Primary structure of apolipoprotein A-II from inbred mouse strain BALB/c

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Abstract The primary structure of apolipoprotein A-II (apoA-II) isolated from the plasma high density lipoprotein (HDL) fraction of the inbred mouse strain BALB/c is described in this work. The complete 78 amino acid protein sequence was determined by proteolytic fragmentation, gas-phase microsequence analysis, and fast atom bombardment (FAB) mass spectrometry. The apolipoprotein has a calculated molecular weight of 8,715 and a net negative charge conveyed by ten acidic and eight basic amino acid residues. There exists a 55% amino acid sequence homology between the BALB/c mouse apoA-II and human apoA-II. Unlike human plasma apoA-II, which exists as a disulfide dimer, BALB/c apoA-II lacks cysteine and is a monomer. BALB/c apoA-II contains one residue each of histidine and arginine, neither of which are found in the human A-II protein. Chou and Fasman analysis of the BALB/c apoA-II primary structure predicts approximately 68% alpha-helical potential compared with a 62% potential for human apoA-II. The alpha-helical domains are structurally amphipathic, generating a polar and an apolar face consistent with the proposed models describing apolipoprotein-phospholipid interaction. — **Miller, C. G., T. D. Lee, R. C. LeBoeuf, and J. E. Shively.** Primary structure of apolipoprotein A-II from inbred mouse strain BALB/c. *J. Lipid Res.* 1987. **28:** 311–319.

Supplementary key words apolipoprotein • high density lipoprotein • amino acid sequence • mass spectrometry • tryptic peptide mapping • protein sequence homology

Apolipoprotein A-II (apoA-II) is the second most abundant protein of high density lipoproteins (HDL) from most mammalian species. The function of this protein is unknown although it probably plays a role in the structural integrity of HDL (1, 2). Further, it may serve as an inhibitor of lecithin:cholesterol acyltransferase (LCAT) since it can displace an LCAT activator, apolipoprotein A-I (apoA-I), from the surface of HDL. ApoA-I is the major protein of HDL, and is usually present as 65% to 95% of the total HDL protein. The molar ratio of apoA-I to apoA-II appears to vary among species and between HDL density fractions of a given species. For instance, it is clear from recent studies that only a subpopulation of HDL contains apoA-II (3). Thus it is tempting to speculate that the abundance of apoA-II may be an indication of particular structural properties or biological activities of HDL.

Genetic polymorphisms in the structure and plasma concentration of apoA-II have been identified among inbred mouse strains (4, 5). Among 40 inbred strains, there appear to be two major structural phenotypes of apoA-II as determined by isoelectric focusing. Further, strains vary by twofold in their plasma apoA-II concentrations. Thus the mouse offers a unique system in which to examine the role of apoA-II in lipoprotein structure and metabolism.

We are interested in the underlying molecular basis for apolipoprotein structural polymorphisms found among inbred mouse strains. This report establishes the complete amino acid sequence of one form of apoA-II, derived from the inbred strain BALB/c.

EXPERIMENTAL PROCEDURES

Materials

Pyroglutamate aminopeptidase (bovine liver) was obtained from the Calbiochem-Behring Co. and TPCK-treated trypsin (bovine pancreas) was purchased from the Sigma Chemical Co. Acetonitrile (HPLC grade) was obtained from the J. T. Baker Chemical Co. HCl (6 N) was obtained from the Pierce Chemical Co. and polybrene was from the Aldrich Chemical Co. Trifluoroacetic acid was distilled over chromium trioxide followed by distillation over alumina. Additional microsequencer reagents were obtained or prepared as described elsewhere (6).

Abbreviations: RP-HPLC, reverse-phase high performance liquid chromatography; TPCK, L-1-tosylamido-2-phenylethylchloromethylketone; PTH, phenylthiohydantoin; FAB/MS, fast atom bombardment-mass spectrometry; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; TFA, trifluoroacetic acid.

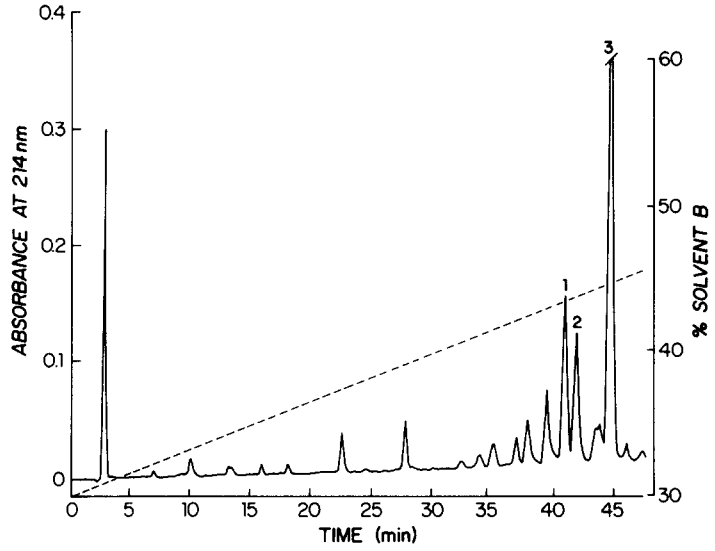

Fig. 1. RP-HPLC fractionation of gel permeation HPLC-purified pyroglutamyl-cleaved BALB/c apoA-II. The sample was eluted off a Vydac C4 reverse-phase HPLC column in peak 3 as described in Experimental Procedures.

Animals

BALB/cBy mice were purchased from the Jackson Laboratory, Bar Harbor, ME. Female mice between the ages of 2 and 4 months were used and maintained on Purina mouse chow. Animals were bled from the orbital sinus into tubes containing 1 mM EDTA. Plasma was collected by low speed centrifugation and used immediately for the isolation of HDL.

Isolation of apolipoprotein

ApoA-II was isolated from mouse plasma HDL as described previously (4). Briefly, the HDL fraction was

TABLE 1. Amino acid compositions of RP-HPLC fractions[a]

Amino Acid Residue	Residue/Molecule[b]	Vydac C4 p3		Vydac C4 p2		Vydac C4 p1	
		Mol %	Residue per mol	Mol %	Residue per mol	Mol %	Residue per mol
Asx	5.3 (5)	6.5	5.3	6.2	4.9	7.3	5.7
Thr	5.6 (6)	6.9	5.6	7.1	5.6	7.5	5.8
Ser	9.2 (8)	11.3	9.2	11.1	8.7	11.8	9.1
Glx	13.5 (14)	16.6	13.5	18.7	14.6	18.6	14.4
Pro	4.2 (4)	5.2	4.2	4.7	3.7	5.6	4.3
Gly	7.8 (3)	9.6	7.8	4.4	3.5	4.6	3.6
Ala	7.6 (7)	9.3	7.6	10.5	8.2	10.9	8.5
Val	2.9 (4)	3.6	2.9	2.9	2.3	2.7	2.1
Met	1.7 (3)	2.1	1.7	3.7	2.9	2.5	1.9
Ile	0.9 (1)	1.1	0.9	0.7	0.5	0.6	0.4
Leu	7.6 (7)	9.3	7.6	10.4	8.2	9.7	7.5
Tyr	2.1 (3)	2.6	2.1	3.6	2.8	3.7	2.9
Phe	4.9 (5)	6.1	4.9	6.3	5.0	6.5	5.0
His	1.5 (1)	1.9	1.5	2.0	1.6	0.8	0.6
Trp	0.0 (0)	0.0	0.0	0.0	0.0	0.0	0.0
Lys	5.6 (6)	6.8	5.6	6.8	5.3	8.5	6.6
Arg	0.8 (1)	1.7	0.8	0.9	0.7	0.8	0.6
Cys	0.0 (0)	0.0	0.0	0.0	0.0	0.0	0.0

[a] Methods for amino acid analysis are described in the Experimental Procedures.
[b] A 0.6-μg sample of reverse-phase HPLC-purified deblocked apoA-II was hydrolyzed in 6 N HCl containing 0.2% mercaptoethanol for 24 hr at 110°C. Cysteine was determined after performic acid oxidation of a second sample. The numbers in parentheses indicate the number of residues determined from microsequence analysis.

TABLE 2. Amino-terminal amino acid sequence of mouse BALB/c apolipoprotein A-II[a]

Cycle	Amino Acid Residue[b]	pmol
1	Ala	578
2	Asp	335
3	Gly	255
4	Pro	178
5	Asp	220
6	Met	275
7	Gln	262
8	Ser	183
9	Leu	246
10	Phe	195
11	Thr	48
12	Gln	214
13	Tyr	145
14	Phe	163
15	Gln	180
16	Ser	88
17	Met	76
18	Thr	91
19	Glu	111
20	Tyr	132
21	Gly	70
22	Lys	201
23	Asp	67
24	Leu	32
25	Val	35
26	Glu	39
27	Lys	46
28	Ala	37
29	Lys	47
30	Thr	6
31	Ser	17
32	Glu	24
33	Ile	15
34	Gln	19
35	Ser	17
36	Gln	16
37	Val	6
38	Lys	14
39	Ala	13

[a]Based on approximately 2 nmol of HPLC-purified deblocked apolipoprotein A-II.
[b]Determined as the PTH-amino acid derivative. PTH-Ser is quantitated as the sum of the PTH-Ser derivative and the DTT-adduct to dehydro-PTH-Ser. PTH-Thr pmol yields are reported as the recovery of the PTH-Thr derivative alone.

isolated based on its density in salt solutions by preparative ultracentrifugation (7). The protein component was delipidated with ethanol–ether extractions and apoA-II was separated from apoA-I and C peptides on a Sephacryl S-200 (Pharmacia) gel permeation column. The equilibration and elution buffer was 10 mM Tris–HCl, 1 mM EDTA, pH 8.6, containing 8 M guanidine hydrochloride. Sample purity was assayed by isoelectric focusing and immunoblot procedures (8).

Amino acid analysis

Approximately 0.2–2.0 μg of peptides was hydrolyzed in 6 N HCl–0.2% 2-mercaptoethanol for 24 hr at 110°C. Hydrolyzates were analyzed on a Beckman 121MB amino acid analyzer (9). Performic acid-oxidized samples were analyzed to detect cysteine as cysteic acid.

Pyroglutamate aminopeptidase treatment

Approximately 200 μg of BALB/cBy apoA-II was dissolved in 300 μl of deblocking buffer (0.1 M NaH_2PO_4, 0.1 M Na_2PO_4, 3% (v) glycerol, 10 mM Na_2EDTA, 5 mM DTT, pH 8.0) (10). The solution was chilled and about 0.5 mg of pyroglutamate aminopeptidase (bovine liver, Calbiochem) was added. The reaction mixture was stirred at 4°C for 18 hr followed by the addition of 0.5 mg of enzyme at room temperature. The reaction was allowed to proceed for an additional 18 hr at room temperature. The cleaved apolipoprotein was recovered from the product mixture by HPLC with a Waters Associates Protein-Pak 300sw gel-permeation column (300 mm × 7.5 mm) eluting isocratically with 0.1% aqueous trifluoroacetic acid at a flow rate of 0.25 ml/min.

Reverse-phase HPLC of apolipoprotein

ApoA-II, isolated from Sephacryl S-200 gel elution, was analytically and preparatively passed over a Vydac-C_4 reverse-phase HPLC column (25 cm × 4.6 mm, 5 μm particle size, 300 Å pore size) eluting with a 90-min linear gradient from 70% solvent A (0.1% aqueous TFA) to 60% solvent B (TFA–H_2O–CH_3CN 0.1:9.9:90 (v/v/v)) at a flow rate of 1 ml/min. The Beckman 344 HPLC system used consisted of Model 112 solvent delivery modules, a Model 421 controller, a Model 160 UV absorbance detector (monitoring at 214 nm), and a Model 340 injector/dynamic mixer organizer.

Tryptic digestion and purification of peptides

Approximately 2.5 nmol of RP-HPLC-purified BALB/c apoA-II was treated with 1 μg of TPCK-treated trypsin (Sigma) in 0.2 M NH_4HCO_3, pH 8, for 18 hr at room temperature. The digest was directly fractionated on a Vydac-C_4 reverse-phase HPLC column (25 cm × 4.6 mm, 5 μm particle size, 300 Å pore size) using a 90-min linear gradient from 100% solvent A (0.1% aqueous TFA) to 70% solvent B (TFA–H_2O–CH_3CN 0.1:9.9:90 (v/v/v)) and a flow rate of 1 ml/min on the Beckman 344 HPLC system.

Amino-terminal and tryptic peptide microsequence analysis

A City of Hope-built gas-phase peptide-protein microsequencer (11) was used for the automated Edman chemical degradations of peptide and pyroglutamate-free protein samples. For the amino-terminal sequence, approximately 2 nmol of deblocked apoA-II was applied to the reaction disk and subjected to 40 cycles of Edman chemistry. Tryptic peptides (0.1–2.0 nmol) were sequenced in a similar fashion. The PTH-amino acid residues were identified by reverse-phase HPLC on an

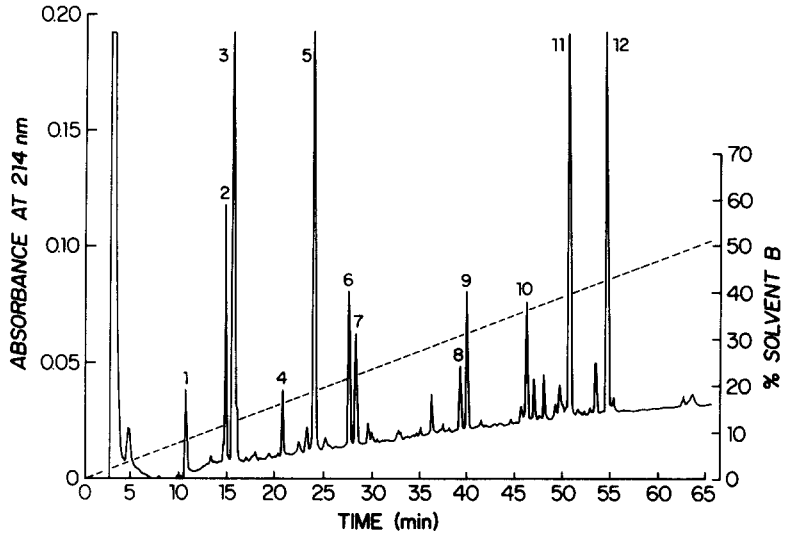

Fig. 2. Tryptic peptide map of BALB/c apoA-II. Approximately 3 nmol of apoA-II was digested with 1 μg of TPCK-trypsin and fractionated on a Vydac C4 reverse-phase HPLC column as described in Experimental Methods.

Ultrasphere ODS column (Altex, 25 cm × 4.6 mm) using Waters Associates solvent pumps (Model 6000A), UV absorbance detector (Model 440), and a WISP autosampler. Recoveries of the PTH-amino acids were determined by an integration program on a Spectra Physics 4000 computer (12).

FAB-mass spectral analysis of tryptic peptides

Samples of 0.05 to 1.0 nmol were either concentrated to 5 μl or to dryness in a polyethylene microfuge tube in a vacuum centrifuge, redissolved in 1–2 μl of 5% aqueous acetic acid, and added to either 1 μl of glycerol or 1 μl of a 50:50 mixture of glycerol and dithiothreitol on a 1.5 mm × 5.0 mm stainless-steel sample stage. Fast atom bombardment spectra were taken with a JEOL HX-100HF mass spectrometer utilizing a 6KV xenon atom primary beam. The instrument was calibrated to mass 4500 at 5 KV using a mixture of potassium iodide and cesium iodide. Data were collected using a JEOL DA5000 data system. Mass assignments were accurate to within 0.2 μ and values for the molecular ion clusters are reported as the nearest integer value of the monoisotopic mass.

RESULTS

ApoA-II, isolated from BALB/c plasma HDL, was treated with bovine liver pyroglutamate aminopeptidase to deblock the amino-terminal pyroglutamyl residue, which precluded direct amino-terminal microsequence analysis. The product mixture was conveniently fractionated by gel-permeation HPLC. The gel-permeation HPLC-purified deblocked protein exhibited one major 214-nm absorbance band when purified over a Vydac-C_4 reverse-phase HPLC column (**Fig. 1**). Several weakly absorbing species collectively amounted to 20 to 25% of the total UV absorbance. Enhanced protein recoveries from the gel-permeation and reverse-phase HPLC columns were achieved by adjusting the sample to 2 M guanidine–HCl prior to injection.

Amino acid analysis of the principal peak isolated from the reverse-phase HPLC fractionation of deblocked and gel-permeation HPLC-purified apoA-II compared favorably with data derived from the complete amino acid sequence (**Table 1**). Discrepancies can be accounted for by the introduction of slight dust contamination resulting in elevated Gly and Ser values and incomplete recovery of branched aliphatic amino acid residues. Performic acid oxidation of a sample prior to hydrolysis indicated the absence of cysteine. Amino acid analysis of two minor ultraviolet-absorbing species isolated from the reverse-phase HPLC purification (peaks 1 and 2 of Fig. 1) resembled the data derived for the major fraction, peak 3 (Table 1), and were not analyzed further.

The amino-terminal microsequence analysis of 2 nmol of deblocked and gel permeation HPLC-purified apoA-II resulted in a unique and unambiguous sequence of 39 amino acid residues (**Table 2**). The calculated initial yield of 21%, based on the recovery of PTH-Ala at cycle-1, is attributed to an approximate 50% yield of enzymatically deblocked protein and the commonly observed initial yield of about 50% obtained in the microsequence analysis of protein samples.

Approximately 3 nmol of intact apoA-II, isolated as the major Vydac C_4 reverse-phase HPLC fraction (peak 3 of

TABLE 3. Microsequence analysis of tryptic peptides[a,b]

Cycle	T-1 (0.6 μg)	T-2 (0.7 μg)	T-3a (1.2 μg)	T-3b (1.2 μg)	T-4 (0.1 μg)	T-5 (1.2 μg)	T-6 (0.5 μg)	T-7 (0.8 μg)	T-8 (0.1 μg)	T-9 (0.5 μg)	T-10 (0.2 μg)	T-11 (0.1 μg)	T-12 (0.5 μg)
1	Asp (482)	Thr (157)	Ala (1400)	Ala (NQ)	Phe (24)	Thr (113)	Ser (113)	Thr (33)	(N.S.)	Ser (43)	Ser (21)	(N.S.)	Ser (59)
2	Leu (404)	Ser (275)	Tyr (1209)	Lys (184)	Gln (28)	His (257)	Ser (68)	Gln (39)		Ala (68)	Leu (54)		Ala (183)
3	Val (204)	Glu (453)	Phe (923)	Thr (NQ)	Ser (5)	Glu (473)	Leu (157)	Tyr (47)		Gly (30)	Phe (38)		Gly (100)
4	Glu (469)	Ile (435)	Glu (829)	Ser (30)	Met (11)	Gln (518)	Met (92)	Phe (22)		Thr (NQ)	Thr (NQ)		Thr (16)
5	Lys (211)	Gln (373)	Lys (362)	Glu (NQ)	Thr (NQ)	Leu (143)	Asn (130)	Gln (38)		Ser (5)	Gln (51)		Ser (46)
6		Ser (154)		Ile (51)	[Glu]	Thr (57)	Leu (113)	Ser (NQ)		Leu (NQ)	Tyr (32)		Leu (84)
7		Gln (192)		Gln (36)	[Tyr]	Pro (170)	Glu (97)	Met (7)		[Val]	Phe (24)		Val (86)
8		Val (65)		Ser (NQ)	[Gly]	Leu (78)	Glu (68)	Thr (NQ)		[Asn]	Gln (27)		Asn (126)
9		Lys (62)		Gln (14)	[Lys]	Val (56)	Lys (116)	Glu (4)		[Phe]	Ser (5)		Phe (73)
10				Val (NQ)		Arg (16)	Pro (56)	Tyr (46)		[Phe]	Met (5)		Phe (73)
11				Lys (NQ)			[Ala]	Gly (7)			[Thr]		Ser (22)
12							[Pro]	Lys (NQ)			[Glu]		Ser (33)
13							[Ala]				[Tyr]		Leu (35)
14							[Ala]				[Gly]		Met (27)
15											[Lys]		Asn (43)
16													Leu (30)
17													Glu (26)
18													Glu (15)
19													Lys (48)
20													Pro (56)
21													Ala (9)
22													Pro (28)
23													Ala (3)
24													Ala (1-2)

[a]Values (μg) indicate mass of peptides sequenced. Numbers in parentheses indicate pmol yields of PTH-amino acids. (NQ) is used for a PTH-amino acid detected but not quantitated. A bracketed residue, [ ], designates a PTH-amino acid not detected by microsequence analysis but assigned based on amino acid analysis, FAB mass spectral analysis, and overlapping tryptic peptide sequences. (N.S.) indicates no sequence data obtained.

[b]Methods for microsequence analysis are described in the Experimental Procedures.

TABLE 4. FAB-mass spectral analysis of tryptic peptides

Tryptic Peptide	Molecular Ion, MH^+ (m/z)	Position in Sequence	Amino Acid Sequence[a]
T-1	603	24-28	D L V E K
T-2	1020	31-39	T S E I Q S Q V K
T-3a	657	40-44	A Y F E K
T-3b	1219	29-39	A K T S E I Q S Q V K
T-4	1090	15-23	F Q S M T E Y G K
T-5	1194	45-54	T H E Q L T P L V R
T-6	1458	65-78	S S L M N L E E K P A P A A
T-7	1483	12-23	T Q Y F Q S M T E Y G K
T-8	1584	1-14	$_p$E A D G P D M Q S L F T Q Y
T-9	1043	55-64	S A G T S L V N F F
T-10	1830	9-23	S L F T Q Y F Q S M T E Y G K
T-11	2655	1-23	$_p$E A D G P D M Q S L F T Q Y F Q S M T E Y G K
T-12	2481	55-78	S A G T S L V N F F S S L M N L E E K P A P A A

[a] $_p$E corresponds to the amino-terminal pyroglutamyl residue.

Fig. 1), was subjected to tryptic digestion. **Fig. 2** illustrates the reverse-phase HPLC tryptic map and **Table 3** and **Table 4** summarize the proteolysis data. Although the amino acid composition of the intact protein suggested seven to eight possible tryptic cleavage sites, twelve major fragments were obtained on reverse-phase HPLC. Except for peak T-3 (Fig. 2), which consisted of two peptides (T-3a, T-3b), each absorbance peak examined accounted for a single amino acid sequence.

Microsequence analysis of the tryptic peptides permitted, in most instances, unambiguous PTH-amino acid assignments through the carboxy-terminal residues. For tryptic peptides T-1, T-2, T-3(a,b), T-5, and T-7 (Fig. 2), the complete amino acid sequences were determined using the gas-phase microsequencer and were confirmed by FAB-mass spectral data. Peptides T-8 and T-11 were amino-terminal peptides deduced from their FAB-mass spectral analyses and their inability to microsequence by Edman chemistry. These sequences were present in the amino-terminal sequence of the deblocked apolipoprotein.

TABLE 5. Amino acid FAB/MS analysis of BALB/c tryptic fragment T-12

		FAB/MS[b]					
		N-Terminal Series			C-Terminal Series		
Sequence	Amino Acid[a] Residue/Mole	No. A	B	C"	No. Z	Z"	Y"
Ser	4.2	1					
Ala	4.0	2			23		2394.2
Gly	1.6	3			22		2323.2
Thr	0.9	4			21 2251.1		2266.1
Ser		5		421.2	20 2150.1		
Leu	2.8	6 4489.3	517.3		19	2078.1	
Val	0.7	7		633.3	18		
Asn	(Asx) 1.7	8			17 1850.9		
Phe	1.9	9	877.4	894.4	16	1736.9	
Phe		10 996.5	1024.5	1041.5	15	1589.8	1604.8
Ser		11 1083.5		1128.5	14 1440.7	1442.7	1457.7
Ser		12 1170.6	1198.5		13	1355.7	1370.7
Leu		13 1283.7			12 1266.7		1283.7
Met	1.0	14	1442.7		11 1153.6		1170.6
Asn		15		1573.8	10 1022.5		1039.5
Leu		16 1641.8		1686.8	9 908.5	910.5	925.5
Glu	2.1	17			8		
Glu		18	1927.9	1944.9	7		683.4
Lys	1.2	19 2028.0			6	593.3	
Pro	2.0	20 2125.1		2170.1	5	411.2	
Ala		21 2196.1			4		
Pro		22 2293.2		2338.2	3		
Ala		23		2409.2	2		
Ala					1		

[a] The sample (0.1 μg) was hydrolyzed and analyzed as described in Experimental Procedures.
[b] FAB/MS methods are described in Experimental Procedures.

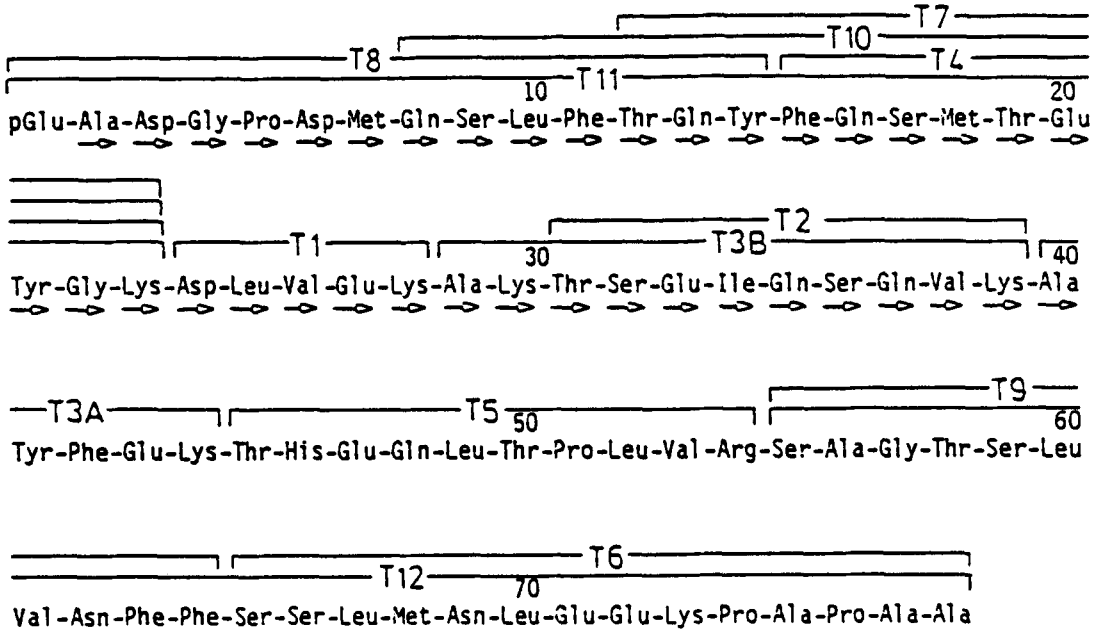

Fig. 3. Amino acid sequence of mouse BALB/c apolipoprotein A-II. The amino-terminal sequence analysis is indicated by →. Results of the tryptic digestion are designated by their respective tryptic peptides, T.

Peptide T-12 corresponded to the carboxy-terminus of the protein and, due to its hydrophobic C-terminus, required both microsequence analysis and FAB-mass spectrometry for conclusive amino acid assignments. Amino acid analysis of this peptide demonstrated, among other residues, the presence of four residues of alanine, two residues of proline, and one residue of lysine. Microsequence analysis of T-12 showed a marked decrease in the PTH-amino acid yields by cycle 18, through which no PTH-Pro and only one PTH-Ala had been identified. The PTH-Lys detected at cycle-19 and the PTH-Pro detected at cycle-20 comprised a peptide bond that was resistant to tryptic digestion and thus located the remaining two prolines and three remaining alanine residues at the carboxy-terminus. The molecular weight of T-12 as determined by the protonated molecular ion at m/z 2481 in the FAB-mass spectrum was consistent with the amino acid composition. Sequence information was contained in six different FAB-mass spectral ion series (**Table 5**) (13). Information from the A and C" series was sufficient to establish the sequence of the carboxy-terminus and corroborate the microsequencing results.

The alignment of peptide sequences utilized overlapping tryptic fragmentations and amino acid sequence homology to rhesus monkey apoA-II, determined by Edelstein et al. (14), and to human apoA-II, as determined by Brewer et al. (15). The complete amino acid sequence of BALB/c apoA-II is shown in **Fig. 3.** With alignments constructed to maximize sequence homology, a 58% homology was achieved with the rhesus A-II and 55% homology was

TABLE 6. Species sequence homology of apolipoprotein A-II

	10										20										30										40		
Mouse BALB/c[a]	pE	A	D	G	P	D	M	Q	S	L	F	T	Q	Y	F	Q	S	M	T	E	Y	G	K	D	L	V	E	K	A	K	T	S	E	I	Q	S	Q	V	K	A
Rhesus monkey[b]	pE	A	E	E	P	S	V	E	S	L	V	S	Q	Y	F	Q	T	V	T	D	Y	G	K	D	L	M	E	K	V	K	S	P	E	L	Q	A	Q	A	K	A
Human[c]	pE	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	E	K	V	K	S	P	E	L	Q	A	Q	A	K	S

	50										60										70												
Mouse BALB/c	Y	F	E	K	T	H	E	Q	L	T	P	L	V	R	S	A	G	T	S	L	V	N	F	F	S	S	L	M	N	L	E	E	K	P	A	P	A	A
Rhesus monkey	Y	F	E	K	S	K	E	Q	L	T	P	L	V	K	K	A	G	T	D	L	V	N	F	L	S	Y	F	V	E	L	R	T	Q	P	A	T	Q		
Human	Y	F	E	K	S	K	E	Q	L	T	P	L	I	K	K	A	G	T	E	L	V	N	F	L	S	Y	F	V	E	L	G	T	Q	P	A	T	Q		

[a] The amino-terminal residue is pyroglutamic acid.
[b] Protein sequence from Ref. 14.
[c] Protein sequence from Ref. 15.

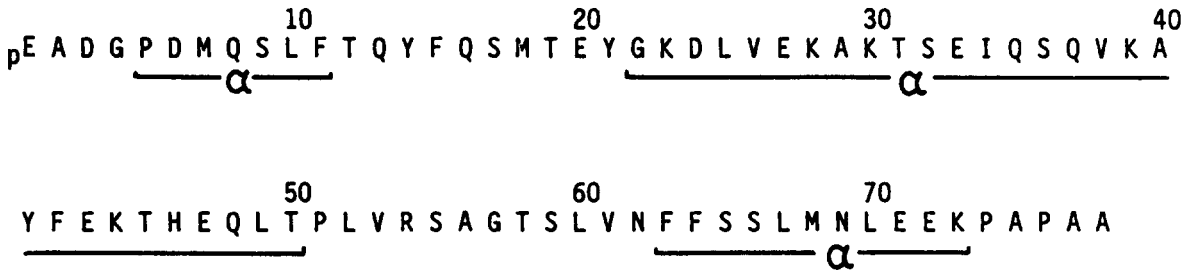

Fig. 4. Alpha-helical content of BALB/c apolipoprotein A-II. Chou and Fasman analysis predicts approximately 68% alpha-helicity from the amino acid sequence and is shown in solid underline.

found with the human A-II protein. Amino acid sequence homology for BALB/c apoA-II with human and monkey A-II proteins is presented in **Table 6.**

Mouse BALB/c apoA-II has a calculated molecular weight of 8,715 and is composed of 78 amino acid residues. The protein exists as a monomer due to the lack of cysteine in its primary structure. The BALB/c A-II protein contains one residue of histidine and arginine which are absent in the human apoA-II. The net acidic charge of the BALB/c A-II protein results from the ten acidic amino acid residues (seven glutamic acids and three aspartic acids) and eight basic residues (seven lysines and one arginine).

DISCUSSION

Chou and Fasman analysis (16) of the primary structure of BALB/c apoA-II predicts four domains of alpha-helical conformation identified by amino acid residues 5 to 11, 15 to 20, 22 to 50, and 63 to 73, resulting in an overall molecular alpha-helical potential of approximately 68% (**Fig. 4**). β-Sheet structure was determined to intervene between residues 12 to 14 and 51 to 62. The BALB/c A-II protein yields a 5% greater alpha-helicity than the human A-II protein based on our calculations.

Segments of the alpha-helical domains are amphipathic in nature allowing for a bifacial polarity along the helical axis. Amino acid residues 18 to 30 and 39 to 47 in human apoA-II exhibit such amphipathy and were identified as phospholipid binding sites (17, 18). The corresponding sequence domains of murine BALB/c apoA-II are highly structurally homologous to human (70% for residues 18 to 30 and 39 to 47) and conserve the charged residues. It is likely that BALB/c apoA-II exhibits a conformational structure of the type predicted to favor apolipoprotein-phospholipid interactions.

The utility of the mouse in genetics is well recognized and its use in studying the genetic control of lipid transport is accelerating. In particular, inbred strains of mice differ in their susceptibility to diet-induced atherosclerosis and this may be related to HDL levels in some strains (19, 20). Mice of the BALB/c strain are resistant and do not develop aortic lesions when fed a high fat diet, whereas mice of strain C57BL/6 develop aortic lesions within 14 weeks (20). Thus, the study of mouse HDL structure and metabolism may prove useful in identifying factors affecting the resistance or induction of atherosclerosis in these strains. A structural polymorphism for apoA-II is among the many variations in the lipid transport system thus described between resistant and susceptible inbred mouse strains. The structural variation is characterized by charge differences on isoelectric focusing gels (5). In this report, we determined the primary structure of apoA-II from the inbred strain BALB/cBy. Comparison of the covalent structure of BALB/c apoA-II with apoA-II of the C57BL/6 strain should reveal a molecular basis for the charge polymorphism and an insight into the biological activities and properties of their respective HDL particles.

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