Isolation and characterization of the major plasma apolipoproteins, A-I and B, in the European badger, *Meles meles*

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Abstract The two major apolipoproteins of badger serum, apoA-I and apoB, have been isolated and characterized. Apolipoprotein A-I was the principal protein of badger lipoproteins with density 1.063–1.21 g/ml and, in addition, was present in the lipoprotein class with density 1.006–1.063 g/ml. This apolipoprotein displayed an M_r of ≅ 27,000–28,000 and was polymorphic (three prominent isoproteins) on isoelectric focusing, with pI values in the range 5.38–5.55. The amino acid profile of badger apoA-I generally resembled those reported in the literature for similar proteins in dog and man. Amino terminal sequence analysis up to the 40th residue showed close homology between the badger, dog, and human proteins; badger and dog apoA-I differed only at residue 24, at which serine in the dog was substituted by glycine in the badger. Several forms of apolipoprotein B were present in badger lipoproteins with densities <1.063 g/ml, their distribution and apparent M_r being unaffected by the presence or absence of 1 mM PMSF during the isolation process. The components of higher M_r were essentially represented by a protein with M_r ≅ 530,000–550,000 ($apoB_H$) as determined by SDS-polyacrylamide electrophoresis; this protein predominated both in lipoproteins with d 1.006–1.063 g/ml and in those with d < 1.006 g/ml. In addition, proteins with approximate M_r values of 490,000, 450,000, and 190,000, respectively, were present as minor components. A lower M_r form (250,000, $apoB_L$), was observed only in lipoproteins with d < 1.006 g/ml. Higher M_r badger B proteins were isolated from d 1.006–1.063 g/ml lipoproteins by gel filtration chromatography on Sephadex G-200 in anionic detergent. The amino acid content of these proteins was indistinguishable from that of human apoB-100. These findings extend our knowledge of the protein moieties of the circulating lipoproteins in the badger, and will allow further development of its use as an animal model for studies of the hormonal regulation of lipid transport. — **Beaubatie, L., P. M. Laplaud, S. C. Rall, and D. Maurel.** Isolation and characterization of the major plasma apolipoproteins, A-I and B, in the European badger, *Meles meles*. *J. Lipid Res.* 1986. **27:** 140–149.

Supplementary key words gel filtration chromatography • preparative electrophoresis • isoelectric focusing • amino acid sequence

During the past few years, studies in our laboratory have progressively provided evidence for the interest in the European badger as a model species for investigation of plasma lipid transport and, in particular, of its endocrine regulation. Indeed, marked quantitative seasonal modifications occur in the plasma concentrations of the different lipid classes in the badger, particularly with regard to cholesterol and phospholipids (1, 2). Such changes lead to the appearance of hypercholesterolemia, hyperphospholipidemia, and to the prominence of particles of density 1.006–1.063 g/ml in the plasma lipoprotein spectrum during late fall and the beginning of winter. This is the period of the year when thyroid activity, determined as plasma thyroxine, reaches its annual minimum (3). By contrast, maximal thyroxine levels occur during spring and concomitantly with both minimal concentrations of plasma lipids and a large decrease in the level of lipoproteins with density in the range 1.006–1.063 g/ml.

Electrophoretic analyses of the protein moieties of badger plasma lipoproteins have shown that the two major apolipoproteins in this species have molecular weights on SDS-polyacrylamide gels that resemble those of human apolipoproteins A-I and B, respectively. However, contrary to findings in normal humans, but similar to those observed in several other animal species, the badger apolipoprotein that corresponds in size to human

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; pI, isoelectric point; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; VLDL, very low density lipoproteins; LDL, low density lipoproteins.

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apolipoprotein A-I was present in large amounts not only in the 1.063–1.21 g/ml region, but also throughout the 1.006–1.063 g/ml density interval (2). On the other hand, reports concerning the presence of up to four different forms of apolipoprotein B in man (4) raise the question as to whether a similar situation may exist in the badger. We therefore undertook the isolation and characterization of counterparts to human apolipoproteins A-I and B in this species, extending our knowledge of lipid transport in *Meles meles*.

MATERIALS AND METHODS

Animals and diets

The adult male badgers, bred in the Centre d'Etudes Biologiques des Animaux Sauvages, were approximately 2–6 years old. They were kept individually in 6-m^2 parks under natural conditions of light, temperature, and rainfall. None of the animals exhibited pathological manifestations during the course of the present study. The badgers used received a diet consisting of a commercial food for dogs (Canina, Duquesne-Purina) that contained the following proportions by weight of the major constituents: protein, 21%; fat, 7%; fiber, 2.5%; ash, 7%; water 10%; nitrogen-free extract, 51.5%; vitamin A, 15,000 I.U./kg; vitamin D_3, 1,500 I.U./kg. Water was provided ad libitum.

Collection and treatment of blood

For each of the successive series of manipulations, blood samples were taken from six animals that had been fasted overnight for approximately 18 hr. Blood was collected on EDTA (final concentration, 1 mM), at approximately 11:00 AM, by puncture of the radial vein. Plasma was then separated by low-speed centrifugation and brought to the laboratory on ice. For evaluation in polyacrylamide-SDS gels of any possible involvement of proteolytic degradation in defining the B protein pattern of badger lipoproteins, the proteolytic inhibitor PMSF was added to half of the volume of two separate plasma pools (final concentration 1 mM) and lipoproteins were isolated (see below) in parallel from each.

Lipoprotein isolation and delipidation

Lipoproteins with hydrated densities < 1.006 g/ml, 1.006–1.063 g/ml, and 1.063–1.21 g/ml, respectively, were isolated by sequential ultracentrifugal flotation according to the procedure of Havel, Eder, and Bragdon (5) in an MSE Prepspin 50 ultracentrifuge (MSE, Crawley, U.K.) using an aluminum fixed-angle rotor (capacity 8 × 14 ml) at 40,000 rpm (100,000 g_{av}) at 17°C. Each lipoprotein fraction was subsequently dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA, exclusion limit 3,500) for 3 × 12 hr at 4°C against a solution containing 0.15 M NaCl, EDTA (0.4 g/l), sodium azide (0.1 g/l), and merthiolate (1 mg/l). Lipoprotein pools prepared with a view to analysis of badger apoB were separated into two halves, and ultracentrifuged and dialyzed as reported above. PMSF (final concentration 1 mM) was added to all the salt solutions used for preparation of one-half of each pool. Finally, lipoproteins were delipidated with ethanol–diethylether 3:1 (v/v) as described by Brown, Levy, and Fredrickson (6); the apoprotein residue was dried under N_2.

Apolipoprotein isolation

Apolipoprotein A-I was isolated from both 1.006–1.063 g/ml and 1.063–1.21 g/ml lipoproteins using preparative electrophoresis according to the methodology of Stephens (7). For this purpose, apolipoproteins were dissolved to a concentration of 3 mg/ml in a phosphate buffer 0.05 M, pH 8.2, made 4% by wt in SDS; a 100-μl aliquot was dansylated using dansyl chloride according to the technique of Talbot and Yphantis (8). The dansylated aliquot was mixed with the bulk of the sample, and the mixture was heated at 90°C for 3 min. The apolipoprotein solution was then dialyzed against 2.5 mM Tris–glycine, 0.1% (w/v) SDS, pH 8.3, at room temperature. After addition of 50 μl of a solution of sucrose and 50 μl of bromphenol blue as a tracking dye, aliquots containing 1 mg of protein were placed on polyacrylamide gels (7.5% monomer concentration and 0.1% in SDS, 1.5 cm diameter and 10 cm long). Electrophoresis was then carried out at 300 V (about 7 mA/gel) and 15°C; the progress was monitored both by the migration of bromphenol blue dye and by UV illumination in the dark. Gels were subsequently removed from the glass tubes, and bands corresponding to apolipoprotein A-I were sliced; slices were stacked in a similar glass tube which was then tied to a dialysis bag of Spectrapor tubing (exclusion limit 3,500). Electrodialysis was carried out in the same electrophoresis chamber as above, for approximately 3–4 hr at 150 V, using 2.5 mM Tris–glycine, 0.1% SDS buffer. On completion, the fluorescent content of the dialysis bag was recovered and exhaustively dialyzed against a solution of 5 mM NH_4HCO_3.

Apolipoprotein B was isolated by the technique of Herbert et al. (9) as adapted by Chapman and Goldstein (10). For this purpose, dry apolipoproteins from d 1.006–1.063 g/ml lipoproteins were dissolved in 0.01 M sodium phosphate buffer containing 1% (w/v) SDS and 1% β-mercaptoethanol, at pH 8.0 (solution S). Up to 4 mg of apolipoproteins, applied in a maximal volume of 1 ml, were chromatographed at 20°C at flow rates of 10–12 ml/hr on a Sephadex G-200 column (60 × 0.9 cm) equilibrated with a buffer containing 0.01 M Tris (pH 8.0), 2 mM sodium decyl sulfate, 3 mM sodium azide, and 0.001% (w/v) merthiolate. The effluent was monitored at 280 nm.

Column fractions were lyophilized and dissolved in double-distilled water to a final concentration of sodium decyl sulfate between 5 and 15 mM; complete and ready solubilization of all fractions was obtained with this procedure. However, in the case of aliquots taken for SDS-polyacrylamide gel electrophoresis, the SDS concentration was adjusted to 1% (w/v) by addition of a suitable volume of threefold concentrated solution S.

Isoelectric focusing and electrophoresis

Analytical isoelectric focusing in the pH range 4–6.5 was performed according to Pagnan et al. (11). Apolipoprotein samples were dissolved in a solution containing 6 M urea, 20 mM ethylmorpholine, and 20% sucrose at pH 8.6. The electrolyte solutions were 0.1 M glycine (upper buffer, pH 6.7) and 0.01 M HEPES, pH 5, and focusing was carried out at constant voltage (400 V) for 5.5 hr at 10°C.

The apolipoproteins of d < 1.006 g/ml, 1.006–1.063 g/ml, and 1.063–1.21 g/ml lipoproteins were electrophoresed according to the modification of Kane (12) of the alkaline polyacrylamide disc gel system of Davis (13). The molecular weights of apolipoproteins were estimated by electrophoresis in SDS-polyacrylamide gels of either 10% monomer concentration as described by Weber and Osborn (14), or 3% monomer concentration according to the modification of Weisgraber et al. (15) of the methodology of Stephens (7). Calibration curves for estimation of molecular weights were constructed from a series of molecular weight markers ranging in size from 14,000 to 94,000 (Low Molecular Weight electrophoresis calibration kit, Pharmacia Fine Chemicals), and from 56,000 to 280,000 (BDH Biochemicals, Poole, U.K.). On completion of electrophoresis, gels were stained with Coomassie brilliant blue R 250. The staining technique of Karlson et al. (16) was used in the case of isoelectric focusing and SDS-polyacrylamide gels with 3% monomer concentration.

Amino acid and sequence analysis

Amino acid analyses were carried out on a Beckman 121 MB analyzer (Beckman Instruments, Fullerton, CA) after hydrolysis of samples in 6 N HCl for 20 hr at 110°C in sealed, evacuated tubes.

Partial sequence analysis was carried out on 35 or 40 nmol of badger apolipoprotein A-I after the addition of polybrene (2 mg in 0.5 ml of H_2O); the protein, dissolved in 0.5 ml of 50% acetic acid, was applied wet to the cup of a Beckman 890 C sequencer. The first cycle was double-coupled; otherwise, a standard 0.1-mol Quadrol program (no. 122974) was used. After conversion, phenylthiohydantoin amino acids were identified and quantified on a Beckman 322 high performance liquid chromatograph, equipped with a CR 1 A integrator-recorder. Chromatography was carried out using the procedure of Somack (17).

Protein determination

Lipoprotein protein and purified apolipoproteins were quantitated by the procedure of Lowry et al. (18) using bovine serum albumin (Sigma) as the working standard.

RESULTS

Apolipoprotein A-I

As noted previously in this report, apolipoprotein A-I was isolated separately from d 1.006–1.063 g/ml and from d 1.063–1.21 g/ml lipoproteins. Electrophoresis patterns of pure apolipoproteins are presented in **Fig. 1** and compared with patterns of total apolipoproteins of each of the three density classes of lipoproteins (d < 1.006 g/ml, 1.006–1.063 g/ml, and 1.063–1.21 g/ml). Such a comparison provided unequivocal evidence for the presence of elevated amounts of apoA-I throughout the lipoprotein density spectrum in the badger. Indeed, in SDS-polyacrylamide gels of 10% monomer concentration, the purified apoA-I consistently migrated as a single band with M_r approximately 27,000–28,000, irrespective of the density class of lipoproteins from which it originated (Fig. 1, gel D). A protein with similar M_r was present in all three lipoprotein classes studied, although its relative amount decreased with density (Fig. 1, gels A, B, and C). This observation was consistent with those obtained using alkaline-urea gels (Fig. 1, gels E, F, and G) with regard to band IV (mobility 0.24, similar to that of human apoA-I in the same system).

Similarly, isoelectric focusing data on apoA-I were independent of the lipoprotein origin of this polypeptide. Indeed, apoA-I from both 1.006–1.063 g/ml and 1.063–1.21 g/ml lipoproteins was consistently found to be polymorphic, exhibiting four to five bands with isoelectric points 5.65 ± 0.06, 5.52 ± 0.07, 5.44 ± 0.10, 5.38 ± 0.11, and 5.27 ± 0.09 (results obtained when analyzing apoA-I originating from 1.063–1.21 g/ml lipoproteins, n = 6), or 5.59 ± 0.08, 5.55 ± 0.07, 5.47 ± 0.06, 5.41 ± 0.06, and 5.34 ± 0.07 (apoA-I originating from 1.006–1.063 g/ml lipoproteins, n = 10) (Fig. 1, gel K). Clearly then, results regarding apoA-I from the two different density classes of lipoproteins were comparable. Among the bands observed, those with mean pI 5.52–5.55, 5.44–5.47, and 5.38–5.41 were consistently prominent. These latter values for the main isoforms of apoA-I are slightly more acidic than those noted for the most intensely stained bands in the upper portion of the patterns obtained when examining the whole apolipoprotein content of 1.006–1.063 g/ml lipoproteins (i.e., 5.74, 5.65, 5.54, and 5.48) and 1.063–1.21 g/ml lipoproteins (i.e., 5.75, 5.66, and 5.58) by the same technique (Fig. 1, gels I and J). Taking into account the prominence, observed in 10% SDS-polyacrylamide gels, of apoA-I in such apolipoprotein material,

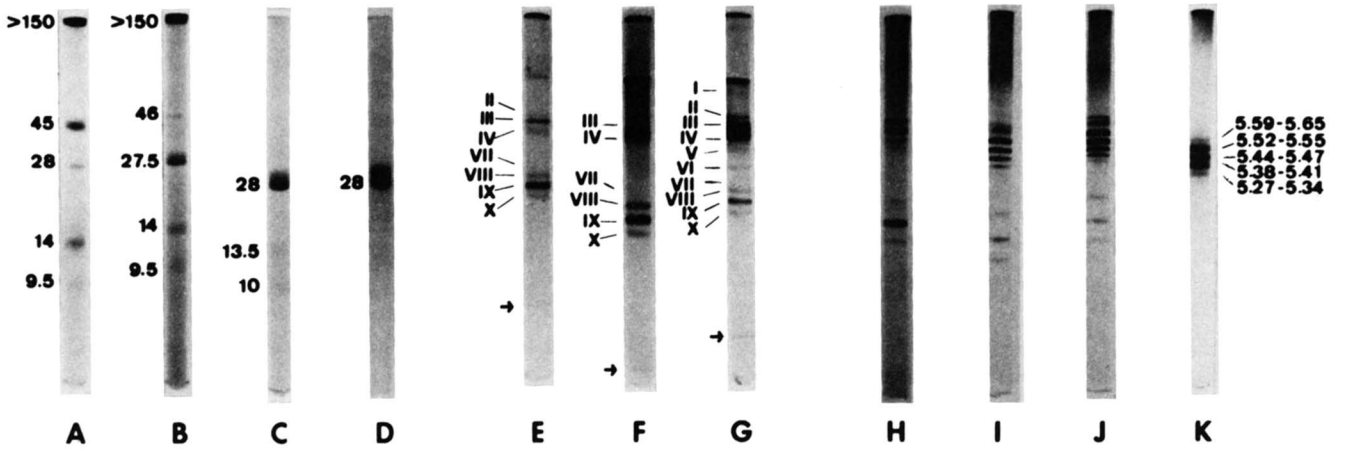

Fig. 1. Electrophoretic studies on the density distribution of badger apoproteins and characterization of purified badger apoA-I. Gels A to D: electrophoresis in 10% SDS-polyacrylamide gels. Molecular weights are expressed in kD. Gels E to G: electrophoresis in alkaline-urea gels. Arrows indicate the position of the dye front. Gels H to K: isoelectric focusing in the pH range 4–6.5. In each of the three series of gels, the first gel (i.e., gels A, E, and H), the second gel (i.e., gels B, F, and I), and the third gel (i.e., gels C, G, and J) show typical results obtained when examining the apolipoprotein content of lipoproteins with d < 1.006 g/ml, 1.006–1.063 g/ml, and 1.063–1.21 g/ml, respectively. Gels D and K are representative of results obtained when examining badger apoA-I, purified either from d 1.006–1.063 g/ml or d 1.063–1.21 g/ml lipoproteins. Approximately 100 μg of protein was applied in each case. Gels were stained with Coomassie brilliant blue R-250.

these latter bands could reasonably be attributed to apoA-I isoforms. Therefore, a ready explanation of the observed discrepancy between the respective pI's of apoA-I isoforms, whether contained in whole 1.006–1.063 g/ml or 1.063–1.21 g/ml lipoprotein apolipoproteins, or in purified apoA-I, is not at hand. Indeed, our preparative procedure for apoA-I consists of SDS-polyacrylamide electrophoresis and thus avoids the use of urea-gel electrophoresis, which could lead to carbamylation of lysine residues in apoA-I isoforms and consequently to the appearance of more acidic bands (10). However, Nestruck, Suzue, and Marcel (19), when studying human apolipoprotein A-I, showed that "exposure of the isolated polymorphs to alkaline pH (8.6–8.9) resulted in the refocussing of more than one band," the newly appearing bands being consistently more acidic than the parent one. Thus, our preparative electrophoresis procedure, which subjects badger apoA-I to pH in the 8.2–8.3 range, could have led to deamidation. In addition, the different steps of concentration, dialysis, and lyophilization to which the apolipoprotein is subjected in the process of its purification could also have allowed oxidation or deamidation to occur, as has been suggested in the case of the isolation of human apoA-I (20, 21).

Table 1 shows the amino acid composition of badger apolipoprotein A-I. Data from the protein isolated from d 1.006–1.063 g/ml and from d 1.063–1.21 g/ml lipoproteins are very similar and thus clearly relate to the same protein. Badger apoA-I contained elevated amounts (>10 mol of amino acid/mol of protein) of lysine, arginine, aspartic and glutamic acids, serine, glycine, alanine, valine, and leucine. Comparison of our data in the badger with previously published data for the dog (22) and man (23) provides evidence for the overall similarity of apolipoprotein A-I in the three species, although several differences were detected. These included *1*) the presence of one to two isoleucine residues in badger apoA-I, a fea-

TABLE 1. Amino acid composition of apolipoprotein A-I from badger, dog, and man[a]

Amino Acid	Badger Apolipoprotein A-I Origin: d 1.006–1.063 g/ml Lipoproteins (3)	Badger Apolipoprotein A-I Origin: d 1.063–1.21 g/ml Lipoproteins (4)	Canine ApoA-I	Human ApoA-I
Lys	18.9 ± 0.5	18.1 ± 1.4	19	21
His	3.5 ± 0.2	3.9 ± 0.2	2	5
Arg	15.9 ± 0.1	16.3 ± 0.1	17	16
Asp	22.2 ± 0.3	21.9 ± 0.1	22	21
Thr	9.8 ± 0.2	9.9 ± 0.1	7	10
Ser	12.1 ± 0.1	11.9 ± 0.5	15	15
Glu	45.5 ± 1.7	45.4 ± 1.5	46	46
Pro	9.5 ± 0.1	9.7 ± 0.2	9	10
Gly	11.9 ± 1.3	12.9 ± 0.4	11	10
Ala	24.9 ± 0.2	24.0 ± 0.3	25	19
Val	14.6 ± 0.8	15.4 ± 0.3	15	13
Met	0.9 ± 0.3	1.2 ± 0.2	1	3
Ile	1.3 ± 0.2	1.8 ± 0.4	2	0
Leu	36.1 ± 0.6	34.7 ± 0.7	37	37
Tyr	5.7 ± 0.1	6.0 ± 0.3	6	7
Phe	5.5 ± 0.2	5.3 ± 0.2	4	6
Cys	n.d.[b]	n.d.	0	0
Tryptophan	n.d.	n.d.	4	4

[a] Values for badger apolipoprotein A-I are expressed as mol of amino acid/mol of A-I protein and are means ± SD of the number of preparations in parentheses; each sample was analyzed in duplicate or triplicate. Calculation was made on the assumption that badger apolipoprotein A-I contains 238 amino acid residues as in dog (22), but excluding tryptophan. For human apolipoprotein A-I, the assumed number of residues is 239 excluding tryptophan (23).

[b] n.d., Not determined.

ture shared with the dog but not with man (23); *2*) similar proportions of histidine and threonine in badger and human apoA-I, while the content of these amino acids was lower in dog apoA-I; and *3*) by contrast, a closer relationship between badger and dog, rather than between badger and man, with regard to the respective proportions of alanine and methionine in apolipoprotein A-I.

The results of our partial sequence determination of badger apoA-I, isolated from 1.063–1.21 g/ml lipoproteins, are summarized in **Fig. 2** for five amino acids, i.e., aspartic acid, valine, proline, alanine, and glycine. The amino acid sequence to the fortieth residue is presented in **Table 2** and compared to that for the homologous protein from dog and man. A single difference was noted between the sequence in badger and in dog; this was represented by the substitution of serine in the dog by glycine in the badger at position 24.

Apolipoprotein B

In the present experiments, we isolated lipoproteins with hydrated densities < 1.006 g/ml and 1.006–1.063 g/ml from the plasma of fasting animals. This ultracentrifugal isolation was conducted either in the presence or absence of the proteolytic inhibitor PMSF. After delipidation, the resulting apolipoprotein material was subjected to electrophoretic analysis in 3% monomer SDS gels (**Fig. 3**). It is of note that no difference could be observed between the patterns obtained, according to the presence or absence of PMSF in the course of the isolation of lipoproteins.

Electrophoretic data indicate that the high M_r apolipoprotein material primarily consisted of a protein with M_r approximately 530,000–550,000 in both < 1.006 g/ml and in 1.006–1.063 g/ml lipoproteins (Fig. 3, gels B and C). By analogy with the nomenclature proposed for the rat B proteins (24), we denote this apolipoprotein "apoB_H." When examined in the same gel system under similar conditions, the apoB-100 protein of human apoLDL exhibited a slightly lower M_r (500,000–510,000) (Fig. 3, gel A). In addition, other high M_r components of minor quantitative importance were noted. The distribution of these components differed according to the lipoprotein density class considered, but was reproducible in all of the six series of procedures performed. Indeed, a protein with M_r 280,000 ("apoB_L") was consistently observed as a faint band in lipoproteins with d < 1.006 g/ml (Fig. 3, gel B). Application of the centile nomenclature of Kane, Hardman, and Paulus (4) shows that this protein bears a 52:100 relationship with the prominent high M_r form and thus suggests that it could be analogous to the human B-48 protein. In contrast, this M_r 280,000 component was no longer observable in d 1.006–1.063 g/ml lipoproteins, while this latter material appeared to contain two other components of minor quantitative importance and with M_r values of approximately 450,000 and 190,000, respectively (Fig. 3, gel C). Again,

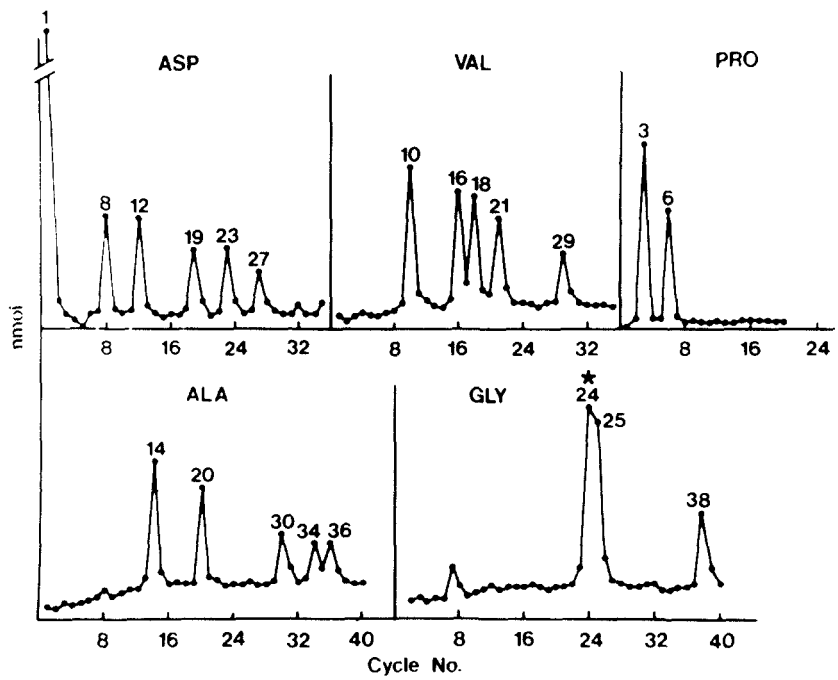

Fig. 2. NH_2-terminal sequence analysis of apolipoprotein A-I isolated from badger plasma d 1.063–1.21 g/ml lipoproteins. Data are plotted as nmol of amino acid (in the PTH form) recovered at each degradative cycle, and are shown for five representative amino acids including the residue (glycine at position 24) at which a difference with the dog sequence was found (see Table 2); this is denoted by an asterisk. Degradation was performed on 35 nmol of apoA-I and the initial yield of Asp was 11 nmol.

TABLE 2. NH_2-terminal sequence of apolipoprotein A-I from badger, dog, and man

Species	Residue Number									
	. . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 40									
Badger	D E P		Q S P W D R V K D L A T V Y Y V D	A V	K D	G	G R D Y V	A	Q F	E A	S A L G K Q
Dog	D E P		Q S P W D R V K D L A T V Y Y V D	A V	K D	S	G R D Y V	A	Q F	E A	S A L G K Q
Man	D E P	P	Q S P W D R V K D L A T V Y Y V D	V L	K D	S	G R D Y V	S	Q F	Q G	S A L G K Q

The dog and human sequences are taken from references 22 and 23, respectively. Homologous regions in badger, canine, and human apoA-I are indicated by rectangles, taking into account the deletion of proline at residue 4 in the animal apoA-Is.

application of the nomenclature of Kane et al. (4) showed that these latter proteins bear a 83:100 and 35:100 relationship with $apoB_H$. In addition, a very faint band representative of a component with M_r 490,000 was also consistently noted.

Two points should be emphasized at this stage. Firstly, due to non-availability of commercial standard proteins of higher M_r, we have employed as markers a set of proteins whose upper M_r limit was 280,000 (see Materials and Methods section). This is similar to the experimental conditions used by Kane et al. (4) in the original report of the existence of human apoB-48. However, such a lack of standard proteins with M_r in the 500,000 region means that the M_r proposed here for badger $apoB_H$ and the supplementary large protein components of minor quantitative importance are subject to some inaccuracy. Secondly, despite the presence of PMSF throughout the process of isolation of certain lipoprotein samples, it is most likely that at least some of the minor components observed are the products of partial degradation of $apoB_H$ by proteases.

The upper portion of these 3% gels consistently showed the presence of several discrete bands of very high M_r (> 1,000,000). These bands could be representative of aggregates due to the relatively heavy loading of our gels (70–100 μg), as compared to that of other authors such as Kane et al. (4) (20 μg).

Finally, in the lower portion of the gels, two supplementary bands were observable. The one with the higher M_r (50,000–55,000) was present as a component of both d < 1.006 g/ml and 1.006–1.063 g/ml lipoproteins. On the other hand, the lower M_r peptide (migrating to the end of the gel, M_r < 40,000) was a quantitatively significant component of the latter lipoproteins only.

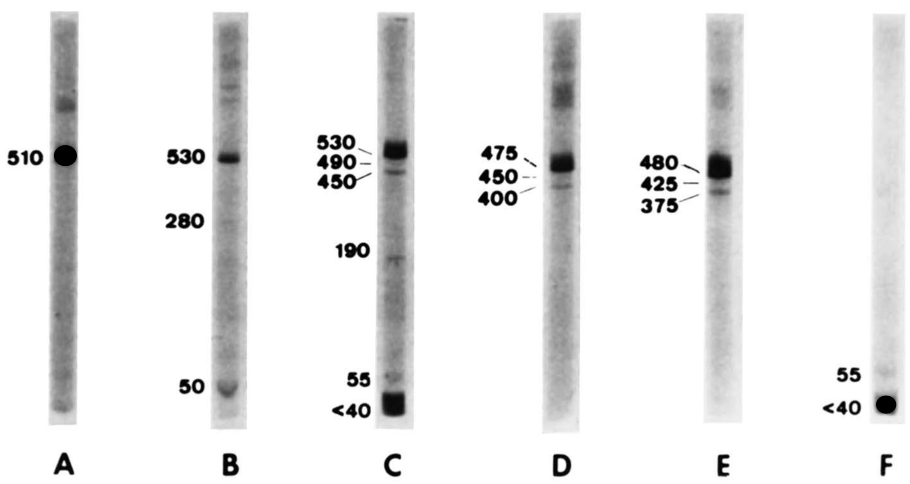

Fig. 3. Electrophoretic patterns in SDS-polyacrylamide gels of the apolipoprotein content of badger lipoproteins with respective hydrated densities < 1.006 g/ml and 1.006–1.063 g/ml, and the corresponding column fractions I and II obtained by gel filtration chromatography in anionic detergent on a Sephadex G-200 column. Patterns: A, human apoLDL (d 1.019–1.063 g/ml) isolated in the absence of PMSF; B, apolipoproteins from badger lipoproteins with d < 1.006 g/ml, isolated either in the absence or presence of PMSF; C, apolipoproteins from badger lipoproteins with d 1.006–1.063 g/ml, isolated either in the absence or presence of PMSF; D and E, fraction I, containing badger apoB-like proteins chromatographically isolated, using buffers lacking PMSF, from the apolipoproteins of 1.006–1.063 g/ml lipoproteins. (The starting material, plasma lipoproteins with d 1.006–1.063 g/ml, was separated ultracentrifugally in the absence (D) or presence (E) of PMSF.) F, fraction II obtained by the same technique; the gel shown is representative of results obtained both in the presence or in the absence of PMSF during the process of lipoprotein isolation. The acrylamide monomer concentration in all gels was 3%; 70–100 μg of protein was applied. Gels were stained with Coomassie brilliant blue R-250.

As previously reported (25), the pattern obtained upon gel filtration chromatography of the apolipoprotein content of badger d 1.006–1.063 g/ml lipoproteins consisted of two well-resolved peaks, thereafter termed peaks I and II. Electrophoretic analysis of their respective content was conducted using the same system (Fig. 3, gels D, E, and F). Peak I was constituted mainly of an M_r 475,000 protein ($apoB_H$). In addition, two other minor bands were observed with apparent M_r 425,000–450,000 and 375,000–400,000, respectively. Based on the ratio of their M_r values to that of $apoB_H$ isolated by this chromatographic technique, i.e., 0.91 and 0.82, respectively, these latter components most probably represent the same proteins as those exhibiting apparent M_r of 490,000 and 450,000, respectively, and observed in the d 1.006–1.063 g/ml lipoproteins. Again, the limited M_r of the marker proteins employed could lead to some inaccuracy in the determination of the proposed M_r. This is possibly responsible for the apparent discrepancy observed between the values of the M_r or $apoB_H$ obtained when analyzing either the apoproteins of lipoproteins with d < 1.006 g/ml or 1.006–1.063 g/ml, or the material constituting peak I from chromatography of the apoprotein moiety of 1.006–1.063 g/ml lipoproteins.

For its part, peak II contained the components migrating to the region of the lower end of the gels, showing approximate M_r values of 55,000 and < 40,000, respectively (Fig. 3, gel F). It is of note that the protein with M_r of approximately 190,000 was only observed as a trace component in one gel representative of the content of peak I from gel filtration chromatography. This is probably a consequence of the fact that only the fractions corresponding to the central portions of the peaks were pooled for subsequent electrophoretic analysis, while an M_r of 190,000 could lead to elution of the corresponding protein in the trailing portion of peak I and/or in the region between peaks I and II. The M_r 190,000 band was consistently seen upon SDS gel electrophoresis of the protein moiety of 1.006–1.063 g/ml lipoproteins.

We have further characterized the badger apoB-like proteins by amino acid analysis. However, the low concentration of lipoproteins with d < 1.006 g/ml in badger plasma prevented us from obtaining data on apoB in this class of lipoproteins. Results obtained by analysis of the content of column peak I from gel filtration chromatography of the apolipoprotein content of 1.006–1.063 g/ml lipoproteins are presented in **Table 3** and compared with data for human apoB-100 (4). Data in the two species were quite comparable, showing only minor discrepancies in the lysine and leucine content.

TABLE 3. Amino acid composition of apolipoprotein B from badger and human serum lipoproteins[a]

Amino Acid	Badger ApoB from d 1.006–1.063 g/ml Lipoproteins	Human B-100	
		VLDL	LDL
Lys	7.3 ± 0.1	8.1	8.0
His	2.6 ± 0.1	2.6	2.6
Arg	3.1 ± 0.1	3.5	3.4
Asp	10.7 ± 0.1	10.6	10.7
Thr	6.9 ± 0.1	6.6	6.6
Ser	8.2 ± 0.1	8.4	8.6
Glu	12.2 ± 0.1	11.7	11.6
Pro	3.8 ± 0.1	3.8	3.8
Gly	5.0 ± 0.4	4.8	4.7
Ala	5.7 ± 0.1	6.1	6.1
Val	5.5 ± 0.1	5.5	5.6
Met	1.7 ± 0.1	1.7	1.6
Ile	5.9 ± 0.1	6.1	6.0
Leu	12.4 ± 0.1	11.8	11.8
Tyr	3.6 ± 0.1	3.3	3.4
Phe	4.9 ± 0.1	5.0	5.1
Cys	0.5 ± 0.1	0.4	0.4

[a] Values are expressed as mol per 100 mol. Values for badger apoB are means ± SD of three different preparations; each sample was analyzed in duplicate or triplicate. Data in man are from Kane et al. (4). Badger apoB was isolated by gel filtration chromatography from lipoproteins of d 1.006–1.063 g/ml.

DISCUSSION

As a first step in the determination of their functional role(s), it was of considerable interest to establish the structural characteristics of the major circulating apolipoproteins in the badger. We presently describe, therefore, the physicochemical characteristics of apoA-I and apoB which, in the absence of noticeable amounts of apoA-II, are the two prominent apolipoprotein components of badger lipoproteins.

In most lipoprotein studies conducted to date, either in man or in different animal species, it has been customary to use the value of 1.063 g/ml as a cut-off between LDL and HDL. In the case of the plasma lipoprotein spectrum of the European badger, the use of this density limit may be inappropriate. Indeed, in this animal, we have previously shown that lipoproteins containing a peptide of M_r of approximately 28,000 (similar to that of human apoA-I) as their principal apolipoprotein component are present in both the 1.006–1.063 g/ml and 1.063–1.21 g/ml regions (1). However, the distribution of the apolipoprotein with M_r > 150,000, which might be considered as an equivalent to human apoB, does not extend to densities higher than 1.063 g/ml (1). Thus, in the badger as in man, this latter value is of physiological significance in that it is the upper limit of the density distribution of "true LDL," i.e., lipoproteins containing apoB, or its equivalent, as their prominent apolipoprotein constituent. On the other hand, our previous studies (1, 2) have shown that the 1.006–1.063 g/ml region of the plasma lipoprotein spectrum in the badger is affected by considerable seasonal changes which include the cyclic increase and decrease of the concentration of lipoproteins with electrophoretic mobility closely resembling that of lipoproteins containing the peptide with M_r corresponding to human apoA-I.

Consequently, it appeared of interest to determine whether an identity existed between the peptides with M_r of approximately 28,000 contained respectively in *1*) the d 1.063–1.21 g/ml lipoproteins present as major and permanent constituents of the plasma lipoprotein spectrum in the badger throughout the year, and *2*) certain lipoproteins belonging to the 1.006–1.063 g/ml region and subjected to important seasonal modifications in concentration (1, 2).

Results obtained by the different techniques in use were consistent with the hypothesis that apoA-I isolated from either 1.006–1.063 g/ml or 1.063–1.21 g/ml lipoproteins was the same protein, whose physicochemical properties appeared very similar to those of human apoA-I. Indeed, the apparent M_r of badger apoA-I, as determined by SDS-gel electrophoresis, was the same (approx. 27,000) as that determined by this procedure for the corresponding human protein. A comparison of the amino acid content of badger apoA-I with those of its canine and human counterparts allowed us to observe, in this respect, a close overall resemblance between these three species. However, some differences were of note between the badger and human proteins, as the respective proportions of lysine, serine, and methionine in the animal apoA-I were lower than the corresponding ones in man. By contrast, glycine, alanine, valine, and isoleucine were present in greater proportions in badger apoA-I than in the human protein. With regard to glycine, an increase in the proportion of this particular amino acid is consistent with results from our partial sequence determination, which showed the substitution of glycine for serine at position 24 when comparing badger apoA-I with the dog or human apoA-I. This mutation is compatible with a single change in a nucleotide base in the respective codons.

Two points are of note at this stage. First, as one could have expected considering the phylogenetic proximity of the badger and the dog, the differences listed above in the amino acid content were equally observed when comparing dog and human apoA-I, with the exception of the serine content which was similar in these latter species. Second, the presence, in badger apoA-I, of isoleucine residues was an additional similarity with the dog protein but was in contrast with the situation in most human subjects whose apolipoprotein A-I lacks this amino acid. It is, however, known that a mutant form of human apoA-I, i.e., apoA-I Milano, possesses one isoleucine residue/mol (15).

Isoelectric focusing showed that badger apoA-I was polymorphic, a feature shared with several other species studied to date, e.g., man (24, 26), nonhuman primates (27, 28), rat (29), and mouse (30). The isoelectric points determined for the different isoforms (range 5.30–5.55 approx.) were comparable with those reported for human apoA-I when studied in the same system (range 5.27–5.41) (26). This is in accord with the similar number of acidic residues (i.e., 67) in the A-I proteins in the two species, while their respective contents of basic amino acids were slightly lower in the badger (i.e., 39) than in man (i.e., 42). In addition, it is possible that the more basic isoform (pI 5.60–5.65) may correspond to a precursor form of the mature apolipoprotein (pro-apoA-I), as was suggested by Miller et al. (31) for the mouse. On the other hand, the bands noted for apolipoproteins of 1.006–1.063 g/ml and 1.063–1.21 g/ml lipoproteins, exhibiting pIs in the range 5.70–5.90, could be attributed to badger apolipoprotein E. Indeed, the presence of such an apolipoprotein is suggested by the observation in SDS-polyacrylamide gels (Fig. 1, gels A and B), of a band with apparent M_r 45,000 and thus rather similar to that originally determined for human apoE (M_r 39,000) (32).

Thus we have documented the presence of badger apoA-I in considerable amounts throughout the lipoprotein spectrum. However, as shown previously (1, 2), the respective concentrations of lipoproteins belonging to both the d 1.006–1.063 g/ml and 1.063–1.21 g/ml intervals vary largely according to season, by approximately 400% to 500% for d 1.006–1.063 g/ml lipoproteins, and by approximately 100% for d 1.063–1.21 g/ml lipoproteins; such variations inevitably lead to concomitant large seasonal differences in the plasma concentration of badger apoA-I.

The second major apolipoprotein in badger plasma lipoproteins exhibited physicochemical characteristics (molecular weight, distribution in lipoprotein density classes, elution profile on gel filtration chromatography, and amino acid composition), comparable to those of human apoB-100. Using analytical ultracentrifugation and compositional analysis, our earlier studies have established the respective concentrations of d < 1.006 g/ml and d 1.006–1.063 g/ml lipoproteins, and have shown that, at any season, the former components are present at low concentrations (< 50 mg/100 ml) (1). Thus, whatever the date of the year considered, most of $apoB_H$ should belong to 1.006–1.063 g/ml lipoproteins.

In our animals, the profile of high molecular weight apolipoproteins in 3% SDS-polyacrylamide gels was independent of the presence or absence of the proteolytic inhibitor PMSF during the isolation process. This is in contrast with the findings reported in other species (30). In human LDL, fragments of apoB-100 have been reported and denoted apoB-26 and apoB-74 on the basis of the relationship existing between their respective M_r and that of apoB-100 (4). In the badger, we have consistently observed the presence of proteins with M_r bearing an 83:100 and 35:100 relationship, respectively, to the M_r of $apoB_H$. These two proteins may be distinct from $apoB_H$. However, we suggest that the component with M_r bearing the 35:100 relationship to that of $apoB_H$ could be the result of dimerization of one with an M_r ratio of 17:100

relatively to apoB_H. Thus, badger apoB_H could be cleaved preferentially into two fragments, or be made up from two components, with M_r bearing, respectively, an 83:100 and a 17:100 relationship with apoB_H. This hypothesis is in accordance with a semi-quantitative evaluation of the appearance of SDS-acrylamide gels (Fig. 3, gel C), showing that the intensity of staining of the band with M_r of approximately 190,000 (M_r ratio 35:100, compared with that of apoB_H) was consistently lower than that of the band representative of the protein with M_r of approximately 450,000 (M_r ratio 83:100 compared with that of apoB_H).

For its part, the faint band with M_r of approximately 490,000 (M_r ratio 93:100 relative to apoB_H) could be related to another B protein in the rat, termed apoB-95 or P II (33, 34). In the rat, apoB-95 has recently been shown to have a metabolism distinct from that of apoB-100 (35).

The high molecular weight apolipoproteins in lipoproteins with d < 1.006 g/ml included small amounts of apoB_L. This latter protein exhibited an apparent M_r of approximately 280,000 and was thus analogous, in its relationship to apoB_H, to the relationship of human apoB-48 to B-100. In man, apoB-48 was originally described as an apoprotein component of lymph and plasma chylomicrons, therefore originating from the gut, but was also observed as a minor constituent of some VLDL (4). A similar protein has been reported in the rat, where it appears to originate both from the gut and the liver (36). In mouse VLDL and LDL, Forgez et al. (30) observed an equal content of apoB_L with apparent M_r 250,000–280,000 (30). Both in this latter species and in the badger, whether apoB_L is merely a fragment of apoB_H or is actually a distinct protein awaits determination of its amino acid sequence.

The present study has delineated the main structural characteristics of the two major circulating apolipoproteins in the badger. These data will form the basis for further studies dealing with important points such as the metabolic origin(s) and fate(s) of the different lipoprotein species containing apoA-I and the various forms of apoB, as well as the endocrine regulation of their metabolism. Such work is now in progress in our laboratory, concomitant with studies regarding other badger apolipoproteins, including possible counterparts to apoE and the different apoC's.

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