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Homologues of the Human C and A Apolipoproteins in the *Macaca fascicularis* (Cynomolgus) Monkey†

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ABSTRACT: We used antisera to human A and C apolipoproteins to identify homologues of these proteins among the high-density lipoprotein apoproteins of *Macaca fascicularis* (cynomolgus) monkeys, and NH_2-terminal analysis was used to verify the homology. The NH_2-terminal sequence of the *M. fascicularis* apoA-I is identical with that of another Old World species, *Erythrocebus patas*, and differs from human apoA-I at only 4 of the first 24 residues. *M. fascicularis* apoA-II contains a serine for cysteine replacement at position 6 and is therefore monomeric like the apoA-II from all species below apes. Human and monkey apoA-II are not otherwise different through their first 25 residues. About 20% of *M. fascicularis* apoC-I aligns with human apoC-I through residue 22, and 80% lacks an NH_2-terminal dipeptide. Otherwise, the monkey apoC-I differs from the human protein at only 2 of 25 positions. Two forms of *M. fascicularis* apoC-II were identified. ApoC-II_1 is highly homologous with human apoC-II, whereas an NH_2-terminal hexapeptide is absent from apoC-II_2. ApoC-II_2 was the predominant species, and apoC-II_1 appears to represent a propeptide from which a hexapeptide prosegment is cleaved at a Gln–Asp bond. Both forms of monkey apoC-II are potent activators of lipoprotein lipase. There are two polymorphic forms of *M. fascicularis* apoC-III, and their electrophoretic mobilities become identical after treatment with neuraminidase. Except for a glycine for serine substitution at position 10, the first 15 NH_2-terminal residues of *M. fascicularis* and human apoC-III are the same.

Subhuman primates are widely employed in studies of lipoprotein metabolism, but their apolipoproteins remain incompletely characterized. The A apoproteins of high-density lipoproteins (HDL)[1] have received the most attention (Edelstein et al., 1973, 1976; Blaton et al., 1974, 1977; Mahley et al., 1976), and these have been shown to be highly homologous to their human counterparts (Brewer et al., 1972, 1978).

Little is known of the properties of the lower molecular weight C apolipoproteins in subhuman primates. Parks and Rudel (1979) were unable to isolate a homologue of apoC-I from vervet HDL, and they found apoC-II to differ markedly in amino acid composition and sialic acid content from human apoC-II. They identified only a single homologue of apoC-III, which in humans is found in at least three forms differing in sialic acid content.

We have been using the relatively abundant *Macaca fascicularis* (cynomolgus) species of Old World monkey in studies of lipoprotein metabolism.[2] We undertook these studies to identify and characterize the HDL apolipoproteins in this species, particularly the C apoproteins, and to establish their homology with human HDL proteins. Identification was first based on immunologic cross-reactivity between the monkey and human proteins and was verified by NH_2-terminal sequence analysis in each case.

Materials and Methods

Isolation of Lipoproteins. Sera were obtained at the time of tuberculin testing from the majority of the *M. fascicularis* monkeys in the New England Regional Primate Research Center colony. The animals were not fasting and were sampled throughout the working day. They were permitted unlimited access to monkey chow containing approximately 5% fat. HDL were isolated by preparative ultracentrifugation (Havel et al., 1955) at d = 1.080–1.21 g/mL after sequential removal of the very low density (d = 1.019 g/mL) and low-density lipoproteins (d = 1.063 g/mL). The HDL were recentrifuged

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[1] Abbreviations: HDL, high-density lipoproteins; apo, apolipoprotein; SDS, sodium dodecyl sulfate; DEAE, diethylaminoethyl; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

[2] Animals studied were housed at the New England Primate Regional Research Center, Southboro, MA.

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once at $d = 1.21$ g/mL, dialyzed against 0.15 M NaCl, and brought to a concentration of 8–9 mg of protein/mL by dehydration in dialysis sacks coated with Aquacide IIA (Calbiochem-Behring Corp., American Hoescht Corp., San Diego, Ca). A total of 520 mg of protein was recovered from 680 mL of serum. Human lipoproteins were prepared as detailed elsewhere (Herbert et al., 1978).

Fractionation of Apolipoproteins. Monkey HDL were delipidated with methanol–diethyl ether (1:3) (Herbert et al., 1978). Initial fractionation was performed on a 5.0 × 140 cm column of Bio-Gel A 0.5 M eluted with 5 M guanidine hydrochloride. The proteins in the major low molecular weight fraction (pool 6, Figure 1) were further fractionated on a 1.2 × 40 cm column of DEAE-Sephacel (Pharmacia) equilibrated with 0.03 M Tris-HCl and 6 M urea, pH 8.2. The column was eluted with a 500-mL linear gradient from 0.03 to 0.2 M Tris-HCl. The protein in pool 7 (Figure 1) was applied to a 1.2 × 5.0 cm column of DEAE equilibrated with 0.01 M Tris-HCl and 6 M urea, and the material eluting in the break-through volume was further characterized. Column fractions were exhaustively dialyzed against 5 mM NH_4HCO_3 and lyophilized. Human apolipoproteins were purified by conventional methods (Herbert et al., 1978).

Automated Amino-Terminal Analyses. Lyophilized proteins were solubilized either in 10% acetic acid and dialyzed against 1% acetic acid (apoC-I) or in 0.05 M NH_4HCO_3 and dialyzed against 1 mM Na_2CO_3 (all other proteins) and then lyophilized. Proteins were resolubilized in 100% trifluoroacetic acid. A Beckman Model 890C sequencer was used for automated Edman degradation with either Beckman Quadrol protein program 122974 or 121078. Conversion of thiazolinone to phenylthiohydantoin (PTH) amino acid derivatives was accomplished by incubation in 1 N HCl for 10 min at 80 °C. PTH-amino acids were identified by high-pressure liquid chromatography on a Waters Model 204 apparatus with the solvent system of Bhown et al. (1978) or by amino acid analysis after HI hydrolysis (Smithies et al., 1971).

Analytical Methods. Chromatographic fractions were assessed by anionic (Reisfeld & Small, 1978), cationic (Jovin et al., 1970), and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (Weber et al., 1972). Amino acid analyses were performed on a Beckman 119 CL instrument with a single-column system (Beckman). Proteins were hydrolyzed in sealed, evacuated tubes with either 6 N HCl containing 2-mercaptoethanol (1:2000, v/v) for 24 h or 4 N methanesulfonic acid for 20 h. Cysteic acid was determined after performic acid oxidation (Hirs, 1967). Neuraminidase digestion was performed as described by Morell et al. (1971), and polyacrylamide gel bands were qualitatively assessed for carbohydrate with the periodate–Schiff stain (Kapitany & Zebrowski, 1973). Reaction of cynomolgus apoA-II with pyroglutamyl aminopeptidase (1:20 weight ratio) (Sigma) was performed in 0.1 M ammonium acetate containing 5 mM dithiothreitol. After incubation for 12 h at 37 °C, the reaction mixture was lyophilized, the pyroglutamate extracted with acetone, and glutamic acid generated by hydrolysis in 6 N HCl for 20 h at 100 °C. Cholesterol (Allain et al., 1974) and triglyceride (Buccolo & David, 1973) concentrations were determined on a Gilford System 3500 computer-directed analyzer by enzymatic methods. Protein in lipoprotein fractions was estimated by the technique of Lowry et al. (1951) and phospholipid by the method of Eibl and Lands (1969).

Immunologic Methods. Precipitating antisera to human apolipoproteins and to cynomolgus apoA-I were prepared in goats as previously described (Herbert et al., 1978). Antisera were harvested when they produced precipitin lines with the immunogen on double diffusion in 1% agarose containing 50 mM veronal buffer, pH 8.0. The double-antibody radioimmunoassay for apoA-I has been reported (Henderson et al., 1978).

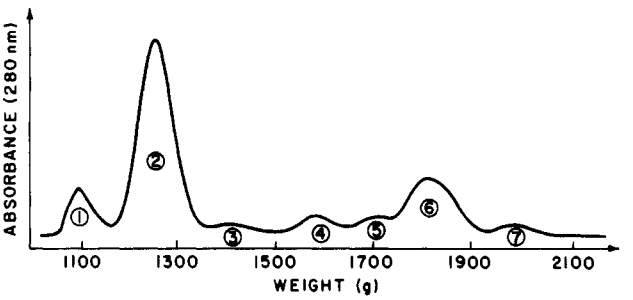

FIGURE 1: Chromatographic separation of 75 mg of *M. fascicularis* HDL apoproteins on a 5.0 × 140 cm column of Bio-Gel A 0.5 M eluted with 5 M guanidine hydrochloride. Column fractions were pooled as indicated by circled arabic numbers.

Lipoprotein Lipase Cofactor Activity. The assay of lipoprotein lipase cofactor activity employed rat epididymal fat pads for the enzyme source as described previously (Krauss et al., 1973). Final concentrations in the assay mixture were as follows: triolein, 4.24 μmol/mL; 1-α-phosphatidylcholine (egg yolk; Sigma), 0.28 μmol/mL; bovine fatty acid free albumin (Miles), 24.4 mg/mL Tris-HCl, 0.16 M; glycerol tri-[9,10(n)-^{3}H]oleate, 3.4 μCi/mL; glycerol, 62.3 mg/mL; protein activator, 0.1–4 μg/mL.

RESULTS

HDL Chemical Composition. A 680-mL pool of *M. fascicularis* serum contained 119 mg/dL total cholesterol, 107 mg/dL triglycerides, and 43 mg/dL HDL cholesterol. The isolated HDL contained 22% cholesteryl esters, 4% unesterified cholesterol, 3% triglycerides, 35% phospholipids, and 36% protein. Lipoprotein electrophoresis demonstrated light contamination with lipoproteins of pre-β mobility, presumably LP (a). After preparative ultracentrifugation and organic solvent extraction, 520 mg of HDL proteins was recovered.

Gel Chromatography of the HDL Apoproteins. Chromatography on Bio-Gel A 0.5 M generated seven partially resolved peaks (Figure 1). The first peak, 6% of the total protein, contained aggregated apoproteins. This material showed bands comigrating with apoA-I and proteins of higher molecular weight on SDS gel electrophoresis, but very little protein was visualized in the separating gel in acid and alkaline gel electrophoresis systems containing urea (Figure 2). Peak 2 contained the major protein of HDL and comprised an average of 55% of the protein recovered. As documented below, this apoprotein was apoA-I.

The third, fourth, and fifth gel chromatography peaks each accounted for 4–6% of the HDL apoprotein and generated multiple bands when examined in the three gel electrophoresis systems (Figure 2). These proteins were not further characterized in these studies. The sixth peak from Bio-gel A 0.5 M (Figure 1) contained 32% of the protein recovered. Proteins in this fraction included apoproteins A-II, C-II, and C-III, and these were further purified by DEAE chromatography. Their characterization is described below. ApoC-I was the major protein in the seventh peak. It was identified by its characteristic mobility in gel electrophoresis at pH 3.5 (Figure 2) and accounted for about 2% of the HDL apoproteins.

Apolipoprotein A-I. The amino acid composition of *M. fascicularis* A-I was very similar to those of other primate species (Table I). It contains no isoleucine or cysteine. Its

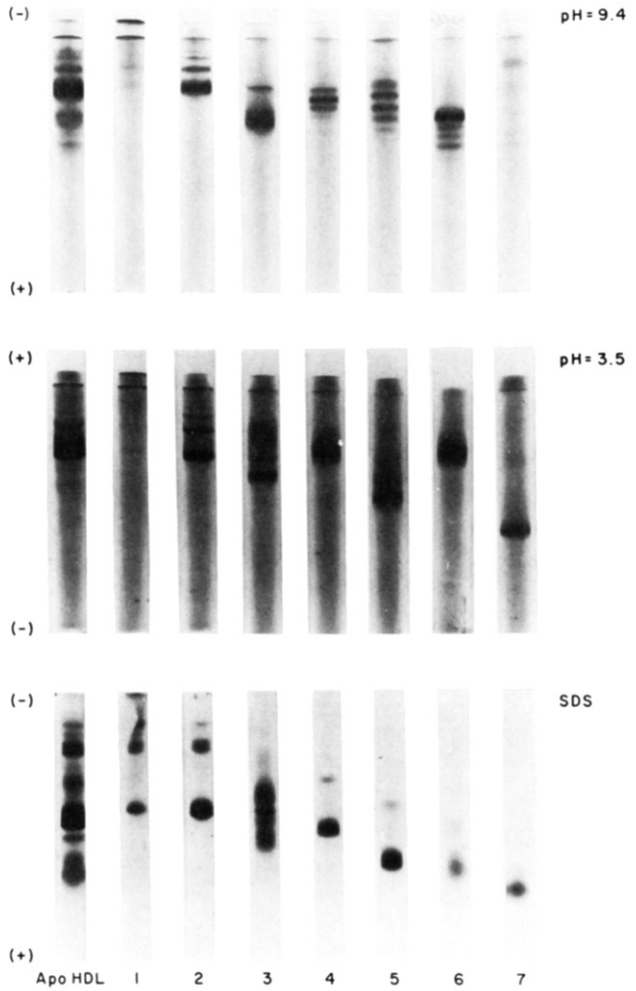

FIGURE 2: Polyacrylamide gel electrophoretic analysis of column fractions indicated in Figure 1. The separating gel acrylamide concentration was 7.5% in each system, and the anionic (Reisfeld & Small, 1966) and cationic (Jovin et al., 1970) gels contained 8 M urea.

		RESIDUE 1 5 10 15 20 25
A-I	Hs	D E P P Q S P W D R V K D L A T V Y V D V L K D
	M_F	- - - - - T - - - - - - - - V - - - - E A - - -
A-II	Hs	Z A K E P C V E S L V S Q Y F Q T V T D Y G K D L
	M_F	- - Q - - S - - - - - - - - - - - - - - - - - -
C-I	Hs	T P D V S S A L D K L K E F G N T L E D K A
	M_F	(A -) -
C-II	Hs	T Q Q P Q Q D E M P S P T F L T Q V K E S L S S Y W E
$C\text{-}II_1$	M_F	A - - - - - - - P - X - A L
$C\text{-}II_2$	M_F	- - P - - - A L - S - - Q - - - X - - - -
C-III	Hs	S E A E D A S L L S F M Q G Y M
	M_F	- - - - - T - - - G - - - - - -

FIGURE 3: Comparison of NH_2-terminal sequences of the A and C apolipoproteins of humans (Hs) and *M. fascicularis* (Mf). The symbol Z denotes pyrrolidonecarboxylic acid and X a residue that was not identified. The human sequences are taken from the following: A-I, Brewer et al. (1978); A-II, Brewer et al. (1972); C-I, Jackson et al. (1974) and Shulman et al. (1975); C-II, Hospattanker et al. (1984) and this paper; C-III, Brewer et al. (1974).

content of aspartic acid/asparagine, alanine, and glutamic acid/glutamine residues more closely resembles those of human, rhesus, and chimpanzee apoA-I than patas and vervet apoA-I (Table I).

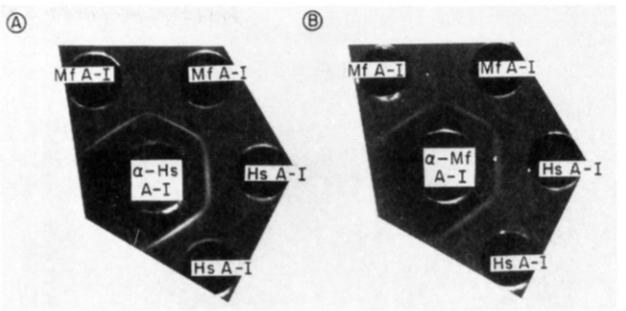

FIGURE 4: Ouchterlony double diffusion comparing the reactivity of *M. fascicularis* (Mf) and human (Hs) apoA-I with antisera to these apoproteins.

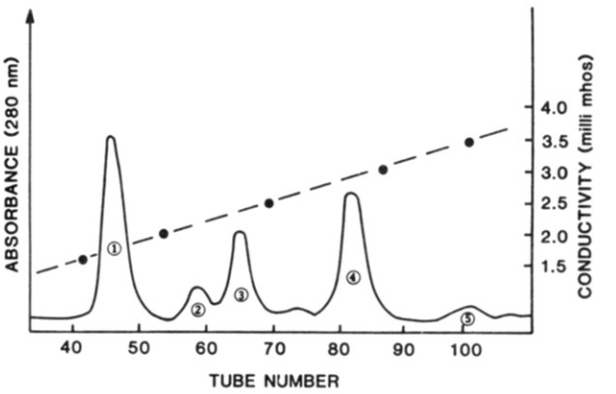

FIGURE 5: Chromatographic pattern from DEAE-Sephacel of the major low molecular weight peak (pool 6, Figure 1) of *M. fascicularis* HDL apoproteins. A 2.5 × 40 cm column equilibrated in 6 M urea was eluted with a 500-mL linear gradient from 0.03 to 0.2 M Tris-HCl. Fractions were pooled as indicated.

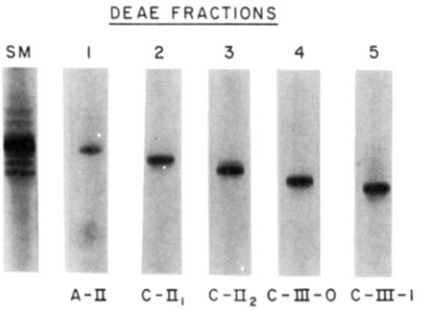

FIGURE 6: Anionic urea–polyacrylamide gel electrophoretic analysis of fractions eluted from DEAE-Sephacel (Figure 5).

The NH_2-terminal sequence of *M. fascicularis* apoA-I is compared with that of human apoA-I in Figure 3. There are substitutions of threonine for serine at position 6, valine for alanine at position 15, and glutamic acid and alanine for aspartic acid and valine at positions 20 and 21. Human and *M. fascicularis* apoA-I, therefore, differ at about 20% of the residues sequenced.

On double diffusion against both anti-human apoA-I and anti-*M. fascicularis* apoA-I, the human and monkey homologues generated precipitin lines of identity (Figure 4). However, in a radioimmunoassay employing human tracer, standards, and antiserum, monkey serum was only 10% as reactive as human serum, and its displacement curve did not parallel those of human serum and human apoA-I (not shown).

Apolipoprotein A-II. DEAE chromatography (Figure 5) of pool 6 from the Bio-Gel A 0.5 M chromatographic step (Figure 1) yielded monkey apoA-II in homogeneous form (Figure 6). Like its human, vervet, and rhesus homologues,

Table I: Comparisons of Amino Acid Compositions of Primate ApoA-I

	human (mol/100 mol of amino acids)[a]	chimpanzee (mol/100 mol of amino acids)[b]	Old World monkeys (mol/100 mol of amino acids)				
			baboon[c]	cynomolgus[d]	patas[e]	rhesus[f]	vervet[g]
Lys	8.6	8.6	8.3	8.6	6.8	8.7	10.4
His	2.1	2.1	3.0	2.6	3.0	2.6	3.0
Arg	6.6	6.9	6.3	6.1	5.3	5.7	6.1
Asx	8.6	9.4	8.0	8.3	8.7	7.9	6.9
Thr	4.1	4.2	4.5	4.7	3.8	4.4	3.5
Ser	6.2	6.6	6.5	5.9	7.2	5.7	5.2
Glx	18.9	19.4	18.8	19.2	19.7	19.2	16.4
Pro	4.1	4.0	4.2	4.4	3.9	4.4	5.2
Gly	4.1	4.5	4.5	4.7	4.6	4.4	4.8
Ala	7.8	7.8	7.4	7.6	8.1	7.4	10.0
Val	5.3	5.7	6.1	6.2	6.6	6.1	6.5
Met	1.2	ND[h]	1.2	0.8	0.9	0.9	0.4
Ile	0	tr	0	0	0	0	0
Leu	15.2	15.1	15.2	15.4	16.1	15.7	14.7
Tyr	2.9	2.9	2.7	2.9	3.0	2.6	2.6
Phe	2.5	2.8	2.1	2.4	2.3	2.2	2.2
Cys	0	ND	0	0	ND	0	ND
Trp	1.6	ND	1.3	1.4	ND	2.2	2.2

[a] Calculated from Brewer et al. (1978). [b] From Blaton et al. (1974). [c] Calculated from Blaton et al. (1977). [d] This paper. [e] From Mahley et al. (1976). [f] Calculated from Edelstein et al. (1973). [g] Calculated from Parks and Rudel (1979). [h] ND = not determined.

Table II: Comparisons of Amino Acid Compositions of Primate ApoA-II

	human (mol/100 mol of amino acids)[a]	chimpanzee (mol/100 mol of amino acids)[b]	Old World monkeys (mol/100 mol of amino acids)				
			baboon[c]	cynomolgus[d]	patas[e]	rhesus[f]	vervet[g]
Lys	11.7	9.6	9.7	10.9	10.2	10.4	10.1
His	0	1.1	0	0	0	0	0
Arg	0	3.1	1.3	1.3	1.1	1.3	1.3
Asx	3.9	8.6	5.4	5.5	5.5	5.2	6.3
Thr	7.8	6.4	7.6	8.2	7.2	7.8	6.3
Ser	7.8	6.9	8.1	7.1	7.3	7.8	5.1
Glx	20.8	19.0	22.1	21.7	21.0	20.8	20.2
Pro	5.2	5.0	5.0	5.0	5.3	5.2	5.1
Gly	3.9	4.9	2.7	3.3	3.7	2.6	2.5
Ala	6.5	7.2	8.0	8.3	7.6	7.8	8.9
Val	7.8	6.3	8.4	9.0	9.6	9.1	8.9
Met	1.3	ND[h]	1.8	0.9	1.2	1.3	1.3
Ile	1.3	1.6	0	0	0	0	0
Leu	10.4	12.4	10.3	10.9	10.3	10.4	10.1
Tyr	5.2	3.7	4.5	5.4	4.8	5.2	3.8
Phe	5.2	4.3	4.9	5.0	5.3	5.1	5.2
Cys	1.3	+[i]	0	0	0	0	ND
Trp	0	ND	0	0	0	0	0

[a] Calculated from Brewer et al. (1978). [b] From Blaton et al. (1974). [c] Calculated from Blaton et al. (1977). [d] This paper. [e] From Mahley et al. (1976). [f] Calculated from Edelstein et al. (1973). [g] Calculated from Parks and Rudel (1979). [h] ND = not determined. [i] (+) = present but not quantified.

M. fascicularis apoA-II contains no histidine or tryptophan (Table II). It also lacks isoleucine and cysteine, which are present in human apoA-II. In contrast to human apoA-II but like the apoA-II from other subhuman primates, it contains arginine. Otherwise, the amino acid compositions of all primate apoA-II species are very similar (Table II).

Pyrrolidonecarboxylyl peptidase released glutamic acid from the blocked NH_2 terminus of *M. fascicularis* apoA-II as demonstrated by acid hydrolysis of the acetone extract of the enzymatic digestion. Automated Edman degradation through the next 24 residues (Figure 3) documented differences between human and monkey apoA-II only at position 3 where glutamine replaces lysine and position 6 where a serine replaces cysteine. Neuraminidase digestion of *M. fascicularis* apoA-II did not alter its mobility in alkaline–urea gel electrophoresis, suggesting the absence of sialic acid, and the protein did not stain with the periodate–Schiff reagent.

Cross-reactivity of monkey and human apoA-II was demonstrated by Ouchterlony double diffusion (Figure 7). Lines of partial identity were generated with spurring, indicating recognition of additional antigenic sites in human apoA-II by its homologous antisera.

Apolipoprotein C-I. The lowest molecular weight pool from the Bio-Gel A 0.5 M column (pool 7, Figure 1) contained primarily apoC-I, and the protein was recovered in homogeneous form by batch elution from DEAE. *M. fascicularis* apoC-I lacks tyrosine and is very rich in lysine like its human counterpart (Table III). It does contain histidine but no methionine and differs from human apoC-I in its content of aspartic acid/asparagine and serine. Other minor compositional differences probably also exist (Table III). *M. fascicularis* apoC-I produced a precipitin line of partial identity on reaction with anti-human apoC-I (Figure 7).

About 80% of *M. fascicularis* apoC-I contained NH_2-terminal aspartic acid and aligned with the third position of human apoC-I. The remaining apoC-I contained a dipeptide NH_2-terminal extension (Ala-Pro). No other differences between human and monkey apoC-I were identified through

Table III: Amino Acid Compositions of Primate C Apoproteins

	C-I (mol/100 mol of amino acids)		C-II (mol/100 mol of amino acids)				C-III (mol/100 mol of amino acids)		
				cynomolgus[b]					
	human[a]	cynomolgus[b]	human[c]	C-II$_1$	C-II$_2$	vervet[d]	human[e]	cynomolgus[f]	vervet[d]
Lys	15.8	15.4	7.6	7.2	8.1	10.5	7.6	7.9	7.1
His	0	0.7	0	tr	tr	1.8	1.3	1.3	1.2
Arg	5.3	5.6	1.3	2.4	2.6	5.3	2.5	1.5	3.5
Asx	8.8	12.6	6.3	5.8	6.3	5.2	8.9	8.4	8.2
Thr	5.3	4.3	11.4	7.6	8.1	7.0	6.3	8.6	9.4
Ser	12.2	5.9	11.4	9.0	9.2	8.7	13.9	8.8	8.2
Glx	15.8	13.6	17.7	17.3	15.3	15.8	12.7	15.6	15.3
Pro	1.8	3.1	5.1	7.7	7.1	7.0	2.5	3.2	3.5
Gly	1.8	2.7	2.5	4.4	4.1	3.5	3.8	7.0	9.4
Ala	5.3	6.5	7.6	11.4	11.2	12.3	12.7	11.8	14.1
Val	3.5	5.4	5.1	5.5	5.6	7.0	7.6	5.4	5.9
Met	1.8	0	2.5	1.2	1.1	0	2.5	1.8	1.2
Ile	5.3	5.7	1.3	tr	tr	1.8	0	0	0
Leu	10.5	8.0	10.1	11.9	11.7	12.3	6.3	9.2	8.2
Tyr	0	0	6.3	5.8	6.2	3.5	2.5	3.0	1.2
Phe	5.3	7.5	2.5	2.2	2.4	1.8	5.1	3.9	3.5
Cys	0	0	0	0	0	ND	0	0	ND
Trp	1.8	2.9	1.3	0.7	0.9	0	3.8	2.9	ND

[a] Calculated from Jackson et al. (1974) and Shulman et al. (1975). [b] This paper. [c] From Musliner er al. (1979). [d] Calculated from Parks and Rudel (1979). [e] Calculated from Brewer et al. (1974). [f] Mean of C-III-0 and C-III-1; this paper. [g] ND = not determined.

22 steps of automated Edman degradation (Figure 3).

Apolipoprotein C-II. The two proteins eluting from DEAE-cellulose in the incompletely resolved peaks 2 and 3 (Figure 5) reacted with anti-human apoC-II (Figure 7) but generated bands of different mobility on alkaline–urea gel electrophoresis (Figure 6). Digestion with neuraminidase did not change the mobility of either electrophoretic species, indicating that sialic acid did not account for the charge heterogeneity. Neither band stained with the periodate–Schiff reagent.

The amino acid compositions of these two forms of monkey apoC-II are similar, but minor differences are apparent, particularly in the content of glutamine/glutamic acid (Table III). Relative to the composition reported for vervet apoC-II, *M. fascicularis* apoC-II contains less arginine and valine and greater proportions of threonine and tyrosine. Histidine is absent from both human and monkey apoC-II, but these homologues differ in their content of a number of other amino acids (Table III).

Automated Edman degradation demonstrated that the *M. fascicularis* apoC-II$_1$ protein contains an hexapeptide extension absent from apoC-II$_2$ (Figure 3). The NH_2 terminus of apoC-II$_1$ aligns with that of human apoC-II. Confident identification of residues after position 14 in apoC-II$_1$ was not possible, and this may have been due to cyclization of glutamine residues during automated degradation. ApoC-II$_2$, in contrast, was degraded with interpretable yields through 21 steps.

The sequence found for human apoC-II (Figure 3) agrees with that reported by Hospattankar and co-workers (Hospattankar et al., 1984). *M. fascicularis* apoC-II contains a proline for methionine substitution at position 9, and five other documented substitutions in the first 27 residues.

Monkey apoC-II$_1$ and apoC-II$_2$ are both potent activators of rat adipose tissue lipoprotein lipase (Table IV), and their dose–response patterns are indistinguishable. The maximal activation they produced was as great as that of human apoC-II and human serum.

Apolipoprotein C-III. The apolipoproteins in the last two DEAE-cellulose peaks (fractions 4 and 5, Figure 5) contained two forms of apoC-III. Like human apoC-III, the *M. fascicularis* proteins lacked cysteine and isoleucine, but they contained more glycine and less serine than their human homologues (Table III). The amino acid compositions of the two monkey apoC-III proteins were indistinguishable, but only

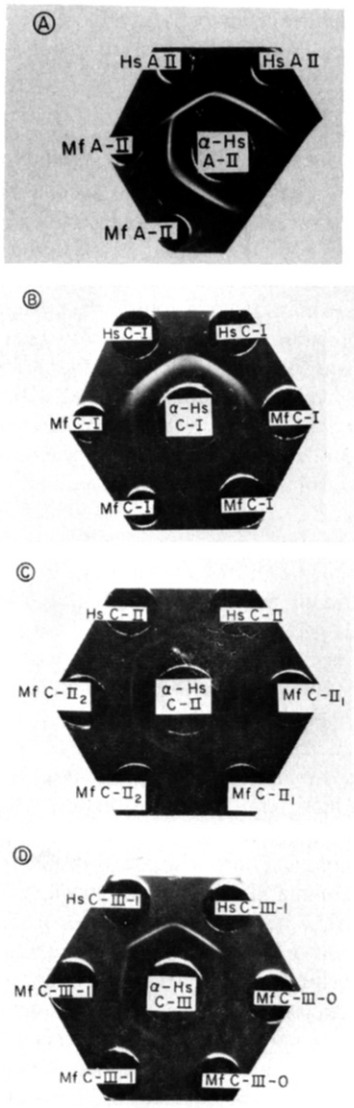

FIGURE 7: Cross-reactivity of human (Hs) and *M. fascicularis* (Mf) apoA-II and C apoproteins with antisera to the human proteins demonstrated by Ouchterlony double diffusion.

Table IV: Activation of Rat Adipose Lipoprotein Lipase by *M. fascicularis* Apolipoproteins C-II_1 and C-II_2

apoprotein concn (μg/mL)[a]	% of base-line activity[b]	
	C-II_1	C-II_2
0.10	159	168
0.25	173	182
0.75	197	200
1.50	223	216
2.00	196	199
3.00	214	208
4.00	193	193

[a] Proteins were quantified by amino acid analysis, and values represent final concentration in assay mixture. [b] Base-line activity is that without added apoprotein activator.

that form designated apoC-III-1 stained with the periodate–Schiff reagent. Treatment of apoC-III-1 with neuraminidase reduced the electrophoretic mobility of this protein to that of apoC-III-0, providing evidence that sialic acid accounts for the charge heterogeneity. Both forms of *M. fascicularis* apoC-III generated precipitin lines on double diffusion against an antiserum to human apoC-III (Figure 7). The first five NH_2-terminal residues of monkey apoC-III-0 and human apoC-III are identical (Figure 3), and there are only two amino acid substitutions among the first 16 residues.

Discussion

The close phylogenetic relationship of human and subhuman primates is reflected in the degree of homology of their major apolipoproteins. ApoA-I in primates accounts for 55–65% of the total HDL protein. In all species examined to date, apoA-I is a single-chain protein of about M_r 28 000 capable of activating the enzyme lecithin:cholesterol acyltransferase (Fielding et al., 1972). A mutant form containing cysteine and able to form intermolecular disulfide bonds has been identified in humans (Weisgraber et al., 1980). *M. fascicularis* apoA-I has an NH_2-terminal sequence identical with that reported for another Old World species, *Erythrocebus patas* (Mahley et al., 1976), and differs from human apoA-I at only 5 of the first 28 NH_2-terminal residues. All differences, moreover, are conservative and attributable to single-base substitutions in a codon.

ApoA-II, which typically constitutes 20–30% of the HDL protein, is a monomeric protein of about M_r 8500 and lacks cysteine in all species studied except for humans (Brewer et al., 1972) and apes (Blaton et al., 1974; Edelstein et al., 1976). The NH_2 terminus of apoA-II is pyrrolidonecarboxylic acid. A cysteine at position 6 in humans and apes is replaced by serine in *M. fascicularis* and rhesus apoA-II (Edelstein et al., 1976). Altogether, rhesus and human apoA-II differ at only 6 of 77 residues, and all substitutions are conservative (Edelstein et al., 1976).

The C apolipoproteins, which are found in considerable quantity in human and monkey very low density lipoproteins as well as HDL, account for 10–15% of *M. fascicularis* apoHDL. ApoC-I has not previously been characterized in a subhuman primate species. The electrophoretic mobility, amino acid composition, NH_2-terminal sequence, and immunological cross-reactivity of *M. fascicularis* apoC-I unequivocally define its homology with human apoC-I.

ApoC-II in humans is a protein of 79 residues, containing no carbohydrate, and capable of activating lipoprotein lipase (LaRosa et al., 1970; Havel et al., 1970). Isoelectric heterogeneity of human apoC-II has been documented (Havel et al., 1979), but the two forms identified had virtually identical amino acid compositions and the same NH_2 terminus. The two species of apoC-II isolated from *M. fascicularis* HDL, in contrast, differ in that one contains an NH_2-terminal hexapeptide extension. The less abundant species, apoC-II_1, appears homologous to human apoC-II in alignment of NH_2-terminal residues (Figure 3). The NH_2-terminal amino acid of the more abundant apoC-II_2, in contrast, corresponds to residue 7 of apoC-II_1. It is likely that apoC-II_1 represents a propeptide converted to apoC-II_2 by an extracellular protease as has been proposed for rat (Gordon et al., 1982) and human (Gordon et al., 1983; Zannis et al., 1983) apoA-I.

Sharpe and co-workers (Sharpe et al., 1984) previously called attention to the fact that the sequence of amino acids 5–12 in mature human apoC-II (Q-Q-D-E-M-P-S-P) was remarkably similar to residues −2 to 7 (Q-Q-D-E-P-P-Q-S-P) in human pro-apoA-I. They speculated that amino acids 1–6 in apoC-II may constitute a propeptide that undergoes processing similar to apoA-I. It may be, however, that the presence of methionine instead of proline at position 5 in human apoC-II results in a conformational change that inhibits processing of the proapolipoprotein. The preservation of proline at this position in *M. fascicularis* apoC-II and the fact that the apoC-II_2 species is the predominant plasma form support this argument.

Neither form of *M. fascicularis* apoC-II stained with the periodic acid–Schiff reagent, and their electrophoretic mobility was not altered by incubation with neuraminidase. It has been reported that apoC-II in vervet monkeys contains 1 mol of sialic acid/mol of protein (Parks & Rudel, 1979). Vervet is the only species in which a glycosylated form of apoC-II has been identified.

We isolated two polymorphic forms of apoC-III from cynomolgus HDL. These had identical amino acid compositions but different mobilities on alkaline polyacrylamide gel electrophoresis (Figure 6). The polymorph designated apoC-III-1 stained with the periodic acid–Schiff reagent, and its mobility was converted to that of the apoC-III-0 form after treatment with neuraminidase. The two major species of apoC-III in humans, C-III-1 and C-III-2, contain 1 and 2 mol of sialic acid, respectively, and a minor species containing no sialic acid has been identified (Albers & Scanu, 1970). Only one form of apoC-III was found in vervet HDL, and this was reported to contain 2 mol of sialic acid/mol of protein (Parks & Rudel, 1979).

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