corresponding to an increase in α-helicity. Thus, analysis of the new CD spectrum indicated an α-helical content of 57.5%; β-sheet, 18%; β-turn, 4%; and random coil, 20.5%.

Activation of lecithin:cholesterol acyl transferase

The ability of hedgehog apoA-I to activate LCAT was assayed in two different series of experiments. First, whole

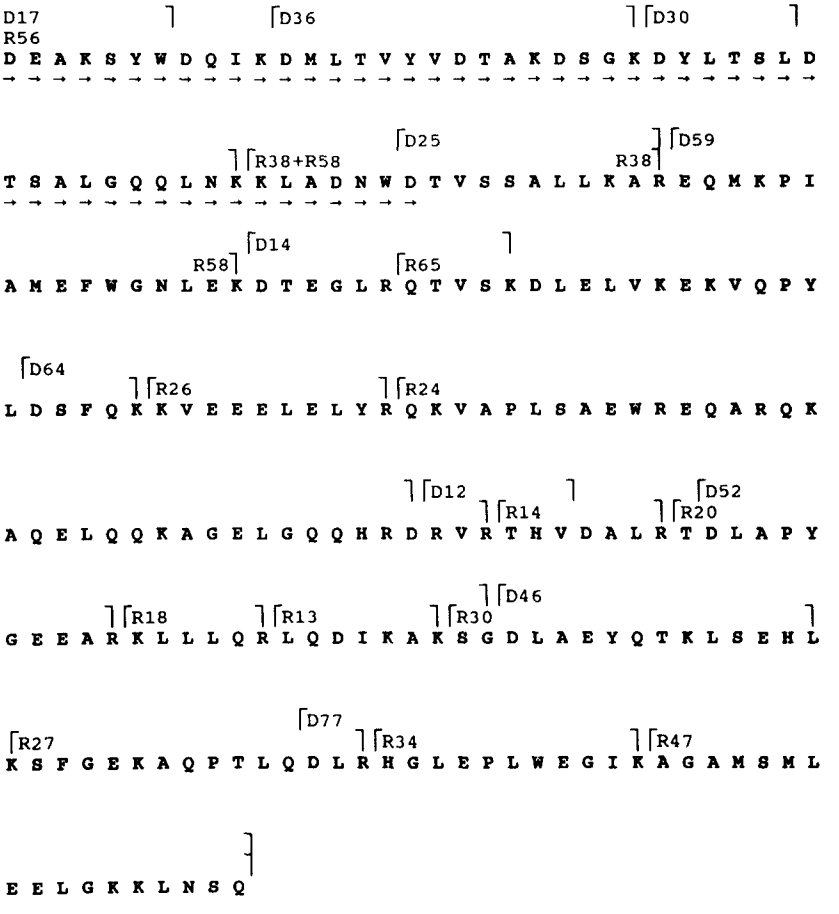

Fig. 1. Primary amino acid sequence of hedgehog apoA-I determined by sequence analysis of peptides generated by arginylendopeptidase and endoproteinase Asp-N digestion and aligned by homology with bovine apoA-I. The N-terminal sequence of the protein was confirmed by automatic gas phase sequencing for 49 cycles. (→) Residues determined by sequence analysis of whole protein. R(n) and D(n), fractions from HPLC separation of the products of arginylendopeptidase digestion and endoproteinase Asp-N digestion, respectively.

hedgehog plasma was used in conjunction with proteoliposomes prepared using either hedgehog or human apoA-I. Results (**Table 1**) showed that in the homologous hedgehog system, the mean rate of formation of cholesteryl esters was about 6.2 nmol/h per ml versus about 2 nmol/h per ml when human apoA-I replaced the animal protein ($P < 0.0005$). However, it is of note that in a series of similar experiments conducted simultaneously, the use of normolipidemic human plasma added with proteoliposomes containing human apoA-I resulted in the formation of 23.2 nmol/h per ml of cholesteryl esters. Thus, when hedgehog and human plasmas were simultaneously incubated with identical proteoliposomes containing human apoA-I, the ratio of their respective activities was found to be about 10% (2 vs. 23.2 nmol/h per ml of cholesteryl esters formed).

Purified human LCAT was subsequently used in activation experiments in the presence of proteoliposomes containing either human or hedgehog apoA-I. Our data (**Fig. 2**) showed that although it was indeed capable of activating the enzyme, the animal protein appeared considerably less efficient in this respect than its human counterpart. Indeed, after a 30-min. incubation, the absolute activity of human LCAT resulted in the formation of 48 nmol/h per ml cholesteryl esters when the enzyme was activated by human apoA-I versus about 12 nmol/h per ml when the activating protein was hedgehog apoA-I.

DISCUSSION

As a first step in the structural and metabolic characterization of HDL and VHDL subfractions in the hedgehog, we have determined the complete amino acid sequence of the prominent protein component of these lipoproteins, apolipoprotein A-I. To perform this sequence determination, we chose to use the arginine-specific protease arginylendopeptidase as amino acid analysis indicated a lower arginine content in the hedgehog protein than in its human counterpart. Using this enzyme, we observed the occurrence of cleavages at the level of lysine residues, a phenomenon already reported by others (25). However, we obtained a majority of the protein sequence from these peptides, although we did not find a hexapeptide, DTEGLR (positions 77–82), resulting from cleavage at a lysine residue and the two dipeptides DR and VR present at positions 149–150 and 151–152, respectively. Due to the small size of these latter arginine cleavage fragments, it became necessary to use the aspartic acid-specific endoprotease, Asp N, to confirm their sequence positions. As was the case with regard to bovine apoA-I (24), we observed cleavage by this latter enzyme at Glu residues in a non-sequence-specific manner.

The amino acid composition of hedgehog apoA-I, derived from the sequence analysis, is presented in **Table 2,** and compared with those of the homologous protein in most of the mammalian species in which the composition of apoA-I has been determined to date, including human (26), baboon (27), rat (28), mouse (29), dog (30), badger (31), rabbit (32), and calf (33) apoA-I. Although such a comparison provided evidence for the overall similarity existing between apoA-I in all these species, hedgehog apoA-I presented several marked differences compared with the corresponding protein in humans and other animals. Indeed, the hedgehog protein was poorer than most of its counterparts in Pro, Val, Leu, Phe, and Arg, while it was markedly enriched in Gly, Lys, and especially Ile (4 mol/mole protein), and Met (5 mol/mole protein). Data regarding Ile are particularly remarkable, as this

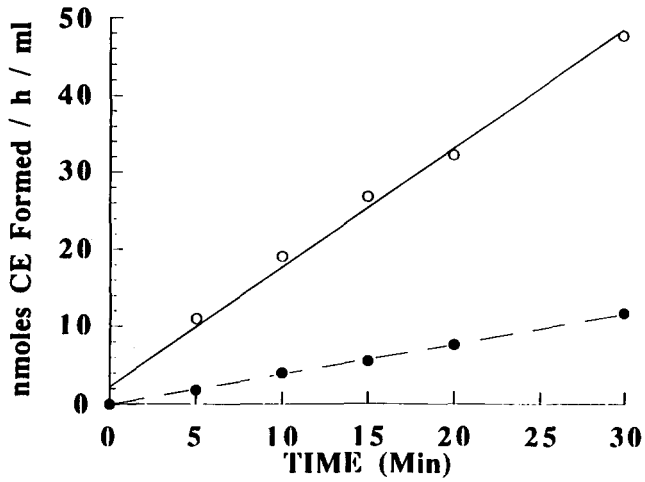

Fig. 2. Activation of human LCAT by human (-O-) and hedgehog (--●--) apoA-I in lecithin/cholesterol proteoliposomes with molar ratios egg lecithin:cholesterol:apoA-I 250:15:0.8. Radiolabel was [^{3}H]cholesterol. After incubation with LCAT, the reaction was stopped and the lipids were separated by TLC. The spot corresponding to cholesteryl esters was scraped off and counted. See Materials and Methods for further details of the experiments.

TABLE 1. Assay of hedgehog plasma LCAT activity with hedgehog and human apoA-I-containing proteoliposomes

Animal Identification	Hedgehog Plasma + Hedgehog ApoA-I	Hedgehog Plasma + Human ApoA-I
	nmol CE formed/h/ml	
249	5.13	2.58
251	7.18	1.80
270	6.03	1.55
319	7.19	1.49
321	6.53	2.20
322	5.25	2.26
	6.22 ± 0.91[a]	1.98 ± 0.43[a,b]

Hedgehog samples were shipped on wet ice and assayed within 24 h of arrival in Halifax. Each proteoliposome contained lecithin (egg):cholesterol:apoA-I in the molar ratios of 250:15:0.8 and was radiolabeled with [^{3}H]cholesterol. The LCAT assay was as described by Jauhiainen and Dolphin (22).

[a] Mean ± SD.

[b] $P < 0.0005$ (paired t-test) versus results obtained using hedgehog apoA-I.

TABLE 2. Amino acid composition of apolipoprotein A-I from hedgehog and various mammals

Amino Acid	Hedgehog	Human (26)	Baboon (27)	Rat (28)	Mouse (29)	Dog (30)	Badger (31)	Rabbit (32)	Calf (33)
Lys	26	21.0	20.3	20.7	16.2	19.7	18.1	19.9	21.1
His	4	4.5	7.3	4.7	4.8	2.1	3.9	1.9	3.4
Arg	12	15.7	15.5	12.2	15.7	16.8	16.3	17.0	14.9
Asp	22	20.5	19.5	31.4	25.2	22.3	21.9	20.4	22.3
Thr	11	9.6	11.0	11.2	13.1	6.4	9.9	10.3	6.9
Ser	14	14.7	15.9	10.1	16.7	14.3	11.9	17.3	14.1
Glu	45	45.7	46.1	41.9	48.6	47.8	45.4	52.2	51.9
Pro	6	10.4	10.2	6.7	9.5	9.7	9.7	8.6	8.7
Gly	13	10.2	11.0	11.2	9.8	11.9	12.9	8.9	11.6
Ala	20	19.0	18.1	18.1	14.6	23.7	24.0	18.0	25.4
Val	10	13.3	14.9	12.6	15.8	15.7	15.4	13.9	12.9
Met	5	3.0	2.9	5.6	4.6	0.8	1.2	0.9	0.7
Ile	4	0	0	4.6	1.0	1.9	1.8	1.8	3.6
Leu	34	37.4	37.1	30.1	31.6	36.5	34.7	33.8	35.2
Tyr	7	7.0	6.5	4.9	5.2	5.9	6.0	5.5	5.4
Phe	3	5.9	5.1	6.7	5.4	4.0	5.3	6.5	4.9
1/2 Cys	0	0	0	1.1	0.4	0	n.d.	0	0
Trp	5	4.0	3.2	2.2	n.d.	4.0	n.d.	4.0	Present

Values for hedgehog apolipoprotein A-I are the actual numbers of residues of each amino acid, derived from complete sequence determination. Data presented for comparison include species for which complete sequence data are available (i.e., human, rat, dog, rabbit, and bovine) or not available (baboon, mouse, and badger).

amino acid is completely absent from human and baboon apoA-Is. However, a number of Ile residues comparable to that found in the hedgehog has been reported in the corresponding proteins in the rat (five residues) (28) and in the calf (33). As regards methionine, values in the 4 to 6 mol/mol protein range have also been noted in some species remote from humans in phylogeny, such as the rat (28), chicken (34), and duck (35).

Determination of the complete amino acid sequence allowed comparison with those of apoA-Is from other species, i.e., baboon (36), bovine (24), chicken (34), dog (30), duck (35), human (37, 38), macaque (39), mouse (40), pig (41), rabbit (32), rat (42), and salmon (43) (**Fig. 3**). The hedgehog protein demonstrated a 58% overall homology to the human protein (142 conserved sites). As far as apoA-I is concerned, the phylogenetic distance between hedgehog and humans thus appeared comparable to that existing between the rat and humans, i.e., about 60% overall homology. This value is therefore much closer than those observed in some species more remote from humans, such as the chicken (34) and the duck (35) (**Fig. 4**). Changes in the percentage homology could be noted according to the region of the protein considered, with the highest proportion of mutations occurring proximal to the N- and C- termini. It is of note that among the 98 mutations coding for an amino acid and existing between the hedgehog and human proteins, only 40 were conservative hydrophobic ones.

Experimental results obtained by CD spectroscopy showed that the percentage of helix structure in hedgehog apoA-I, both in absence or presence of phospholipids, may be somewhat lower than the corresponding one observed by others for human apoA-I, e.g., 50 ± 3% for free apoA-I, and up to 72% for apoA-I included in recombinant HDL (44).

Compared to the corresponding human protein, hedgehog apoA-I lacked Pro_3, a deletion commonly found in most animal proteins studied to date (see Fig. 3). We also observed the absence of an equivalent to Glu_{183} in human apoA-I. In contrast, hedgehog apoA-I lacked the supplementary Gly_{184} only found in the chicken and duck proteins. Finally, it is of note that in the course of the sequence determination of hedgehog apoA-I, we did not observe the presence of a pro-sequence as is the case in many other species. We cannot rule out the possibility that the purification procedure may have eliminated proapoA-I from the examined samples. However, it is of note that we were able to show that approx. 10% of calf apoA-I contained a propeptide, after purifying this latter protein by the same methodology used in the present experiments.

Whatever the species considered, apoA-I has at least three main functions, i.e., association with lipids, binding to HDL binding sites/receptors, and LCAT activation. Lipid binding is related to α-helical structure. Most of apoA-I thus consists of repeats of 22 amino acids (22-mers) (45, 46), each of which being a tandem array of two 11-mers. The succession of 22-mers is punctuated by the strong helix breaker proline (47), these residues being essential for maintaining the normal secondary structure of the protein. In the case of hedgehog apoA-I, it is therefore of interest to note that six (i.e., Pro 65, 98, 120, 164, 207, and 218 of the hedgehog protein) amongst the seven Pro initiating 11-mers and considered as necessary for the correct folding of the protein are present in the protein.

```
 hhapoa-i : DE.AKSYWDQIKDMLTVYVDTAKDSGKDYLTSLDTSALGQ.....................
babapoa-i : --pPQTP--RV--LV----EAL-------VSQFEG----K.....................
bovapoa-i : -D.PQ-S--RV--FA----EAI----R--VAQFEA----K.....................
chiapoa-i : --.PQTPL-R-R--VD--LE-V-A----AIAQFES--V-K.....................
dogapoa-i : --.PQ-P--RV--LA-----AV----R--VAQFEA----K.....................
ducapoa-i : --.PQAPL-RLR-LVD--LE-V-A----AIAQFEA--V-K.....................
humapoa-i : --pPQ-P--RV--LA-----VL----R--VSQFEG----K.....................
macapoa-i : --pPQTP--RV--LV----EAL-------VSQFEG----K.....................
mouapoa-i : --.PQ-Q--KV--FAN----AV----R--VSQFES-S---.....................
pigapoa-i : -D.PQ-P--RV--FA-----AI----R--VAQFEA----K.....................
rabapoa-i : --.PR-S--K---FA------.V----RE-VAQFEA--F-K.....................
ratapoa-i : --.PQ-Q--RV--FA-----AV----R--VSQFES-T--K.....................
salapoa-i : .D.-P-QLEHV-AA-NM-IAQV-LTAQRSIDL--DTEYKEykmqlsqsldnlqqfadsts

 hhapoa-i : QLNKKLADNWDTVSSALLKAREQMKPIAMEFWGNLEKDTEGLRQTVSKDLELVKEKVQPY
babapoa-i : ---L--L----S-T-TVS-L---LG-VTQ---D----E------EM-----E--A-----
bovapoa-i : ---L--L-----LA-T-S-V---LG-VTQ---D----E-AS---EMH----E--Q-----
chiapoa-i : --DL-----L--L-A-AA-L--D-A-YYK-VREMWL----A--AELT----E----IR-F
dogapoa-i : ---L--L----SL--TVT-L---IG-VTQ---D----E--V---EM-----E--Q-----
ducapoa-i : --DL-----L--LGA-AA-L--D-A-YYK-VREMWL----S--AELT----E----IR-F
humapoa-i : ---L--L----S-T-TFS-L---LG-VTQ---D----E------EM-----E--A-----
macapoa-i : ---L--L----S-T-TVS-L---LG-VTQ---D----E------EM-----E--A-----
mouapoa-i : ---LN-LE----LG-TVSQLQ-RLG-LTRD--D----E-DWV--EMN----E--Q-----
pigapoa-i : H--L--L----SLG-TFT-V---LG-VTQ---D----E--A---KM-----E--K-----
rabapoa-i : ---L--L----SL--TVS-LQ--LG-VTQ---D----E------EMN---QE-RQ-----
ratapoa-i : ---LN-L-----LG-TVGRLQ--LG-VTQ---A----E-DW--NEMN----N--Q-M--H
salapoa-i : KSWPPTPRSSAPSCD-TATV-AEVMKDVEDVRTQ--PKRAE--EVLN-HIDEYRK-LE-L

 hhapoa-i : LDSFQKKVEEELELYRQKVAPLSAEWREQARQKAQELQQKAGELGQQHRDRVRTHVDALR
babapoa-i : --D----WQ--M-------E--R--LH-GT---LH--HE-LSP--EEV---A-A------
bovapoa-i : --E----WH--V-I--------GE-F--G----V----D-LSP-A-EL---A-A--ET--
chiapoa-i : --Q-SA-WT----Q---RLT-VAQ-LK-LTK--VELM-A-LTPVAEEA---L-G--EE--
dogapoa-i : --D----WQ--V----------GS-L--G----L----E-LSP--EEL---A--------
ducapoa-i : --Q-SA-WT----Q---RL--VAQ-LK-LTK--VELM---LTPVAEEA---L-G--EE--
humapoa-i : --D----WQ--M-------E--R--LQ-G----LH---E-LSP--EEM---A-A------
macapoa-i : --D----WQ--M-------E--R--LH-GT---LH--HE-LSP--EEV---A-A------
mouapoa-i : --E----WK-DV----------G--LQ-S----L----GRLSPVAEEF---M-----S--
pigapoa-i : --D--N-WQ--M-T----M.--G--F--G----V----E-LSP-AEEL---L-A--A---
rabapoa-i : --E----WQ--V-R-----E--GV-L--S----LT---E-LSP-AEEL--SA-----T--
ratapoa-i : --E--E-WN--V-A----LE--GT-LHKN-K....-M-RHLKVVAEEF---M-VNA----
salapoa-i : IKEHIELRRT-MDAF-A-IE-VVE-M-AKVAVNVE-TKT-LMPIVEIV-AKLTERLEE--

 hhapoa-i : TDLAPYGEEARKLLLQRLQDIK.AK.SGDLAEYQTKLSEHLKSFGEKAQPTLQDLRHGLE
babapoa-i : -H----SD-L-QR-AA--EAL-eNG.GAR----HA-A----STLS---K-A-E---Q--L
bovapoa-i : QQ----SDDL-QR-TA--EAL-.EG.G-S----HA-A--Q--AL----K-V-E---Q--L
chiapoa-i : KN----SD-L-QK-S-K-EE-R.E-gIPQAS---A-VM-Q-SNLR--MT-LV-EF-ER-T
dogapoa-i : AQ----SDDL-ER-AA---AL-eGG.GAS----HARA--Q-SAL----R-A-----Q--L
ducapoa-i : KN----SD-L-QK-S-K-EE-R.E-gIPQA----A-VV-Q-SNLR--MT-LV--FKER-T
humapoa-i : -H----SD-L-QR-AA--EAL-eNG.GAR----HA-AT---STLS---K-A-E---Q--L
macapoa-i : -H----SD-L-QR-AA--EAL-eNG.GAR----HA-A----STLS---K-A-E---Q--L
mouapoa-i : -Q---HS-QM-ES-A---AEL-..S.NPT-N--H-RAKT---TL----R-A-E----S-M
pigapoa-i : QHV---SDDL-QRMAA-FEAL-.EG.G-S-----A-AQ-Q--AL----K-A-E---Q--L
rabapoa-i : -K----SQ-LQQR-AA--ES--eGG.GAS-----A-AR---SVLS---R-A-E---Q--L
ratapoa-i : AKFGL-SDQM-EN-A---TE--..N.HPT-I--H--A-D---TL----K-A-D--GQ--M
salapoa-i : -LA---A--YKEQMFKAVGEVR.E-.........................VAP-SEDFK

 hhapoa-i : PLWEGIKAGAMSMLEELGKKLNSQ
babapoa-i : -VL-SF-VSFL-A---YT---ST-
bovapoa-i : -VL-SL-VSILAAID-AS----A-
chiapoa-i : -YA-NL-NRLI-F-D--Q-SVA..
dogapoa-i : -VL-SF-VSLLAAID-AT----A-
ducapoa-i : -YA-NL-TRFI-L-D--Q-TVA..
humapoa-i : -VL-SF-VSFL-A---YT----T-
macapoa-i : -VL-SF-VSFL-A---YT---ST-
mouapoa-i : -ML-TL-TK-Q-VI-..STPFSEG
pigapoa-i : -VL-NL-VSILAAID-AS----A-
rabapoa-i : -VL-SF--SVQNLVD-AT----T-
ratapoa-i : -VL-AW--KI---ID-AK----A.
salapoa-i : AR-APPPRRPSKSSWLSTRPSARP
```

Fig. 3. Sequence alignment of the different apoA-Is whose primary amino acid sequence has been determined. Upper case letters, aligned non-identical bases; lower case letters, unaligned bases. Gaps are indicated with a . and homology with a -. The successive lines refer respectively to the hedgehog, baboon (36), bovine (24), chicken (34), dog (30), duck (35), human (37, 38), macaque (39), mouse (40), pig (41), rabbit (32), rat (42), and salmon (43) proteins.

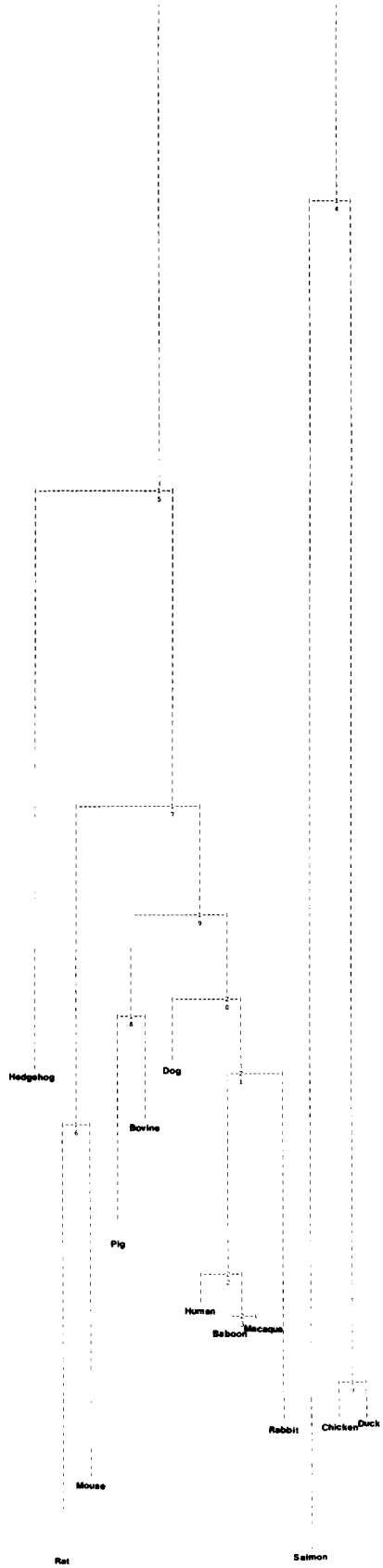

Fig. 4. Phylogenetic tree generated by computer alignment of mammalian apoA-I sequences.

The seventh residue (Pro_{143} in humans) is substituted for by Glu, a mutation not compatible with a single base change in the corresponding codon. It is of note that this particular Pro is conserved in all animal species studied to date, with the exception of the rat in which it is replaced by Val (Fig. 3). According to Chou and Fasman (47), Glu is a "strong α-helix former" while, in addition to breaking α-helix segments, Pro appears to induce reversal of the orientation of the adjacent helices (48). A major change in the overall apoA-I spatial structure may thus occur consecutively, which in turn may have consequences in the functionality of the protein (see below).

These data, as well as those obtained using CD spectroscopy, both suggest that compared to its human counterpart, a smaller portion of hedgehog apoA-I may actually be in the form of α-helix. In this respect, it is of interest to note that recently, Jonas, Steinmetz, and Churgay (49), by reconstituting HDL-like particles from human apoA-I, phospholipids, and cholesterol in different stoichiometric ratios, demonstrated that the number of amphipathic α-helical segments of apoA-I correlated directly with the size of the resulting lipoprotein. Such results are to be considered together with our own findings in the hedgehog. Indeed, we have demonstrated in this species, using a variety of techniques including analytical ultracentrifugation (5) and gel electrophoresis of density gradient fractions (6), the occurrence of higher density, small size, HDL and VHDL subpopulations.

Using polyclonal antibodies, we have not been able to detect any cross-reactivity between human and hedgehog apoA-Is, although prediction of the antigenic structure using the method (50) and algorithm (51) of Hopp and Woods indicated that several potentially antigenic hydrophilic sites may occur in similar regions of the proteins, i.e., near residues 25, 60, 76, 87, 126, 150, 168, 176, and 235. It is, however, of note that at least in the case of the five latter sites, major amino acid differences were present in their close environment. As Ehnholm et al. (52) have demonstrated in their studies on the interaction between human apoA-I and monoclonal antibodies to human apoA-I, the substitution of a single amino acid can cause the loss of an antigenic determinant. Such findings may therefore explain the observed lack of cross-reactivity.

In humans, several domains of apoA-I have been successively suggested to be responsible for the activation of LCAT. Using synthetic peptides, Anantharamaiah et al. (53) have suggested that the LCAT-activating domain of apoA-I is located between residues 66 and 121, a region containing two 22-mers exhibiting the Glu residue at position 13 and separated by an intervening 11-mer. Results from these authors emphasized the importance of this latter polar residue in the LCAT-activating efficiency of the domain. Comparison of the respective 66–121 regions in human and hedgehog apoA-I provided evidence for a much higher degree of homology (75%) than exists in the

rest of the protein. In addition, among the two proposedly essential Glu residues in the human protein, the first one is replaced in the hedgehog by a similarly polar Asp and the second one is conserved. Even more recently, Banka et al. (54) and Meng et al. (55) have confirmed the implication of residues 95–121 in the LCAT-activating function of apoA-I. This latter region appears extremely well conserved between hedgehog and humans, with 21 residues identical out of 26, i.e., more than 80% homology. Despite these noticeable similarities, our own data show that hedgehog apoA-I was able to activate homologous LCAT by only 25% of the corresponding values obtained in humans. A partial and hypothetical explanation for these results may be suggested from a comparison between hedgehog apoA-I and a mutant of the human protein, apoA-I Giessen (56). Indeed, this particular form of apoA-I exhibits a mutation at residue 143 by which the usual Pro is replaced by Arg, and its ability to activate LCAT is consequently reduced by 30–40%. In the hedgehog, Pro_{143} is the only Pro residue situated at a β-turn position mutated and replaced by Glu (see above). We may thus speculate that a consecutive change in the spatial structure of this part of the protein may partly explain the observed low potential of hedgehog apoA-I with regard to activation of this enzyme. This hypothesis is supported by the findings of Sparrow and Gotto (57), and Fukushima et al. (58) who have shown, using synthetic peptides, that the region between residues 124 and 185 contained at least one and possibly two LCAT-activating domains, i.e., 124–167 and 143–185. These results have been recently partially corroborated by Meng et al. (55) using monoclonal antibodies. It must be emphasized, however, that other recent results, such as those of Minnich et al. (59) and Sorci-Thomas, Kearns, and Lee (60), suggest that several, possibly interacting, apoA-I domains may be required for mediating the interaction of the protein with LCAT.

Finally, in humans, it seems highly probable that most of HDL binding to liver cells, making possible the end steps of reverse cholesterol transport, is at least partly mediated through interaction of apoA-I with specific HDL binding proteins. Recently, immunological studies using Fab fragments from antiA-I (205–220) and antiA-I (230–243) have demonstrated the importance of these regions of the protein in binding to liver plasma membranes (61). Considering the quantitative importance in hedgehog plasma of the various apoA-I-containing HDL and VHDL particles, which can represent as much as 70 to 85% of total circulating lipoproteins according to the season considered, the question of their uptake by cells is essential for the lipid metabolism homeostasis in this species. From the present results, it may be noted that fragments of hedgehog apoA-I corresponding to human residues 205–220 and 230–243 appear relatively similar in general organization to their human counterparts. Indeed, Pro 229 and 240 are also present in the hedgehog protein, and most substitutions in the above-mentioned fragments are conservative ones with respect to hydrophilicity or hydrophobicity. It may thus be speculated that the recognition function towards specific receptors of this part of the protein is also present in the hedgehog.

In the present report, we have established the basic characteristics of hedgehog apoA-I. We are now taking advantage of these data for the characterization of structural and metabolic features of HDL and VHDL subfractions, which is presently in progress in our laboratory.

This research was partially supported by a grant from the National Institutes of Health (HL-27341, SCOR on Atherosclerosis) to J. T. Sparrow, by grant #MT 5999 from The Medical Research Council of Canada to P. J. Dolphin, and by NATO Collaborative research Grant #920745 to P. J. Dolphin and INSERM Unité 321. The authors wish to thank Sue Kelly for preparing the figures.

Manuscript received 4 April 1994 and in revised form 7 October 1994.

REFERENCES

1. Laplaud, P. M., L. Beaubatie, S. C. Rall, Jr., G. Luc, and M. Saboureau. 1988. Lipoprotein [a] is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (*Erinaceus europaeus*). *J. Lipid Res.* **29:** 1157–1170.
2. Utermann, G. 1989. The mysteries of lipoprotein [a]. *Science.* **246:** 904–910.
3. Mbewu, A. D., and P. N. Durrington. 1990. Lipoprotein [a]: structure, properties, and possible involvement in thrombogenesis and atherogenesis. *Atherosclerosis.* **85:** 1–14.
4. Scanu, A. M. 1992. Lipoprotein [a]. A genetic risk factor for premature coronary heart disease. *J. Am. Med. Assoc.* **267:** 3326–3329.
5. Laplaud, P. M., L. Beaubatie, and M. Saboureau. 1983. Distribution and partial characterization of the plasma lipoproteins in the hedgehog (*Erinaceus europaeus* L.), a hibernator with potential use in the study of the hormonal regulation of lipoprotein metabolism. *Biochim. Biophys. Acta.* **752:** 396–406.
6. Laplaud, P. M., M. Saboureau, L. Beaubatie, and B. El-Omari. 1989. Seasonal variations of plasma lipids and lipoproteins in the hedgehog, an animal model for lipoprotein [a] metabolism: relation to plasma thyroxine and testosterone levels. *Biochim. Biophys. Acta.* **1005:** 143–156.
7. Saboureau, M., and J. Boissin. 1978. Variations saisonnières de la testostéronémie et de la thyroxinémie chez le Hérisson (*Erinaceus europaeus* L.). *C. R. Acad. Sci. Paris.* **286D:** 1479–1482.
8. Franceschini, G., P. Maderna, and C. R. Sirtori. 1991. Reverse cholesterol transport: physiology and pharmacology. *Atherosclerosis.* **88:** 99–107.
9. Fielding, C. J. 1993. Molecular aspects of reverse cholesterol transport. *In* High Density Lipoproteins: Physiopathology and Clinical Relevance. A. L. Catapano, F. Bernini, and A. Corsini, editors. Raven Press, New York. 75–85.
10. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-β-migrating high density lipoprotein. *Biochemistry.* **27:** 25–29.

11. Francone, O. L., A. Gurakar, and C. Fielding. 1989. Distribution and functions of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins. Evidence for a functional unit containing these activities together with apolipoproteins A-I and D that catalyzes the esterification and transfer of cell-derived cholesterol. *J. Biol. Chem.* **264:** 7066–7072.
12. Asztalos, B. F., C. H. Sloop, L. Wong, and P. S. Roheim. 1993. Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apoA-I-containing subpopulations. *Biochim. Biophys. Acta.* **1169:** 291–300.
13. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
14. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies on the proteins of human plasma very low density lipoproteins. *J. Biol. Chem.* **244:** 5687–5694.
15. Stephens, R. E. 1975. High-resolution preparative SDS-polyacrylamide gel electrophoresis: fluorescent visualization and electrophoretic elution-concentration of protein bands. *Anal. Biochem.* **65:** 369–379.
16. Talbot, D. N., and D. A. Yphantis. 1971. Fluorescent monitoring of SDS gel electrophoresis. *Anal. Biochem.* **44:** 246–253.
17. Ouchterlony, O. 1964. Gel diffusion techniques. *In* Immunological Methods. J. F. Ackroyd, editor. Blackwell, London. 55.
18. Altschul, S. F., and B. W. Erickson. 1986. Optimal sequence alignment using affine gap costs. *Bull. Math. Biol.* **48:** 603–616.
19. Klotz, L. C., and R. L. Blanken. 1981. A practical method for calculating evolutionary trees from sequence data. *J. Theor. Biol.* **91:** 261–272.
20. Chang, C. T., C. S. C. Wu, and J. T. Yang. 1978. Circular dichroic analysis of protein conformation: inclusion of the β-turns. *Anal. Biochem.* **91:** 13–31.
21. Chen, C., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
22. Jauhiainen, M., and P. J. Dolphin. 1986. Human plasma lecithin:cholesterol acyltransferase. An elucidation of the catalytic mechanism. *J. Biol. Chem.* **261:** 7032–7043.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. S. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Sparrow, D. A., B. R. Lee, P. M. Laplaud, S. Auboiron, D. Bauchart, M. J. Chapman, A. M. Gotto, Jr., C. Y. Yang, and J. T. Sparrow. 1992. Plasma lipid transport in the preruminant calf, *Bos* spp: primary structure of bovine apolipoprotein A-I. *Biochim. Biophys. Acta.* **1123:** 145–150.
25. Schenkein, I., M. Levy, E. C. Franklin, and B. Frangione. 1977. Proteolytic enzymes from the mouse submaxillary gland. Specificity restricted to arginine residues. *Arch. Biochem. Biophys.* **182:** 64–70.
26. Brewer, H. B., Jr., T. Fairwell, A. La Rue, B. Ronan, A. Houser, and T. J. Bronzert. 1978. The amino acid sequence of human apoA-I, an apolipoprotein isolated from high density lipoproteins. *Biochem. Biophys. Res. Commun.* **80:** 623–630.
27. Blaton, V., R. Vercaemst, M. Rosseneu, J. Mortelmans, R. L. Jackson, A. M. Gotto, Jr., and H. Peeters. 1977. Characterization of baboon plasma high-density lipoproteins and of their major apoproteins. *Biochemistry.* **16:** 2157–2163.
28. Swaney, J. B., F. Braithwaite, and H. A. Eder. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **16:** 271–278.
29. Forgez, P., M. J. Chapman, S. C. Rall, Jr., and M. C. Camus. 1984. The lipid transport system in the mouse, *Mus musculus:* isolation and characterization of apolipoproteins B, A-I, A-II and C-III. *J. Lipid Res.* **25:** 954–966.
30. Chung, H., A. Randolph, I. Reardon, and R. L. Heinrikson. 1982. The covalent structure of apolipoprotein A-I from canine high density lipoproteins. *J. Biol. Chem.* **257:** 2961–2967.
31. Beaubatie, L., P. M. Laplaud, S. C. Rall, and D. Maurel. 1986. Isolation and characterization of the major plasma apolipoproteins, A-I and B, in the European badger, *Meles meles. J. Lipid Res.* **27:** 140–149.
32. Yang, C., T. Yang, H. J. Pownall, and A. M. Gotto, Jr. 1986. The primary structure of apolipoprotein A-I from rabbit high-density lipoprotein. *Eur. J. Biochem.* **160:** 427–431.
33. Auboiron, S., D. A. Sparrow, L. Beaubatie, D. Bauchart, J. T. Sparrow, P. M. Laplaud, and M. J. Chapman. 1990. Characterization and amino-terminal sequence of apolipoprotein A-I from plasma high density lipoproteins in the ruminant calf, *Bos* spp. *Biochem. Biophys. Res. Commun.* **166:** 833–839.
34. Yang, C. Y., Z. W. Gu, W. Patsch, S. A. Weng, T. W. Kim, and L. Chan. 1987. The complete amino acid sequence of proapolipoprotein A-I of chicken high density lipoproteins. *FEBS Lett.* **224:** 261–266.
35. Gu, Z. W., Y. H. Xie, M. Yang, J. T. Sparrow, K. Wang, Y. Li, W. H. Li, A. M. Gotto, Jr., and C. Y. Yang. 1993. Primary structure of Beijing duck apolipoprotein A-I. *J. Prot. Chem.* **12:** 585–591.
36. Hixson, J. E., S. Borenstein, L. A. Cox, D. L. Rainwater, and J. L. Vandeberg. 1988. The baboon gene for apolipoprotein A-I: characterization of a cDNA clone and identification of DNA polymorphisms for genetic studies of cholesterol metabolism. *Gene.* **74:** 483–490.
37. Cheung, P., and L. Chan. 1983. Nucleotide sequence of cloned cDNA of human apolipoprotein A-I. *Nucleic Acids Res.* **11:** 3703–3715.
38. Law, S. W., and H. B. Brewer, Jr. 1984. Nucleotide sequence and the encoded amino acids of human apolipoprotein A-I mRNA. *Proc. Natl. Acad. Sci. USA.* **81:** 66–70.
39. Polites, H. G., G. W. Melchior, C. K. Castle, and K. R. Marotti. 1986. The primary structure of cynomolgus monkey apolipoprotein A-I deduced from the cDNA sequence: comparison to the human sequence. *Gene.* **49:** 103–110.
40. Boyle, T. P., and K. R. Marotti. 1992. Structure of the murine gene encoding apolipoprotein A-I. *Gene.* **117:** 243–247.
41. Weiler-Güttler, H., M. Sommerfeldt, A. Papandrikopoulou, U. Mischek, D. Bonitz, A. Frey, M. Grupe, J. Scheerer, and H. G. Gassen. 1990. Synthesis of apolipoprotein A-I in pig brain microvascular endothelial cells. *J. Neurochem.* **54:** 444–450.
42. Poncin, J. E., J. A. Martial, and J. E. Gielen. 1984. Cloning and structure analysis of the rat apolipoprotein A-I cDNA. *Eur. J. Biochem.* **140:** 493–498.
43. Powell, R., D. G. Higgins, J. Wolff, L. Byrnes, M. Stack, P. M. Sharp, and F. Gannon. 1991. The salmon gene encoding apolipoprotein A-I: cDNA sequence, tissue expression and evolution. *Gene.* **104:** 155–161.
44. Jonas, A., K. E. Kédzy, and J. H. Wald. 1989. Defined apolipoprotein A-I conformations in reconstituted high density lipoprotein discs. *J. Biol. Chem.* **264:** 4818–4824.
45. Fitch, W. M. 1977. Phylogenies constrained by the crossover process as illustrated by human hemoglobins and a thirteen-cycle, eleven amino-acid repeat in human apolipoprotein A-I. *Genetics.* **86:** 623–644.
46. Mc Lachlan, A. D. 1977. Repeated helical pattern in apoli-

poprotein A-I. *Nature.* **267:** 465–466.
47. Chou, P. Y., and G. D. Fasman. 1974. Conformational parameters for amino acids in helical, β-sheet, and random coil regions calculated from proteins. *Biochemistry.* **13:** 211–245.
48. Rosseneu, M., B. Vanloo, L. Lins, M. De Pauw, J. M. Ruysschaert, and R. Brasseur. 1992. Analysis of the primary and of the secondary structure of the apolipoproteins. *In* Structure and Function of Apolipoproteins. M. Rosseneu editor. CRC Press, Boca Raton, FL. 159–183.
49. Jonas, A., A. Steinmetz, and L. Churgay. 1993. The number of amphipathic α-helical segments of apolipoproteins A-I, E, and A-IV determines the size and functional properties of their reconstituted lipoprotein particles. *J. Biol. Chem.* **268:** 1596–1602.
50. Hopp, T. P., and K. R. Woods. 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci. USA.* **78:** 3824–3828.
51. Hopp, T. P., and K. R. Woods. 1983. A computer program for predicting protein antigenic determinants. *Mol. Immunol.* **20:** 483–489.
52. Ehnholm, C., M. Lukka, I. Rostedt, and K. Harper. 1986. Monoclonal antibodies specific for different regions of human apolipoprotein A-I. Characterization of an antibody that does not bind to a genetic variant of apoA-I (Glu_{136}→Lys). *J. Lipid Res.* **27:** 1259–1264.
53. Anantharamaiah, G. M., Y. V. Venkatachalapathi, C. G. Brouillette, and J. P. Segrest. 1990. Use of synthetic peptide analogues to localize lecithin:cholesterol acyltransferase activating domain in apolipoprotein A-I. *Arteriosclerosis.* **10:** 95–105.
54. Banka, C. L., D. J. Bonnet, A. S. Black, R. S. Smith, and L. K. Curtiss. 1991. Localization of an apolipoprotein A-I epitope critical for activation of lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **266:** 23886–23892.
55. Meng, Q. H., L. Calabresi, J. C. Fruchart, and Y. L. Marcel. 1993. Apolipoprotein A-I domains involved in the activation of lecithin:cholesterol acyltransferase. Importance of the central domain. *J. Biol. Chem.* **268:** 16966–16973.
56. Utermann, G., J. Haas, A. Steinmetz, R. Paetzold, S. C. Rall, Jr., K. H. Weisgraber, and R. W. Mahley. 1984. Apolipoprotein A-I Giessen (Pro^{143}→Arg). A mutant that is defective in activating lecithin:cholesterol acyltransferase. *Eur. J. Biochem.* **144:** 325–331.
57. Sparrow, J. T., and A. M. Gotto, Jr. 1982. Apolipoprotein/lipid interactions: studies with synthetic polypeptides. *Crit. Rev. Biochem.* **13:** 87–107.
58. Fukushima, D., S. Yokoyama, D. J. Kroon, F. J. Kézdy, and E. T. Kaiser. 1980. Chain length-function correlation of amphiphilic peptides: synthesis and surface properties of a tetratetracontapeptide segment of apolipoprotein A-I. *J. Biol. Chem.* **255:** 10651–10657.
59. Minnich, A., X. Collet, A. Roghani, C. Cladaras, R. L. Hamilton, C. J. Fielding, and V. I. Zannis. 1992. Site-directed mutagenesis and structure-function analysis of the human apolipoprotein A-I. *J. Biol. Chem.* **267:** 16553–16560.
60. Sorci-Thomas, M., M. W. Kearns, and J. P. Lee. 1993. Apolipoprotein A-I domains involved in lecithin:cholesterol acyltransferase activation. *J. Biol. Chem.* **268:** 21403–21409.
61. Allan, C. M., N. H. Fidge, and J. Kanellos. 1992. Antibodies to the carboxyl terminus of human apolipoprotein A-I. The putative cellular binding domain of high density lipoprotein 3 and carboxyl-terminal structural homology between apolipoproteins A-I and A-II. *J. Biol. Chem.* **267:** 13257–13261.