THE AMINO- AND CARBOXYL-TERMINAL SEQUENCES OF CANINE APOLIPOPROTEIN A-I

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1. Introduction

The major constituent polypeptides of mammalian high density lipoproteins (HDL) are apolipoprotein A-I (Apo A-I) and apolipoprotein A-II (Apo A-II) [1]. These two apolipoproteins are now well characterized and the amino acid sequence of both proteins from human is known [2–7]. Partial sequences of Apo A-I have also been determined in other mammalian species including swine [8–10], chimpanzee [11] and avian species including chicken [12] and turkey [13]. In this communication, we report the NH_2- and COOH-terminal sequence of canine Apo A-I.

2. Experimental procedures

2.1. *Protein Purification*

Apo A-I was purified as previously reported [14]. This material was obtained in peak II from Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, New Jersey) column chromatography of Apo HDL_3 and was shown to be homogeneous by several criteria.

2.2. *Automated Edman degradation*

Automated Edman degradations were carried out in a Beckman 890C Sequencer (Beckman Instruments, Inc., Palo Alto, California). The procedures were essentially those of Edman and Begg [15]. A modified program of Beckman No. 102974 (Peptide-DMAA Program) was used. At the end of the period of coupling with phenylisothiocyanate (PTH) and drying, solvent extraction was carried out with a mixture of equal volumes of benzene and ethyl acetate. The phenylthiohydantoins were identified by gas–liquid chromatography [16] in a Beckman GC-65 apparatus with glass columns packed with SP-400. The identifications were done both as PTH-amino acids and as their trimethylsilyl derivatives. Further verification by thin-layer chromatography [17] was carried out for all residues.

2.3. *COOH-terminal sequence*

The COOH-terminal sequence was determined by the rates of release of free amino acids during digestion of Apo A-I by diisopropylfluorophosphate(DFP)-treated carboxypeptidase A or a mixture of DFP-treated carboxypeptidase A and B (ratio 1:1). The digestion was carried out in 0.2 M *N*-ethylmorpholine acetate, pH 8, at 37°C. The carboxypeptidase:Apo A-I ratio was 1:40. The conditions were the same as described by Ambler [18]. The amino acids released were analyzed in an Amino Acid Analyzer (Beckman 120C). The COOH-terminal residue was also determined by hydrazinolysis [19].

3. Results and discussion

The NH_2-terminal sequence of the first 33 canine Apo A-I residues is shown in table 1. Available NH_2-terminal sequences of Apo A-I from other species are also aligned in table 1. Compared with the sequence of human Apo A-I, only four residue changes are present. In human Apo A-I, an insertion at residue 4 and replacements at residues 21, 22 and 31 are

Table 1
The NH_2-terminal sequences of Apo A-I

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Canine[a]	Asp	Glu	Pro	—	Gln	Ser	Pro	Trp	Asp	Arg	Val	Lys	Asp	Leu	Ala	Thr	Val	Tyr	Val	Asp
Human [4]	Asp	Glu	Pro	Pro	Gln	Ser	Pro	Trp	Asp	Arg	Val	Lys	Asp	Leu	Ala	Thr	Val	Tyr	Val	Asp
Baboon [20]	Asp	Glu	Pro	Pro	Gln	Thr	Pro	?	Asp	Arg	Val	Lys	Asp	Leu	Val	Thr	Val	Tyr	Val	Asp
Chimpanzee [11]	Asp	Glu	Pro	Pro																
Swine [8]	Asp	Asp	Pro	—	Gln	Ser	Pro	Trp	Asp	Arg	Val									
Chicken [12]	Asp	Glu	Pro	—	Gln	Pro	Glu	Leu												
Turkey [13]	Asp	Asp	Asn	—	Gln	Thr	Pro	Leu	Asn	Glu	Ile									

	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Canine	Ala	Val	Lys	Asp	Ser	Gly	Arg	Asp	Tyr	Val	Ala	Gln	Phe	Glx
Human	Val	Leu	Lys	Asp	Ser	Gly	Arg	Asp	Tyr	Val	Ser	Gln	Phe	Gln
Baboon	Ala	Leu	?	Asp										

[a] The numbering of residues is based on the sequence of human Apo A-I [4]. The identification of phenylthiohydantoins of amino acids was made with both gas–liquid and thin-layer chromatography except Arg at residues 10 and 27, which was identified by arginine color reactions [25]. Arbitrary deletion on sequence residue no. 4 was made in order to demonstrate homology with the human sequence.

present. The NH_2-terminal sequence of canine Apo A-I, however, is identical with residues 1 and 3-11 of swine Apo A-I [8].

The COOH-terminal residue was demonstrated to be alanine by 72-h hydrazinolysis. Only 3 amino acids were released during the digestion by carboxypeptidase A and B. The relative rates of their release, which were followed to 30 min, approximated the number of residues found at 10 min digestion: Ala 0.71 > Leu 0.43 > Lys 0.37. Long term carboxypeptidase A digestion released one residue each of Leu and Ala. Therefore, the COOH-terminal sequence must be –Lys–Leu–Ala.

The results presented in this communication as well as in studies by other investigators indicate that the NH_2-terminal Asp residue of Apo A-I from different mammalian species is invariant. On the other hand, the COOH-termini can be divided into two groups. Apo A-I proteins from human [4], chimpanzee [11], baboon [20] and rhesus monkey [21] have COOH-terminal Gln while the proteins from dog, rat [22] and turkey [13] have COOH-terminal Ala. There is immunologic cross reactivity between human, chimpanzee, baboon and rhesus monkey [11,20,21]. In contrast, we have found no immunologic cross reactivity between human Apo A-I and canine Apo A-I (unpublished data). These observations suggest that the NH_2-terminal part of Apo A-I may play a common functional role whereas the COOH-terminal end may play a key role as the antigenic site. Such COOH-terminal differences could also be important in cellular recognition of lipoproteins. For example, it has been shown that binding and uptake of both rat low-density lipoproteins and rat HDL by rat aortic smooth muscle cells in culture were significantly higher than that of corresponding human lipoproteins [23,24]. This suggests that amino acid sequence may be important in determining cellular recognition of lipoproteins.

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