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COMPARISON OF THE HUMAN, CANINE AND SWINE E APOPROTEINS

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SUMMARY: A comparative study of the E apoprotein isolated from the d<1.02 lipoproteins of human, canine and swine plasma revealed that the various apo-E preparations had similar molecular weights (37,000 - 39,000) and had similar amino acid compositions in that glutamic acid, alanine, leucine and arginine were present in high concentrations. The various preparations showed partial immunochemical cross-reactivity, demonstrating significant sequence homology between the species. However, determination of the amino-terminal amino acid sequence by automated Edman degradation showed each apo-E was different, demonstrating that the amino-terminal portion of the E apoprotein was a variable region of this protein.

The E apoprotein has been the subject of considerable interest in recent years, and several of its characteristics and metabolic properties have been described. The concentration of apo-E increases in the lower density plasma lipoproteins of several animal models following cholesterol feeding (1-7), and also increases in the d<1.006 plasma lipoproteins of humans with Type III hyperlipoproteinemia (8). The E apoprotein can interact with the same high-affinity cell surface receptor as the B apoprotein of low density lipoproteins (LDL)[2]. Its presence in subclasses of HDL, including those of man, accounts for the ability of HDL to interact with cell surface receptors (9). Lipoproteins containing the E apoprotein interact not only with peripheral cell receptors, but are also recognized and taken up by the liver (10). Because of our interest in the E apoprotein and its metabolism, we have begun a detailed

[1] Correspondence should be addressed to this author.

[2] Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; PTH, phenylthiohydantoin; SDS, sodium dodecyl sulfate

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characterization and comparison of apo-E isolated from man, dog and swine to determine the degree of homology between the various species. We wish to report the preliminary results of this study.

MATERIALS AND METHODS

Human plasma was obtained from fasted normal subjects or patients with Type III or Type V hyperlipoproteinemia. Hyperlipoproteinemic plasma was obtained from dogs fed a semi-synthetic coconut oil-cholesterol diet or from Yucatan miniature swine fed a lard-cholesterol diet (7). Plasma was raised to d=1.02 with potassium bromide, and the lipoproteins were prepared by ultracentrifugation for 16 h at 50,000 rpm in a Beckman 60 Ti rotor. The lipoproteins were purified by recentrifugation at d=1.02. The apo-E from the various species was isolated from the tetramethylurea-delipidated d<1.02 density fractions by G-200 Sephadex chromatography, as previously described (4). Amino acid analysis was performed on a Beckman 121M amino acid analyzer after hydrolysis of the protein in 6N HCl for 22 h at 110°C under nitrogen. Cysteic acid was determined after performic oxidation of the protein as described by Moore (11). Immunodiffusion was performed according to the method of Ouchterlony as described by Clausen (12). Sodium dodecyl sulfate (SDS) gel electrophoresis and isoelectric focusing were performed as previously described (13).

Automated Edman Degradation. Sequencing was carried out with automated stepwise degradation on a Beckman 890C Sequencer using a fast peptide program (102974) which employs dimethylallylamine buffer (14). Conversion of the phenylthiohydantoin (PTH) derivatives was performed in 0.3 ml of 1.0 N HCl at 80°C for 10 min under nitrogen (15). PTH derivatives were extracted with 0.8 ml of ethyl acetate (X2) and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 20 µl of methanol or ethyl acetate, and an aliquot of the sample was analyzed either on a Beckman (model 65) gas-liquid chromatograph (16) or a Waters high-pressure chromatography system (17). In some cases, the remainder of the sample was evaporated to dryness, and the free amino acid was regenerated by hydrolysis in 57% hydroiodic acid (18) and determined by amino acid analysis. PTH-arginine in the aqueous phase was identified by high-pressure liquid chromatography. The PTH derivatives of aspartate, asparagine, glutamate, and glutamine were confirmed by thin-layer chromatography (19).

RESULTS AND DISCUSSION

Examination of the various purified apo-E preparations by SDS gel electrophoresis (Fig. 1) showed that each consisted of a single band. Using standard proteins of known molecular weights, the apparent molecular weights of the human and swine apo-E preparations were 37,000, whereas, the canine apo-E appeared to be slightly larger, with an estimated molecular weight of 39,000. Comparison of the amino acid compositions showed that the compositions were similar and characteristic for the E apoprotein in that glutamic acid, alanine, leucine and arginine were present in high concentrations. One significant difference in the amino acid compositions was the presence of cysteine

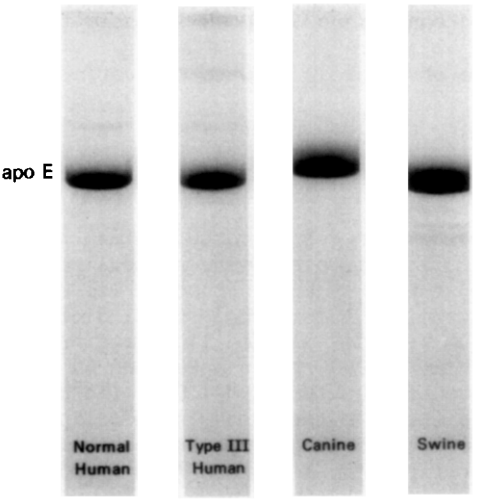

Figure 1. Sodium dodecyl sulfate polyacrylamide gels of the human, canine and swine E apoproteins.

in human apo-E and its absence from the canine and swine preparations (Table 1). Previously, we have reported the presence of two half-cystine residues per mole of Type III apo-E (13). The composition values for both types of the human E apoprotein agreed well with those reported by others (8,20,21). Also, it is interesting to note that amino acid compositions of the E apoprotein from the other two species studied, rat and rabbit, were also similar to the human, canine and swine apo-E (4,5,22).

A functional homology for the various E apoproteins is known to exist. The E apoprotein from man (23), dog (23), swine (24) and rat (25) is capable of binding with high affinity to the cell surface receptor on cultured human fibroblasts. To determine if a structural homology also existed, the immunologic properties of the various apo-E preparations were determined using the technique of double immunodiffusion. All preparations showed the presence of common immunological determinants when tested with antiserum prepared to human Type III apo-E. Normal human and Type III apo-E demonstrated complete immunochemical identity, while the canine and swine gave precipitin lines indicating partial identity with the human preparations (data not shown). A similar demonstration of species cross-reactivity was also obtained with swine apo-E anti-

Table I. Amino Acid Composition of the Various Apo-E Preparations[a]

	HUMAN		CANINE[b]	SWINE[b]
	Normal[b]	Type III[c]		
Asp	5.2	4.7	6.5	5.9
Thr	4.1	3.7	4.1	4.6
Ser	6.8	4.9	4.2	7.4
Glu	23.4	24.2	26.7	24.8
Pro	3.0	2.7	3.8	3.6
Gly	7.3	6.3	4.8	6.4
Ala	11.5	11.6	11.8	8.9
1/2Cys[d]	0.4	0.7	0.0	0.0
Val	6.8	6.9	5.3	5.4
Met	0.7	2.0	1.9	0.5
Ileu	0.7	1.0	0.9	0.5
Leu	13.1	12.7	12.3	13.5
Tyr	1.2	1.5	0.8	1.0
Phe	1.1	1.3	1.1	0.7
Lys	4.2	4.2	4.5	3.7
His	1.0	1.1	0.6	0.7
Arg	9.6	10.7	10.5	12.4

[a] Expressed as mol%.

[b] Average of duplicate determinations on two preparations.

[c] Data from Reference 13.

[d] Determined following performic acid oxidation.

serum. These results demonstrated that the various E apoproteins share extended amino acid sequences in common with each other.

Because of the similarity in amino acid composition, immunoreactivity and binding with high affinity to the cell surface receptor on cultured fibroblasts, it was of interest to compare the isoelectric focusing patterns of the apo-E preparations. Isoelectric focusing on polyacrylamide gels over the pH range 3.5 to 7.0 demonstrated the presence of several bands between pI 5.0 and 6.0 for each of the E apoproteins (Fig. 2). The occurrence of multiple forms of the human apo-E is characteristic of this apoprotein (26,27). The banding patterns for both the Type III and normal apo-E were similar to those previously reported (26,27). Typically, the Type III apo-E pattern was lacking the band at pI$\sim$5.75. The pattern of the normal apo-E isolated from this particular individual contained an additional band (pI$\sim$5.9) which is known to occur in 25% of normal subjects (27). Comparison of the various patterns indicated that the pattern for each species was distinct (Fig. 2).

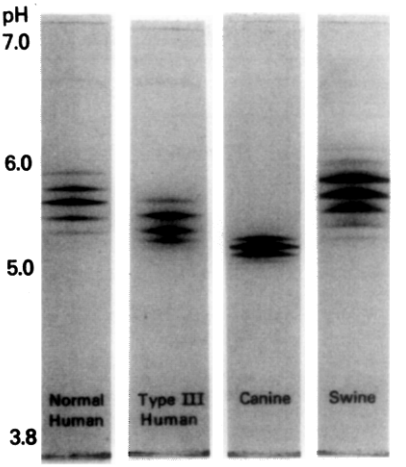

Figure 2. Isoelectric focusing on polyacrylamide gels of the human, canine and swine E apoproteins, pH range 3.5 to 7.0.

The amino acid sequence at the amino terminus of the various E apoproteins was determined by automated Edman degradation (Table II). The detection of lysine as the amino-terminal amino acid of normal human apo-E confirmed an earlier observation (28). It is noteworthy that the apo-E from normal subjects, as well as from patients with Type III and Type V hyperlipoproteinemia, have identical amino acid sequences at the amino terminus. Furthermore, based on the recovery of only one PTH-amino acid at each step, it appeared that a single polypeptide chain was sequenced. This suggested that the multiple bands observed with isoelectric focusing are not due to amino acid substitutions in the amino-terminal region of the human E apoprotein. Sequences of the apo-E from the various species (Table II) showed that there was not a common sequence between human, canine and swine apo-E in the amino terminus. Limited sequence data available for rat apo-E is also consistent with this observation (29). However, there was a short segment shared by the human and canine apo-E (canine residues 4-8 with human 10-14).

In summary, the properties of normal, Type III, and Type V human apo-E have been compared with the apo-E isolated from the dog and swine. Although a significant degree of sequence homology between the various E apoproteins was suggested by the similar amino acid compositions, immunologic cross-reactiv-

Table II. Amino-Terminal Sequence of the Human, Canine and Swine E Apoproteins[a]

	HUMAN			CANINE	SWINE	RAT[b]
Position	Normal	Type III	Type V			
1	Lys	Lys	Lys	Asp	Glu	Glu
2	Val	Val	Val	Val	Asp	Gly
3	Glu	Glu	Glu	Gln	Glu	Glu
4	Gln	Gln	Gln	Pro	Pro	Leu
5	Ala	Ala	Ala	Glu	Gly	Val
6	Val	Val	Val	Pro	Pro	Glu
7	Glu	Glu	Glu	Glu	Pro	
8	Thr	Thr	Thr	Leu	(Pro)	
9	Glu	Glu	Glu	Glu	Gln	
10	Pro	Pro	Pro	Arg	Val	
11	Glu	Glu	Glu	Glu	(Gly)	
12	Pro	Pro	Pro	Leu	X	
13	Glu	Glu	X	Glu	(Leu)	
14	Leu	Leu	Leu	Pro		
15	(Leu)	(Leu)	(Leu)	Lys		
16	Gln	Gln	Gln	Val		
17	Gln	Gln	(Gln)	Gln		
18	Thr	Thr	Thr	Gln		
19	Glu	Glu		Glu		
20				Leu		

[a] Parentheses indicate a suspected residue and X indicates an unidentified residue.

[b] Data from Reference 29.

ities and the ability of each to bind to cell surface receptors, the various preparations had different amino-terminal amino acid sequences. Thus, it appears that the amino-terminal portion of the E apoprotein represents a variable region of this protein.

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