Characterization of Baboon Plasma High-Density Lipoproteins and of Their Major Apoproteins†

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ABSTRACT: Baboon high-density lipoproteins (HDL) were isolated by preparative ultracentrifugation between $d = 1.063$ and 1.215 g/mL. The HDL contains 48.8% protein and a lipid distribution similar to human HDL. The phospholipid distribution shows a low sphingomyelin value (5.9%), and the fatty acid composition of HDL is comparable to the human data except for the 18:1/18:2 ratio as a result of a higher 18:1 content in the CE and a lower 18:2 concentration in the PL. The major HDL apoproteins isolated on diethylaminoethylcellulose had a mobility on sodium dodecyl sulfate–polyacrylamide gel electrophoresis and a molecular weight and an amino acid composition similar to human apoA-I. However, the amino acid sequence of the first 30 residues of baboon apoA-I differed from the human apoprotein in residues 15 and 21. Treatment of apoA-I with carboxypeptidase A indicated a carboxyl-terminal sequence of Leu-Ser-Thr-Gln. Baboon apoHDL contained monomeric apoA-II with the mobility of monomeric human apoA-II and a molecular weight of 8500. The amino acid composition differed from the human apoA-II by the presence of arginine and by the absence of half-cystine and isoleucine. The circular dichroic spectra of apoA-I and apoA-II demonstrated a higher helicity compared to the human apoproteins. Recombination studies by microcalorimetry of apoHDL with dimyristoylphosphatidylcholine (DMPC) indicated similarities in the thermodynamic binding properties of the HDL apoproteins from man and baboon. The maximal-binding enthalpies of DMPC to apoHDL, apoA-I, and apoA-II were lower for the baboon than for the human apoprotein.

In view of their phylogenetic relationship to man, the *Macacus rhesus,* the baboon (*Papio coenocephalus*), and the chimpanzee (*Pan troglodytes* schweinfurti) are of considerable interest as animal models in the study of experimental atherosclerosis (Scott et al., 1971). Previous studies from this laboratory have shown similarities between baboon and chimpanzee lipoproteins to human plasma lipoproteins (Blaton et al., 1970, 1974a; Howard et al., 1972). Both the baboon and the chimpanzee showed a decreased $VLDL_1$ and LDL_2 compared to the human. However, both animal species had increased amounts of HDL and in particular HDL_2 (Howard et al., 1968; Blaton and Peeters, 1976). In both animal species, a hyperbetalipoproteinemia analogous to human type II hyperlipoproteinemia was induced by feeding a high cholesterol diet (Blaton et al., 1970, 1974a). However, the changes in phosphatidylcholine (PC)[1] and sphingomyelin (Sph) were different in the baboon. Blaton et al. (1974b) and Scanu et al. (1974) previously have shown that chimpanzee HDL contains the major apoproteins apoA-I and apoA-II similar to those characterized in the human plasma HDL. However, the ratio of apoA-I/apoA-II (Blaton et al., 1974b) is higher in the chimpanzee than in man.

Scanu et al. (1973) and Edelstein et al. (1973) examined the apoHDL of the *Macacus rhesus* and found significant structural differences between the apoproteins as compared with man.

After delipidation, human apoHDL retains its capacity to bind lipids, especially phospholipids (Scanu and Hughes, 1970; Rosseneu et al., 1974). Recent data suggested that human apoA-I and apoA-II bind PC and Sph to a different extent (Assman and Brewer, 1974), while apA-II seems to have a greater affinity for phospholipids (Kruski and Scanu, 1974), meaning that each apoprotein has distinct lipid-binding properties. To elucidate the contribution of the two major apoproteins to the HDL structure and to the binding capacity for polar lipids, differences in the phospholipid composition and in the apoproteins of man and baboon were compared. The objective of this study was the isolation and the physical and chemical characterization of the baboon HDL apoproteins.

Experimental Procedure

Plasma Samples. Four male baboons (*Papio coenocephalus*) 7-years old were fed a standard control diet (Blaton et al., 1970). Fasting blood was collected in presprayed EDTA-tubes (1 mg/mL) (OVP Plascobel, Belgium) from the femoral artery under Sernylan anesthesia (Parke Davis) as described previously (Mortelmans, 1969). Individual plasma samples (70 mL) were obtained after centrifugation of blood at 3000*g*, and pooled and used within 6 h for lipoprotein isolation.

Preparation and Delipidation of Lipoproteins. Plasma high-density lipoproteins were prepared by ultracentrifugation

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[1] Abbreviations used are: VLDL, very low-density lipoproteins of $d = 1.006$ g/mL; LDL_1, low-density lipoproteins of $d = 1.006$–1.019 g/mL; LDL_2, low-density lipoproteins of $d = 1.019$–1.063 g/mL; HDL, high-density lipoproteins of $d = 1.063$–1.210 g/mL; HDL_2, high-density lipoproteins of $d = 1.063$–1.120 g/mL; apoHDL, lipid-free HDL; apoA-I, major apoprotein of HDL with COOH-terminal glutamine; apoA-II, apoprotein of HDL with COOH-terminal glutamine; dansyl, 8-dimethylamino-1-naphtalenesulfonyl; DMPC, dimyristoylphosphatidylcholine; CD, circular dichroism; PC, phosphatidylcholine; Sph, sphingomyelin; DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

TABLE I: Percentage of Protein-Lipid Distribution and Percentage of Phospholipid Composition of Human and Baboon High-Density Lipoproteins (d = 1.063-1.21 g/mL).

Subject	Protein	TC[d]	FC[d]	CE[d]	PL[d]	TG[d]
Human[a]	47.7	13.3	2.3	18.5	26.9	4.6
Baboon[b]	48.8	12.8	3.6	15.4	28.8	3.4
	PC[d]	OH-PC[d]	Sph[d]	PI[d]	PS[d]	PEt[d]
Human[c]	74.4	2.9	13.2	2.4	0.8	3.1
Baboon[b]	81.2	0.7	5.9	3.6	0.4	8.3

[a] Seidel et al. (1969). [b] Average of duplicate determinations on two pools from four baboons. [c] Skipski et al. (1967). [d] TC, total cholesterol; FC, free cholesterol; CE, cholesteryl esters; PL, phospholipids; TG, triglycerides; PC, phosphatidylcholine: OH-PC, lysophosphatidylcholine; Sph, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PEt, phosphatidylethanolamine.

(IEC ultracentrifuge, A321 rotor) according to the method described by Blaton et al. (1974b).

HDL fractions (d = 1.063-1.21) (5 mg/mL) were dialyzed against 0.1 M NaCl and delipidated. Total HDL was extracted with ether-ethanol (3/1, v/v) for 2 h at −20 °C. After centrifugation (2000g, 10 min), the protein precipitates were repeatedly washed with a total of 240 mL of peroxide-free diethyl ether at −20 °C. The precipitates were finally dried under nitrogen and stored at −20 °C.

Gel Electrophoresis of Lipoproteins and Apoproteins. The homogeneity of the isolated plasma lipoproteins was checked on a modified agarose gel system as previously described (Van Melsen et al., 1974). Analytical polyacrylamide gel electrophoresis of the HDL apoproteins was performed according to Reisfeld and Small (1966). The molecular weight determinations of the approproteins were carried out on polyacrylamide gel electrophoresis in sodium dodecyl sulfate according to Weber and Osborn (1969).

Isolation of the Apoproteins. The apoproteins were isolated on DEAE-cellulose in 6 M urea using a modification of the method of Shore and Shore (1967). Fifty milligrams of apoHDL was dialyzed for 24 h against the starting buffer, 0.01 M Tris-HCl (pH 8.2), containing 6 M urea. Whatman DE-52 cellulose (microgranular, preswollen) was equilibrated with the starting buffer and degassed before packing. The column (0.9 × 60 cm) was eluted with a 1600-mL linear gradient of 0.01 M Tris-HCl (pH 8.2)-6 M urea to 0.10 M Tris-HCl (pH 8.2)-6 M urea. The column was operated at a flow rate of 15 mL/h, and the effluent, collected in 5-mL fractions, was continuously monitored at 280 and 254 nm (LKB, Uvicord III). The column fractions were pooled and exhaustively dialyzed at 4 °C against 5×10^{-3} M NH_4HCO_3. After lyophilization, the apoproteins were stored under nitrogen at −20 °C.

Cyanogen Bromide Cleavage of apoA-II. One micromole of baboon apoA-II was dissolved in 2.0 mL of 70% formic acid and 500 mg of cyanogen bromide. After incubation at 23 °C for 24 h, the solvent was removed by lyophilization. The digest was dissolved in 2.5 mL of 5.4 M urea-0.1 M Tris-HCl (pH 8.0) and fractionated on Sephadex G-50 in the same buffer. The column (2.5 × 200 cm) was operated at 23 °C at a flow rate of 25 mL/h and 5-mL fractions were collected. The peptides were desalted on Bio-Gel P-2 in 0.1 M NH_4HCO_3 (pH 8.0).

NH_2-Terminal Sequence. Edman degradations were performed automatically with a Beckman Sequencer Model 890 B. *N,N*-Dimethylbenzylamine (Pierce Chemicals) was used as the coupling buffer (Hermodson et al., 1972). All of the procedures, including the conversion of the thiazolinones to the phenylthiohydantoin derivatives, were the same as those described in the Beckman Sequencer manual (1969). The phenylthiohydantoin derivatives were identified by gas-liquid chromatography (Pisano et al., 1972) on a support of SP-400 with a Beckman GC65 gas chromatograph. Those derivatives that were not detected by gas-liquid chromatography were identified by thin-layer chromatography (Inagami and Murakami, 1972; Walz and Reuterby, 1975).

Microcalorimetry. The enthalpy changes evolved on mixing apoproteins and phospholipids were measured in a LBK batch microcalorimeter Type 10.700 equipped with 18 carat gold cells. The temperature of the cells was set at 28 °C. Two milliliters of an apoprotein solution at a final concentration of 1×10^{-5} M was mixed with 4 mL of phospholipids at concentrations varying between 1×10^{-4} and 2×10^{-3} M. The enthalpy changes were recorded on a Philips recorder and the area under the curves was quantitated by the use of an electric integrator. An electrical calibration was performed and the enthalpy of reaction was expressed in kcal/mol of apoprotein. All measurements were carried out in a 0.05 M $NaHCO_3$-Na_2CO_3 buffer (pH 9.6).

Other Methods. All biochemical techniques for lipid analysis were described in earlier papers by Peeters et al. (1970), Blaton et al. (1970), and Blaton and Peeters (1971). Protein concentrations were determined by the method of Lowry et al. (1951) with bovine serum albumin (Poviet) as a standard.

Quantitative amino acid analysis was performed by the method of Spackman et al. (1958) on a Beckman Model 121 amino acid analyzer equipped with an automatic sample injector. For the COOH-terminal amino acid sequence analysis, diisopropyl fluorophosphate treated carboxypeptidase A (Sigma) prepared according to Ambler (1967) was used. The ratio of protein to enzyme was 1/50 (w/w). The mixtures were incubated at 37 °C and at scheduled time intervals the reaction was stopped by the addition of 1 volume of 10% trichloroacetic acid. The precipitate was removed by centrifugation and the supernatant was subjected to amino acid analysis. The release of amino acids was quantitatively determined with the Beckman Model 121 amino acid analyzer. The conditions for the amino acid analysis were the same as for the analysis of physiological fluids. Lithium citrate buffers were used for the separation of asparagine and glutamine as described in Beckman Bulletin A-TB-044 (1967).

Circular dichroic spectra were obtained at 23 °C using a Cary 61 spectropolarimeter as described previously (Lux et al., 1972). The mean residue ellipticity was calculated from

$$\theta = \frac{\theta_0 \mathrm{MRW}}{10lc}$$

where MRW is the mean residue weight, l the optical path length of the cell in centimeters, and c the concentration of protein in g/mL. θ_0 was the experimentally measured angle.

Results

Chemical Composition of HDL. Table I summarizes the percentage of protein-lipid distribution and phospholipid composition of human and baboon HDL. Both species have the same percentage of protein concentration without significant difference in the distribution of polar and apolar lipids. The subclasses of phospholipids, however, showed some obvious differences. Phosphatidylcholine is the predominant phos-

TABLE II: Percentage of Fatty Acid Composition of Baboon and Human Total HDL and HDL Lipid Subclasses.

Lipoprotein Fraction	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:3	20:4
Baboon (n = 2)											
HDL	1.0[a]	0.4	23.0	4.2	14.2	22.0	27.4	1.2	0.3	1.0	5.2
HDL-TG	1.1	0.5	23.3	5.2	7.3	38.8	20.3	2.4	0.1	0.1	0.9
HDL-PL	0.5	0.3	22.7	1.6	22.9	15.0	22.7	0.5	0.4	3.8	9.6
HDL-CE	1.5	0.5	11.5	5.2	2.6	28.0	41.7	1.0	0.4	1.5	6.1
Human (n = 4)											
HDL	1.3[a]	0.7	22.3	3.7	10.6	17.3	32.4	1.1	0.6	2.4	7.8
HDL-TG	1.7	0.6	23.6	5.3	8.4	32.8	24.3	1.1	0.3	0.3	1.7
HDL-PL	1.2	0.4	31.0	2.0	9.1	14.2	29.5	0.7	0.1	2.5	9.5
HDL-CE	1.2	0.5	14.6	4.2	2.7	16.4	52.5	0.8	0.6	0.7	6.0

[a] Average of duplicate determinations on individual samples.

TABLE III: Comparative Amino Acid Compositions of apoA-I and apoA-II from Human and Baboon apoHDL.

	Mol of Amino Acid/Mol of Protein			
	apoA-I		apoA-II	
Amino Acid	Human[a]	Baboon	Human[b]	Baboon
Lys	21	20.3	9	7.5
His	5	7.3	0	0
Arg	16	15.5	0	1.0
Trp[c]	4	3.2	0	0
Asp	21	19.5	3	4.2
Thr	10	11.0	6	5.9
Ser	14	15.9	6	6.3
Glu	47	46.1	16	17.1
Pro	10	10.2	4	3.9
Gly	10	11.0	3	2.1
Ala	19	18.1	5	6.2
½-Cystine[d]	0	0	1	0
Val	13	14.9	6	6.5
Met[d]	3	2.9	1	1.4
Ile	0	0	1	0
Leu	39	37.1	8	8.0
Tyr	7	6.5	4	3.5
Phe	6	5.1	4	3.8

[a] Baker et al. (1975). [b] Brewer et al. (1972). [c] Tryptophan was determined according to Liu and Chang (1971). [d] Half-cystine and methionine were determined as cysteic acid and methionine sulfone (Hirs, 1967).

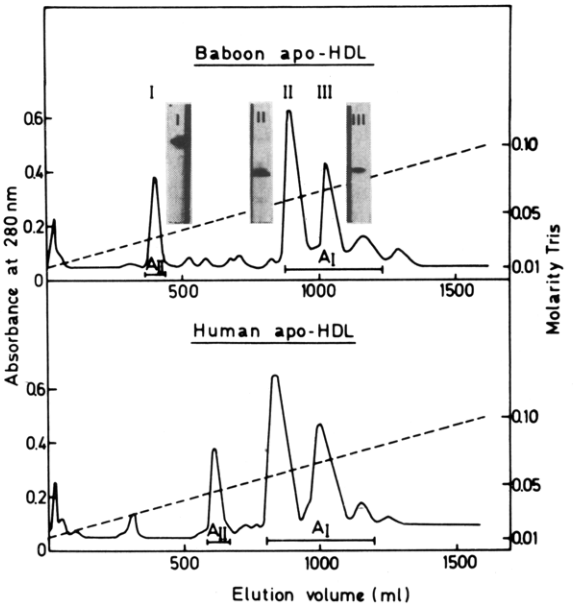

FIGURE 1: The elution profile and the polyacrylamide gel electrophoresis in sodium dodecyl sulfate of human and baboon HDL apoproteins fractionated on DEAE-cellulose.

pholipid with sphingomyelin and phosphatidylethanolamine as minor components. Compared to man, a lower sphingomyelin concentration in the baboon was observed.

The fatty acid pattern of baboon HDL is comparable to human HDL for the 18:1/18:2 ratio, which is higher in the baboon (Table II). Such a difference cannot be related to the fatty acid composition of the diet, as the ratio of polyunsaturated to saturated fatty acids (P/S, 1/1) is similar in normal human diet and in the diet of the baboon. The unsaturated fatty acids, oleic and linoleic acids, were predominant in baboon HDL triglycerides and cholesteryl esters, whereas the saturated fatty acids, palmitic and stearic acids, were predominant in the HDL phospholipids. However, the fatty acid composition of baboon HDL lipids showed significant differences to man, especially the very high percentages of stearic acid in the phospholipids and of oleic acid in the cholesteryl esters.

Isolation and Characterization of HDL Apoproteins. The apoproteins from apoHDL were fractionated by chromatography on DEAE-cellulose in Tris-HCl buffer (pH 8.2)–6 M urea. The elution profile of baboon apoHDL compared to that of man is shown on Figure 1. Fractions I to III were further characterized by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Fractions II and III corresponded to the major apoprotein with a mobility of human apoA-I.

The major differences between the HDL apoprotein elution profiles of baboon and man were the absence of a protein with an elution volume of the human apoA-II and the appearance of a protein fraction (DEAE fraction I) in the baboon with an elution volume different from that of human apoA-II.

Baboon DEAE fraction I migrated on polyacrylamide gel electrophoresis as a single band with a mobility corresponding to a molecular weight of 8500.

The amino acid composition of baboon DEAE fraction I differed from the human apoA-II by the presence of arginine and by the absence of isoleucine and half-cystine (Table III). Based on molecular weight, mobility, and amino acid composition, baboon DEAE fraction I resembles the human monomeric form of apoA-II. In the same table, the amino acid data of apoA-I from man and baboon were also compared,

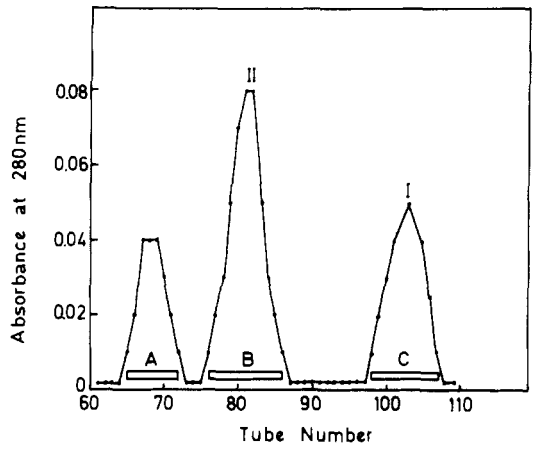

FIGURE 2: Filtration of cyanogen bromide fragments of baboon apoA-II on Sephadex G-50 in 5.4 M urea.

suggesting a complete resemblance of the major HDL apoprotein between both species.

Terminal Amino Acid Residues of apoA-I. For the NH_2-terminal sequence analysis, baboon apoA-I was subjected to 30 cycles of the automatic Edman degradation. The phenylthiohydantoin derivatives were identified by gas-liquid chromatography and, in addition, each step was positively identified by thin-layer chromatography using two different chromatographic techniques. The amino acid sequence for the 30 first residues is: Asp-Glu-Pro-Pro-Gln[5]-Ser-Pro-Trp-Asp-Arg[10]-Val-Lys-Asp-Leu-Val[15]-Thr-Val-Tyr-Val-Asp[20]-Ala-Leu-Lys-Asp-Ser[25]-Gly-Arg-Asp-Tyr-Val[30]. For the first 30 residues, baboon and human apoA-I have identical sequences with the exception of residues 15 and 21. In the human apoA-I (Baker et al., 1975), there is alanine at residue 15 and valine at residue 21.

Treatment of baboon apoA-I with carboxypeptidase A indicated glutamine, the amino acid most rapidly released, as the COOH-terminal residue. Threonine and serine were also rapidly liberated but complete release after 30 min of digestion was not achieved; leucine was released at a slower rate. The kinetics of release of the amino acids suggest a COOH-terminal sequence of Leu-Ser-Thr-Gln.

Isolation and Characterization of Cyanogen Bromide Fragments of apoA-II. Chromatography on Sephadex G-50 in 5.4 M urea of the mixture after cyanogen bromide fragmentation yielded three peaks (Figure 2).

Based on the amino acid content of 1 mol of methionine per mol of apoA-II, cleavage with cyanogen bromide was expected to produce two fragments. Fraction A had an amino acid composition indistinguishable from that of the unfractionated protein and probably represented an aggregate of the starting material. Cyanogen bromide fragments I and II contained no methionine or methionine sulfoxide, had unique amino acid composition, and accounted for the total amino acid content of the protein (Table IV). CNBr I contained 26 amino acid residues and was lacking arginine; CNBr II contained 52 amino acid residues and was devoid of homoserine but did have the single residue of arginine.

Circular Dichroism. The circular dichroic spectra of baboon apoA-I and apoA-II are shown on Figure 3. The structure of native baboon apoA-I is highly ordered with a helical content of 67% compared to 55% in human apoA-I. In order to investigate whether monomeric baboon apoA-II presented the tendency to aggregate reported for human apoA-II (Gwynne et al., 1975), the concentration dependence of the mean residue

TABLE IV: Amino Acid Composition of CNBr Peptides of apoA-II.

Amino Acid	CNBr-I	CNBr-II
Lys	1.06 (1)	6.74 (7)
Arg		1.00 (1)
Asp	2.22 (2)	2.24 (2)
Thr	1.99 (2)	3.16 (4)
Ser	3.02 (3)	2.82 (3)
Glu	6.63 (6)[a]	10.49 (11)
Pro	0.99 (1)	2.84 (3)
Gly	1.07 (1)	1.10 (1)
Ala	1.10 (1)	4.76 (5)
½-Cystine		
Val	3.30 (3)	4.10 (4)
Met	(1)[b]	
Ile		
Leu	1.91 (2)	6.01 (6)
Tyr	1.70 (2)	1.88 (2)
Phe	0.98 (1)	2.88 (3)
Total	26	52

[a] Glu + homoserine. [b] As homoserine.

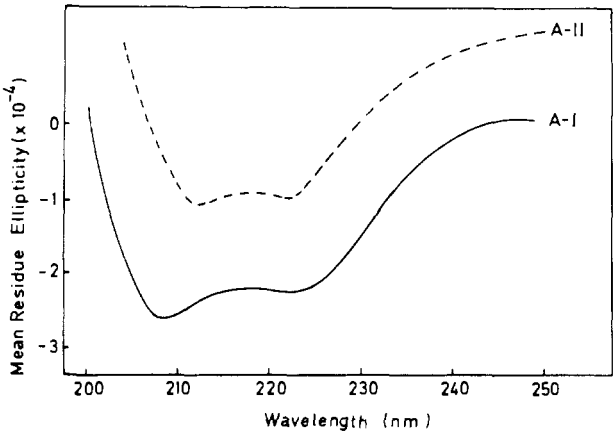

FIGURE 3: Circular dichroic spectra of baboon apoA-I (—) and apoA-II (- - -).

ellipticity was followed over a 100-fold concentration range, as shown on Figure 4a. The negative mean residue ellipticity at 222 nm of monomeric baboon apoA-II is concentration dependent and increases when the monomer to dimer equilibrium is shifted towards higher molecular weight species by increasing the concentration. The maximum value $\theta_{222} = -10\,240$ agrees with that measured for the human apoprotein and corresponds to an α-helical content of 37% (Lux et al., 1972).

Upon titration with increasing quantities of DMPC vesicles, the mean residue ellipticity shows a twofold increase with a maximum at a ratio of 50 mol of DMPC/mol of apoA-II. The maximal value corresponds to an α-helical content of 54% (Figure 4b).

Microcalorimetric Studies of apoA-I and apoA-II with Dimyristoylphosphatidylcholine. Microcalorimetric data on the reassembly of human and baboon apoHDL with DMPC are shown in Figure 5a. The enthalpy changes are very close at any phospholipid/protein ratio, suggesting that the affinity of the two apoproteins for phospholipid is very similar.

The enthalpy changes which evolved on binding DMPC liposomes to human and baboon apoA-I are plotted on Figure 5a as a function of the phospholipid/protein molar ratio.

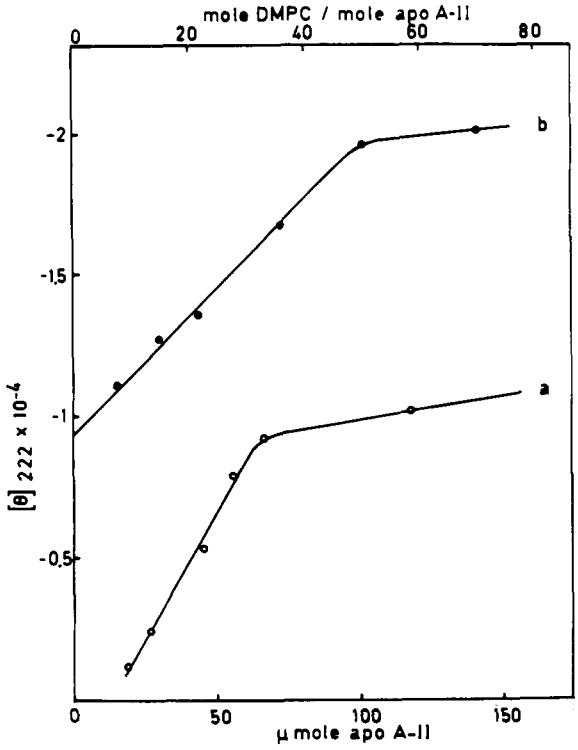

FIGURE 4: (a) The influence of baboon apoA-II concentration on the mean residue ellipticity. (b) The influence of the baboon DMPC/apoA-II ratio on the mean residue ellipticity.

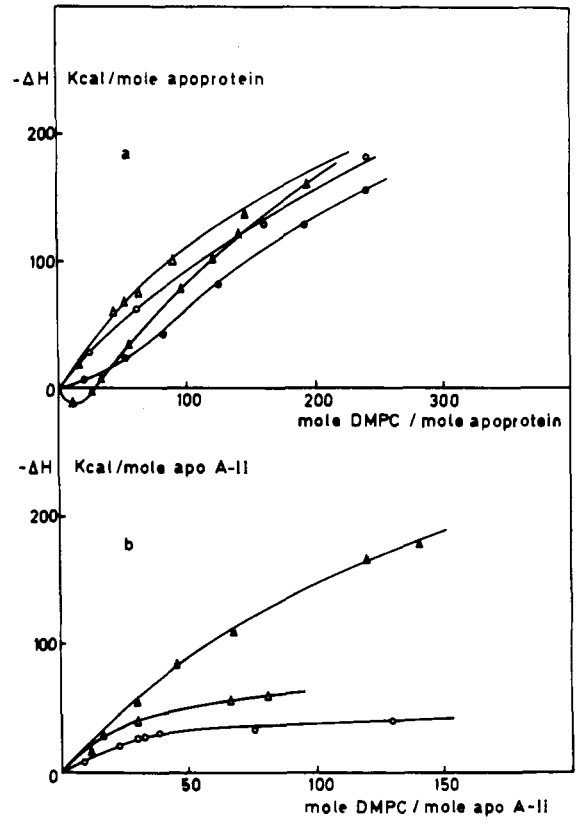

FIGURE 5: Recombination of human and baboon apoproteins with DMPC; (a) human apoHDL (△), baboon apoHDL (○), human apoA-I (▲), baboon apoA-II (●). (b) Human dimeric apoA-II (▲), human monomeric apoA-II (△), baboon apoA-II (○).

DMPC binding to baboon apoA-I is an exothermal process at any phospholipid to protein ratio. The maximal enthalpy change is slightly lower for the baboon than for the human apoprotein, while the complex composition is very similar for both species (Table V).

The enthalpy changes which evolved on binding DMPC liposomes to native baboon monomeric and human dimeric apoA-II are depicted on Figure 5b; the results for human apoA-II calculated in terms of monomeric unit are also shown on the same figure. The complex formed between DMPC and either human or baboon apoA-II has a maximal composition of 35 mol of DMPC/mol of monomeric apoA-II (Table V).

The maximal enthalpy value for baboon apoHDL, apoA-I, and apoA-II is lower than for the human apoproteins.

TABLE V: Characterization of the Complexes Formed between DMPC Liposomes and Baboon Apoproteins.

Apoprotein		n^a	$-\Delta H_{max}$
apoHDL	Human	97	220
	Baboon	90	210
apoA-I	Human	132	215
	Baboon	126	190
dimeric apoA-II	Human	75	230
monomeric apoA-II	Human	38	70
	Baboon	35	40

[a] n = normal complex composition (mol of DMPC/mol of apoprotein). [b] ΔH_{max} = enthalpy change expressed in kcal/mol of apoprotein.

Discussion

The percentage of HDL lipid composition in man and baboon is similar, though the phospholipid subclasss and the fatty acid composition of HDL show obvious differences. Baboon HDL contains only negligible amounts of lysolecithin, and sphingomyelin is significantly less than in human HDL. Similar results were also described for the *Macacus rhesus* HDL (Edelstein et al., 1973). The variation in sphingomyelin to phosphatidylcholine ratio in the baboon, under atherogenic diet, was also different from that of type II hyperlipoproteinemia in man (Blaton et al., 1970, 1974a). These data suggest that differences in HDL lipids and fatty acids may be related to more fundamental differences in the structure of HDL in man and nonhuman primates.

Based on molecular weight, electrophoretic mobility, and amino acid composition, the major apoprotein of baboon HDL is similar to human apoA-I. The presence of apoA-I was also described in the chimpanzee (Blaton et al., 1974b), the *Macacus rhesus* (Edelstein et al., 1973), the pig (Davis et al., 1974; Fidge, 1973; Jackson et al., 1973), the chicken (Jackson et al., 1976), the rat (Koga et al., 1971; Herbert et al., 1974; Swaney et al., 1974), the cow (Jonas, 1972), and the salmon (Nelson and Shore, 1974).

However, the partial NH_2-terminal residue analysis of baboon apoA-I is different from the amino acid sequence of human apoA-I. In both species glutamine is the COOH-terminal residue, followed by threonine in the penultimate position, whereas in baboon apoA-I serine is linked to threonine instead of asparagine in the human. An identical terminal residue was also reported in apoA-I from chimpanzee (Scanu et al., 1974) and Rhesus monkey (Edelstein et al., 1973), whereas alanine is the COOH-terminal residue in dog (Swaney

et al., 1974) and turkey (Kelley and Alaupovic, 1976).

In spite of differences in the amino acid data of apoA-I within the different species, these results suggest that this apoprotein is a major constituent of the high-density lipoproteins.

Among the species studied, only the chimpanzee, representing the great apes, contains the dimeric form of human apoA-II (Blaton et al., 1974b). Baboon HDL contains a monomeric apoprotein, not present in man, with a molecular weight of 8500 and the mobility of human monomeric apoA-II. The amino acid composition of this apoprotein component differs from human apoA-II by the presence of arginine and by the absence of half-cystine and isoleucine. An identical protein was isolated from apoHDL in the *Macacus rhesus* and was described as a monomeric apoA-II (Edelstein et al., 1973).

The helicity content in baboon apoA-I is slightly higher than the value for the human apoA-I (57%) (Lux et al., 1972). Titration of baboon apoA-II with increasing quantities of DMPC vesicles increases the α-helix content up to about 54%. This figure is higher than that reported for the titration of human apoA-II with egg PC (Assman and Brewer, 1974), suggesting that the structure of baboon apoA-II is stabilized by phospholipid to a larger extent than that of the human apoprotein. The cysteine-6 residue in human apoA-II seems not to be involved in self-association nor in phospholipid binding, as its substitution with serine in the baboon does not significantly affect the behavior of the apoprotein.

Thermodynamics of binding DMPC to either human or baboon apoHDL are similar. The differences in the apoprotein composition do not seem to affect significantly the binding properties of apoHDL, suggesting that apoA-I is mainly responsible for the phosphatidylcholine binding capacity of apoHDL. Stoffel et al. (1974) have reported a high affinity of apoA-I for PC in agreement with our results.

The enthalpy changes which evolved on binding DMPC to baboon apoA-I are exothermal at low lipid/protein ratios, in contrast with the behavior of human apoA-I. The enthalpy titration curve for human apoA-I–DMPC was interpreted as resulting from the superposition of an endothermal process occurring at low DMPC levels and an exothermal process predominant at high phospholipid/apoprotein molar ratios. The sigmoidal shape of the curve for baboon apoA-I indicates that these two effects are also present but that the magnitude of the endothermal process is less than for the human apoprotein. This endothermal process was attributed to a lipid-induced disaggregation of apoA-I, in agreement with other reports (Vitello et al., 1975; Jonas, 1975). The enthalpy changes induced on binding DMPC liposomes to either baboon or human monomeric apoA-II are very similar.

The binding experiments and the differences in the apoprotein composition of human and baboon apo-HDL provide useful information on the function of the HDL apoproteins and on the related evolutionary problems.

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Characterization of Antibodies Specific for N^6-Methyladenosine and for 7-Methylguanosine†

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ABSTRACT: Antibodies specific for N^6-methyladenosine (m^6A) and for 7-methylguanosine (m^7G) were prepared by immunization of rabbits with nucleoside conjugates of bovine serum albumin (i.e, m^6A-BSA and m^7G-BSA). Specificity of each antibody was assessed by inhibition of the homologous precipitin reaction with various nucleosides. These analyses revealed that the antibodies elicited in response to m^6A-BSA were specific for the N^6-methyl moiety of adenosine with minimal or no cross-reactivity with BSA, adenosine, and guanosine. Although a major fraction of antibodies elicited in response to m^7G-BSA were specific for m^7G, considerable cross-reactivity was observed with BSA. These latter antibodies were removed by affinity chromatography utilizing BSA-Sepharose adsorbent. In similar fashion, antibodies specific for m^6A and m^7G were isolated by immunospecific adsorption to antigen-coupled Sepharose (e.g, m^6A-BSA-Sepharose), eluted, and coupled to Sepharose. The ability of these antibody-coupled adsorbents to retain specific methylated [*methyl*-^{3}H]nucleosides derived from [*methyl*-^{3}H]tRNA digests was assessed. Both the anti-m^7G and anti-m^6A antibody adsorbents quantitatively and exclusively retained 7-[^{3}H]methylguanosine and N^6-[^{3}H]methyladenosine, respectively. The application of these adsorbents to fractionate oligonucleotides and nucleic acids is discussed.

The use of affinity chromatography as a means of fractionating RNA populations on the basis of their content of specific nucleotide sequences or modified nucleotides has been documented. For example, mRNA containing poly(A)[1] has been isolated following chromatography on either poly(dT)-cellulose (Aviv and Leder, 1972) or poly(U)-Sepharose (Lindberg and Persson, 1972). In a somewhat different system, $tRNA^{Arg}$ and yeast $tRNA^{Phe}$ have been purified via affinity-antibody chromatography utilizing antibodies which react immunospecifically with inosine (Inouye et al., 1973) and the Y nucleoside (Fuchs et al., 1974), respectively. While other antibodies which recognize a variety of minor constituents found in RNA have been characterized (Erlanger and Beiser, 1964; Karol and Tanenbaum, 1966; Sawicki et al., 1971; Levine et al., 1971), their application for fractionating RNA populations

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[1] Abbreviations used are: BSA, bovine serum albumin; EDTA, (ethylenedinitrilo)tetraacetic acid; DEAE, diethylaminoethyl; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; poly(A), poly(adenylic acid); poly(dT)-cellulose, poly(thymidylic acid)-coupled cellulose; poly(U)-Sepharose, poly(uridylic acid)-coupled Sepharose; A, adenosine; m^1A, 1-methyladenosine; m^2A, 2-methyladenosine; m^6A, N^6-methyladenosine; m^6_2A, N^6,N^6-dimethyladenosine; A^m, 2′-*O*-methyladenosine; m^6A^m, 2′-*O*,N^6-dimethyladenosine; m^1I, 1-methylinosine; G, guanosine; G^m, 2′-*O*-methylguanosine; m^1G, 1-methylguanosine; m^7G, 7-methylguanosine; m^2G, N^2-methylguanosine; m^2_2G, N^2,N^2-dimethylguanosine; m^3C, 3-methylcytidine; m^5C, 5-methylcytidine; C^m, 2′-*O*-methylcytidine; m^3U, 3-methyluridine; m^5U, 5-methyluridine; U^m, 2′-*O*-methyluridine; m^7G^*, 2-amino-4-hydroxy-5-(*N*-methylcarboxamido)-6-ribosylaminopyrimidine (i.e., the ring-opened structure of m^7G following treatment with alkali). For spectral data, A_{nm} and E_{nm} are the absorbance (1-cm light path) and molar extinction coefficients, respectively, at a particular wavelength (nm).