ISOLATION AND PARTIAL CHARACTERIZATION OF APOLIPOPROTEIN D AND LIPOPROTEIN D FROM BABOON PLASMA

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1. Introduction

Apolipoprotein D (ApoD), a minor protein constituent of the human plasma lipoprotein system was first isolated and characterized in [1,2]. Although the metabolic role of ApoD is not known, a relationship has been suggested between ApoD and lecithin:cholesterol acyltransferase (LCAT): ApoD may be a cofactor for LCAT [3]; Lipoprotein D (LP-D) may serve to remove products of the LCAT reaction [4]; ApoD could be identical to a plasma factor active in the transfer of cholesteryl esters from high density lipoproteins (HDL) to very low density and low density lipoproteins [5].

ApoD has not been detected in any other animal species. If ApoD has an important metabolic role in lipid transport or metabolism one would expect to find an analogous apolipoprotein in plasma of non-human primates. Here we report the isolation and partial characterization of an apolipoprotein from baboon plasma which is analogous to human ApoD.

2. Material and methods

Blood from baboons was obtained under ketamine HCl sedation. Heparinized plasma was recovered after low speed centrifugation and stored at 4°C until analyses were performed. Lipoprotein density classes were separated by preparative ultracentrifugation as in [6].

Immunoelectrophoresis and immunodiffusion were performed in 1% agarose gels employing veronal buffer (pH 8.5) [6,7]. Monospecific antisera to human apolipoproteins A-I, A-II, C-I, C-II, C-III, D and E, and to lipoproteins A and B were prepared and tested as in [1,2,8–10]. Crossreactivity of antisera to human apolipoproteins with baboon apolipoproteins has been reported [6].

The immunoabsorber for ApoD and the hydroxylapatite columns were prepared as in [1,2]. Polyacrylamide gel electrophoresis was performed essentially as in [11] except with the addition of 8 M urea to the gels. Lipid and protein analyses were performed as in [7]. Neutral and amino sugars were determined as in [12].

3. Results and discussion

The isolation of ApoD from baboon plasma was possible because of the immunochemical crossreactivity of human antiserum to ApoD with baboon plasma [6]. HDL were used as the starting material for the purification of baboon LP-D and ApoD. HDL were first dialyzed against buffer containing 0.15 M NaCl, 0.05 M Tris–HCl (pH 7.3) and chromatographed on an immunoabsorber which contained antibodies to human ApoD. The retained fraction was eluted with 3 M NaSCN. The retained material from the immunoabsorber was rechromatographed on the immunoabsorber twice, then dialyzed against 0.001 M KH_2PO_4 (pH 8.0) and chromatographed on hydroxylapatite. The unretained fraction from the hydroxylapatite column eluted with 0.001 M KH_2PO_4 and showed a single diffuse band on 7% basic polyacrylamide gel electrophoresis with a mobility similar to that of human ApoD. This preparation gave a positive reac-

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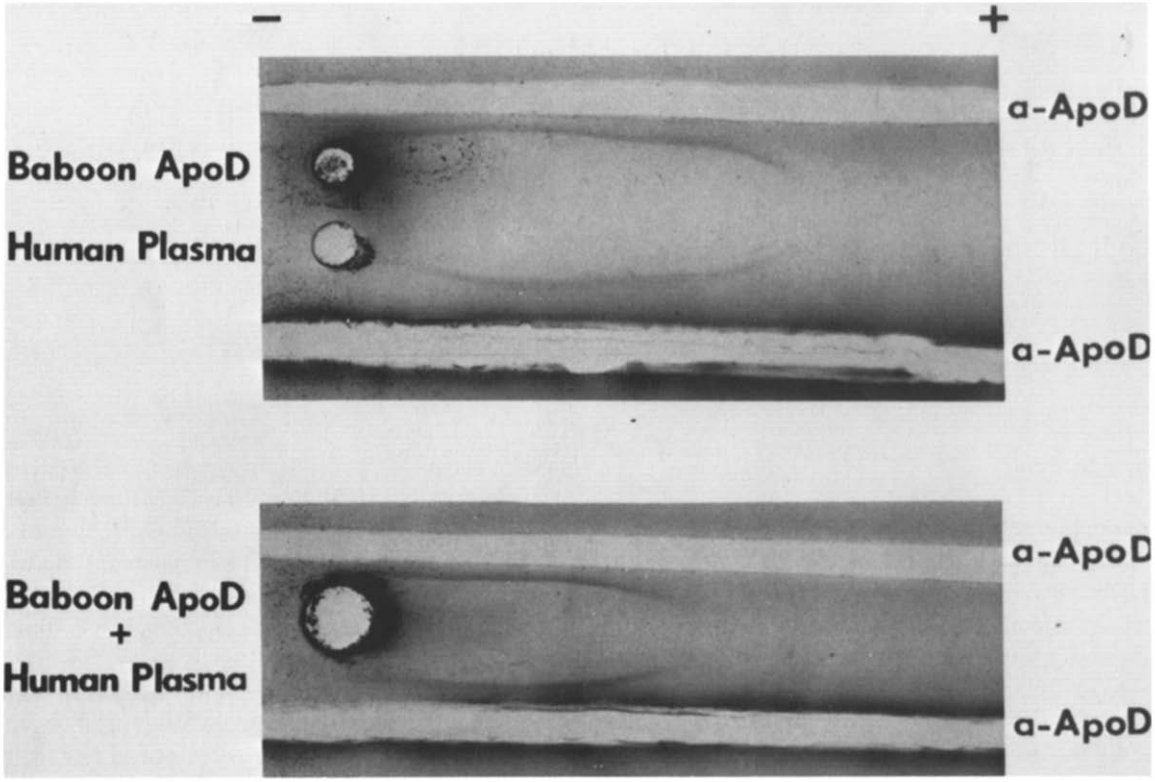

Fig.1. Immunoelectrophoretic analysis of baboon and human ApoD. Baboon ApoD and human plasma reacted separately with human anti-ApoD (top pattern). A mixture of baboon ApoD and human plasma showed a single precipitin line against human anti-ApoD (bottom pattern). The antigens were placed in the wells and the antisera in the troughs in each pattern.

tion with antisera to human ApoD on immunodiffusion and immunoelectrophoresis (fig.1), but did not react with antisera to any other human plasma apolipoproteins. A mixture of purified baboon ApoD and human plasma showed a single precipitin line when tested with an antiserum to human ApoD (fig.1).

LP-D consisted of 52% protein and 48% lipid. The major lipid constituents (table 1) were phospholipids and cholesterol esters. However, in comparison with the human LP-D, the lipid composition of baboon LP-D was characterized by a higher percentage of glycerides and a lower percentage of free cholesterol. Phosphatidyl choline was the major and sphingomyelin, lysophosphatidyl choline and phosphatidyl ethanolamine were the minor phospholipids (table 2). Baboon ApoD consisted of 15% carbohydrate by weight and contained more mannose than human ApoD (table 3). The amino acid composition of

Table 1
Lipid composition of LP-D from baboon plasma (%)

	Triglycerides	Diglycerides	Cholesterol		Phospholipids
			Free	Ester	
Baboon (n = 2)	19.13	14.85	10.85	27.41	27.75
Human[a] (n = 3)	11.68	n.d.	18.08	27.67	46.45

[a] From [2]

Table 2
Phospholipid composition of LP-D from baboon plasma (%)

	Phosphatidyl ethanolamine	Phosphatidyl choline	Sphingo-myelin	Lysophosphatidyl choline
Baboon[a]	4.6	57.2	47.0	11.2
Human[b]	tr	40.1	33.3	26.4

[a] The values represent the average of 3 separate preparations of LP-D
[b] From [2]

Table 3
Carbohydrate composition of ApoD from baboon plasma (wt %)

	Mannose	Galactose	Glucosamine	Neuraminic acid
Baboon[a]	7.1	4.2	4.2	n.d.[c]
Human[b]	3.0	4.4	4.5	4.8

[a] These values represent the average of 2 separate preparations of ApoD
[b] From [2]
[c] n.d., not determined

Table 4
Amino acid composition of ApoD from baboon plasma (mol/1000 mol)

	Baboon ApoD[a]	Human ApoD[b]
Lysine	76	82
Histidine	27	14
Arginine	60	26
Aspartic acid	99	134
Threonine	63	67
Serine	50	46
Glutamic acid	147	126
Proline	42	70
Glycine	52	43
Alanine	81	62
Half-cystine	n.d.[c]	26
Valine	66	74
Methionine	15	13
Isoleucine	42	64
Leucine	94	86
Tyrosine	43	35
Phenylalanine	40	35
Tryptophan	17	24

[a] These values represent the average of 2 independent samples extrapolated to 'zero' time
[b] From [2]
[c] n.d., not determined

baboon ApoD was similar to human ApoD except that baboon ApoD contained more basic amino acids (table 4). The minimum molecular weight calculated from the amino acid and carbohydrate composition was 15 400 compared to 22 100 for human ApoD.

These results show that baboon plasma contains a minor apolipoprotein analogous on the basis of its immunologic and chemical properties to human ApoD. It occurs in plasma in the form of LP-D. The present identification of ApoD and LP-D in plasma of a non-human primate should further stimulate studies on the functional role of this apolipoprotein in lipid transport and metabolism.

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