Isolation and Partial Characterization of a Polypeptide Belonging to Apolipoprotein B from Low-Density Lipoproteins of Human Plasma†

Sven-Olof Olofsson,* Kristina Boström, Ulf Svanberg, and Göran Bondjers

ABSTRACT: A method to solubilize the apolipoproteins of the low-density lipoproteins (LDL) of human plasma has been developed. The recovery of LDL protein during the delipidization and solubilization procedures was 93%. More than 95% of apoLDL could be solubilized with the solubilization procedure. ApoLDL appeared as several protein staining bands on basic polyacrylamide gel electrophoresis. A polypeptide corresponding to one of these protein staining bands (band I) has been isolated and partially characterized. The polypeptide is homogeneous as judged from basic, acidic, and $NaDodSO_4$–polyacrylamide gel electrophoresis, immuno double diffusion, and amino-terminal amino acid analysis. Band I differed from apoA-I, A-II, C-I, C-II, C-III, apoD, apoE, apoF, the protein moiety of Lp(a), and albumin as judged from polyacrylamide gel electrophoresis, immuno double diffusion, immunoelectrophoresis, amino acid analysis, molecular weight estimation, and amino-terminal amino acid analysis. The molecular weight of band I was 72 000–76 000 as estimated from $NaDodSO_4$–polyacrylamide gel electrophoresis. Estimation from gel chromatography in the presence of 8 M urea and β-mercaptoethanol gave a molecular weight of 63 000. Amino acid analysis indicated the presence of all commonly found amino acids. The amino-terminal amino acid was phenylalanine. By immuno double diffusion and immunoelectrophoresis, band I could be shown to be a part of Lp-B and thus by definition belong to apoB.

The protein moiety of plasma lipoproteins consists of several nonidentical polypeptides (Shore & Shore, 1968; Brown et al., 1969, 1970; McConathy & Alaupovic, 1973; Shelburne & Quarfordt, 1974; Olofsson et al., 1978). According to the terminology of the lipoprotein family concept (Alaupovic, 1972), these polypeptides are referred to as apoA-I,[1] A-II, apoB, C-I, C-II, C-III, apoD, apoE, and apoF, and the corresponding lipoprotein families are Lp-A, Lp-A-I, Lp-B, Lp-C, Lp-D, Lp-E, and Lp-F.

So far, no unequivocal characterization of the protein component of the density region LDL (1.006–1.063) has been presented. The presence of apoA-I, A-II, apoC, apoE, and apoF has been reported (Curry et al., 1976a,b; Curry, 1976; Olofsson et al., 1978), but these polypeptides constitute only a minor portion of the total protein content of LDL. Studies on the protein component of Lp-B, the major lipoprotein family of LDL, are contradictory (Scanu et al., 1968; Shore & Shore, 1969; Kane et al., 1970; Simons & Helenius, 1970; Smith et al., 1972; Chen & Aladjem, 1974, 1978). Parts of these problems are related to the insolubility of apoB in aqueous solutions (Helenius & Simons, 1971).

In the present report we present a method to solubilize a major portion of apoLDL as well as the isolation and partial characterization of one of its polypeptide components.

Materials and Methods

Preparation of Low-Density Lipoproteins (LDL). A starting volume of at least 2 L of either pooled blood bank plasma or serum from normolipidemic healthy volunteers was used. Plasma/serum was adjusted to solution density 1.21 g/mL by addition of solid potassium bromide and subjected to ultracentrifugation at 168000*g* for 24 h at +4 °C in a Prepspin 50 preparative ultracentrifuge (MSE, Crawley, West Sussex, England) utilizing a 6 × 100 mL rotor. The supernate was recovered by aspiration and adjusted to a solution density of 1.063 g/mL by dialysis against doubly distilled water for approximately 20 min and recentrifuged under the same conditions. The supernate was recovered with aspiration and subjected to three recentrifugations at density 1.063 g/mL under the same conditions. After recovery, the final supernate was overlayered with 0.05 M Tris buffer, pH 7.5, with a density of 1.006 g/mL (the ratio between lipoprotein solution and buffer was 1:3). Ultracentrifugation was carried out at 120000*g* for 24 h at +4 °C in a Superspeed 65 preparative ultracentrifuge (MSE, Crawley, West Sussex, England) utilizing an 8 × 35 mL rotor. The supernate (the upper half of the centrifuge tube) was removed by aspiration, and the clear yellow infranate was recovered. The infranate appeared as a single lipid staining band with β migration on agarose gel electrophoresis. The lipid composition agreed well with that reported for the human low-density lipoproteins (Oncley, 1963; Skipski et al., 1967).

Delipidization of LDL. LDL were dialyzed against doubly distilled water. The water was carefully deaerated before use, and the glass vessel used for dialysis was flushed with nitrogen before the water was added. The space not filled with water was filled with nitrogen and the vessel was sealed. Dialysis was carried out at +4 °C for 6 h with 20 changes of a 40-fold excess of water. After dialysis the LDL were lyophilized.

One hundred milliliters of chloroform (analytical grade, Merck, Darmstadt, West Germany) was added to the dry LDL, and the mixture was carefully shaken until a fine suspension was obtained. Two hundred milliliters of methanol was added, and the mixture was stored at −20 °C for 30 min. The protein was recovered by low-speed centrifugation, and the organic solvent was removed by aspiration. The procedure was repeated twice, followed by four extractions with chloroform–methanol, 1:1 (v/v), and one extraction with chloroform–methanol, 2:1. In each step the chloroform was added before the methanol to the sample. Finally, the protein pellet

† From the Arterial Biology Group, Department of Medical Biochemistry and Medicine I, University of Göteborg, Sweden. *Received June 11, 1979; revised manuscript received October 31, 1979.* This study was supported by grants from the Swedish Medical Research Council, No. 4531, the Swedish Oleo-Margarine Foundation for Nutritional Research, and the Swedish National Association against Heart and Lung Diseases.

[1] Abbreviations used: VLDL, very low density lipoproteins (*d* 0.96–1.006 g/mL); LDL, low-density lipoproteins (*d* 1.006–1.063 g/mL); LDL_2, low-density lipoprotein-2 (*d* 1.019–1.063 g/mL); HDL, high-density lipoproteins (*d* 1.063–1.21 g/mL); Lp, lipoprotein; apo, apolipoprotein; DEAE, diethylaminoethyl; $NaDodSO_4$, sodium dodecyl sulfate; SD, standard deviation; K_d, distribution coefficient.

0006-2960/80/0419-1059$01.00/0

was washed twice with diethyl ether.

Solubilization of ApoLDL. After the final diethyl ether wash, the protein pellet was recovered by low-speed centrifugation. The diethyl ether was removed by aspiration, leaving enough diethyl ether to cover the surface of the protein pellet. The protein and the diethyl ether were rapidly mixed with 2 M acetic acid (0.5 mL/mg of protein). In this way a two-phase system was obtained. Protein which was not immediately solubilized remained in the interface. After slow evaporation of the diethyl ether (for 12–20 h at +16 to +20 °C), a clear solution of protein in 2 M acetic acid was obtained. Disturbance of the interface by shaking, stirring, or blowing nitrogen on the surface led to aggregation of the protein and impaired solubilization.

Solubilization could also be carried out at 0 °C. Under these conditions evaporation of diethyl ether could be achieved by sucking off the air from a position 5 cm above the surface by a water-driven aspirator. Great care was undertaken to avoid too rapid evaporation of the diethyl ether phase or boiling of the ether.

Occasionally a fine precipitate of apoLDL was obtained when solubilization was carried out at 0 °C. The temperature of the solution was increased to 16–20 °C and urea was added to a concentration of 4 M in order to solubilize this precipitate.

Chromatography on Sephadex G-100. ApoLDL, 150–200 mg, solubilized in 2 M acetic acid, was mixed with urea (analytical grade, Merck, Darmstadt, West Germany) to a final concentration of 4 M, filtered through glass wool, and applied to a Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (170 × 5 cm) equilibrated with 2 M acetic acid. The chromatography was run at +20 °C. Fractions of 10 mL were collected and the optical density at 280 nm was measured in a spectrophotometer (Zeiss Spektralphotometer PMQ II, Zeiss, Oberkochen/Württemberg, West Germany).

Ion-Exchange Chromatography. Fractions from Sephadex G-100 chromatography were dialyzed against 0.01 M Tris buffer, pH 7.5, in 8 M urea. The dialysis was carried out until pH and conductivity of the sample equaled that of the buffer.

DEAE-cellulose (Servacel, 0.63 mequiv/g, Serva, Heidelberg, West Germany) was treated with 0.5 M sodium hydroxide, washed with doubly distilled water until neutrality, treated with 0.5 M hydrochloric acid, and again washed with doubly distilled water until neutrality. The DEAE-cellulose slurry was adjusted to pH 7.5. Prior to use the slurry was carefully equilibrated with 0.01 M Tris buffer, pH 7.5, in 8 M urea. A column of 25 × 1.5 cm was used, and the sample, 80–150 mg of protein, was applied in a volume of 400 mL. The ion-exchange column was eluted with the following: (I) 0.01 M Tris buffer, pH 7.5, in 8 M urea; (II) a gradient from 0 to 0.1 M sodium chloride in 0.01 M Tris buffer, pH 7.5, in 8 M urea; (III) 0.01 M Tris buffer, pH 7.5, in 8 M urea and 1 M sodium chloride.

Fractions of 3 mL were collected and the optical density was measured as described above.

All urea used in these experiments was deionized on a mixed-bed ion-exchange resin (Amberlite MB-3, Mallinckrodt, MS) prior to use.

Chromatography on Sepharose 6B CL. Fractions from the ion-exchange chromatography were dialyzed extensively against doubly distilled water, lyophilized, and resolubilized in 0.01 M Tris buffer, pH 7.5, in 8 M urea. Samples of 10–20 mg in 10 mL of buffer were chromatographed on a Sepharose 6B CL (Pharmacia Fine Chemicals, Uppsala, Sweden) column (195 × 2.5 cm) equilibrated with 0.01 M Tris buffer, pH 7.5, in 8 M urea. Fractions of 4 mL were collected. The optical density at 280 nm was measured as described above. Fractions were also analyzed with electrophoresis on basic and acidic polyacrylamide gels. Homogeneous fractions were combined and rechromatographed under the same conditions on a Sepharose 6B CL column (95 × 2.5 cm). Samples of 7–10 mg of protein in 5 mL of buffer were applied to the column.

Electrophoresis. Basic (Davis, 1964) and acidic (Reisfeldt et al., 1962) polyacrylamide gel electrophoreses were carried out on 7% acrylamide gels containing 8 M urea. The gels were stained with Coomassie Brilliant Blue (Reisne et al., 1975).

Agarose gel electrophoreses were carried out in 0.95% agarose gels (Rapp & Kahlke, 1968). Gels were stained with Oil red O.

Molecular weight estimation was made from electrophoresis on polyacrylamide gels containing $NaDodSO_4$. The acrylamide monomer concentration was either 5 or 10% (Weber & Osborn, 1969). Samples were dissolved in phosphate buffer containing 2.5% $NaDodSO_4$ and 5% β-mercaptoethanol and boiled for 10 min. Standards (Electrophoretic Calibration Kit, Pharmacia Fine Chemicals, Uppsala, Sweden) with known molecular weight were treated in the same way and run on parallel gels. The following standards were used: thyroglobulin (330 000), ferritin half unit (220 000), phosphorylase *b* (94 000), human serum albumin (67 000), catalase (60 000), ovalbumin (43 000), carbonic anhydrase (36 000), lactate dehydrogenase (30 000), trypsin inhibitor (20 400), ferritin monomer (18 500), and α-lactalbumin (14 400).

Molecular weight was also estimated from chromatography on a Sepharose 6B CL (Pharmacia Fine Chemicals, Uppsala, Sweden) column (95 × 1.5 cm) equilibrated with 0.01 M Tris buffer, pH 7.5, in 8 M urea and 5% β-mercaptoethanol. K_d (Gelotte, 1960) was calculated for samples and standards. The following standards of known molecular weight were used: phosphorylase *b* (94 000), transferrin (76 600), human serum albumin (67 000), ovalbumin (43 000), and chymotrypsinogen (25 700).

Amino acid analysis was carried out with methods used earlier (Olofsson et al., 1977).

Amino- and Carboxy-Terminal Amino Acid Analysis. Amino-terminal amino acids were analyzed with the dansyl chloride method by Woods & Wang (1967). Dns-labeled amino acids were chromatographed on polyamide thin-layer plates (Pierce, IL). The plates were developed with *n*-heptane–1-butanol–glacial acetic acid (3:3:1 v/v/v) in the first dimension and benzene–glacial acetic acid (9:1 v/v) in the second dimension. Dns-labeled amino acids were identified by comparison with pure standards (Sigma Chemical Co., MO).

Carboxy-terminal amino acids were determined after hydrazinolysis (Braun & Schroeder, 1967). Anhydrous hydrazine was obtained from Pierce (IL). Amino acids were separated from the hydrazides by chromatography on an Amberlite CG 50 Type II column (15 × 1 cm) (Rohm & Co., Philadelphia, PA) (Braun & Schroeder, 1967). Identification was performed with an automatic amino acid analyzer.

Protein quantification was carried out with the method of Lowry et al. (1951). Lipid analyses were carried out with methods used earlier (Olofsson et al., 1975).

Immunological Methods. Immuno double diffusion and immunoelectrophoresis were carried out in 1% agar gels utilizing barbital buffer with pH 8.6 and ionic strength 0.05. Immunoelectrophoresis was carried out at 5 V/cm for 90 min.

ApoA-I, A-II, C-III, apoD, apoE, and apoF were prepared with methods used earlier (Olofsson et al., 1978). The purity

of the preparations was assessed by electrophoresis on basic, acidic, and $NaDodSO_4$–polyacrylamide gels and with amino acid analysis. Antiserum was raised in New Zealand White rabbits. Antigens (0.1–0.5 mg) dissolved in 0.5 mL of 0.9% sodium chloride were mixed with equal volumes of Freund's incomplete adjuvants and injected into the footpads of the rabbits. After 5 weeks the rabbits were boostered by multifocal subcutaneous injections of 0.1–0.5 mg of antigen in 0.5 mL of 0.9% sodium chloride mixed with equal volumes of Freund's incomplete adjuvants and by intramuscular injection of 0.1–0.5 mg of antigen in 1 mL of 0.9% sodium chloride. The animals were then boostered every tenth day by intramuscular injections of 0.1–0.5 mg of antigen in 0.9% sodium chloride. Three to five injections were necessary to obtain a sufficient titer. Antiserum to Lp(a) (Berg, 1963, 1964) was kindly supplied by Dr. K. Berg, University of Oslo, Norway.

Preparation of Lp-B. Serum was obtained from healthy normolipidemic volunteers. LDL were recovered by ultracentrifugation as described above and dialyzed extensively against 0.05 M Tris buffer, pH 7.5, containing 0.001 M calcium chloride and manganese chloride and 0.1% sodium azide (the start buffer).

LDL were adsorbed onto concanavalin A–Sepharose (McConathy & Alaupovic, 1974) (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer. Unretained material was removed by elution with the start buffer. The elution volume corresponded to 4 times the volume of the column. The retained material was eluted with 0.4 M α-methyl mannoside in the start buffer. This fraction was then subjected to ultracentrifugation at d 1.040 g/mL in a Superspeed 65 preparative ultracentrifuge (MSE, Crawley, West Sussex, England) at 120000g for 24 h at +4 °C. The supernate was removed by aspiration, and the infranate was recovered and dialyzed extensively against the start buffer and rechromatographed on concanavalin A–Sepharose under the same conditions. The retained fraction was subjected to ultracentrifugation at d 1.063 g/mL under the conditions given above. The supernate was recovered with aspiration and dialyzed against 0.05 M Tris buffer, pH 7.5, containing 1% sodium azide. This fraction showed β migration on agarose electrophoresis and on immunoelectrophoresis. Electrophoresis on basic polyacrylamide gels after delipidization with tetramethylurea (Kane, 1973) revealed no protein staining bands in the separating gel.

Antiserum to Lp-B was raised as described above. An electroimmunoassay utilizing the antiserum and Lp-B (prepared as described above) as standards was developed in order to ascertain that this antiserum recognized a major lipoprotein family in human plasma. The concentration of apoB was determined in serum from 76 men, randomly selected from the population of 41–48-year-old men in Göteborg, Sweden (Wiklund et al., 1979). An apoB concentration of 1.13 ± 0.20 g/L (mean ● SD) was obtained. This is in agreement with data on apoB obtained by others (Schonfeld et al., 1974; Albers et al., 1975; Bautovich et al., 1975; Bedford et al., 1976; Thompson et al., 1976; Curry et al., 1978).

^{125}I-Labeling of ApoLDL. LDL were labeled with ^{125}I by the method of McFarlane (1958). ApoLDL was isolated as described above. No free ^{125}I activity could be found in association with apoLDL with paper chromatography (Billheimer et al., 1972).

Results

Solubilization of ApoLDL. After centrifugation of solubilized apoLDL at 120000g for 60 min at +15 °C, all the protein was recovered in the clear supernate while no pellet could be found. The total recovery of protein from LDL was 93 ± 6% (mean ± SD; n = 6), and the recovery of ^{125}I labeled apoLDL was 95 ± 8% (mean ● SD; n = 10).

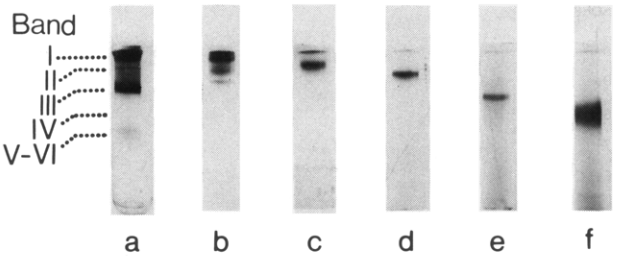

FIGURE 1: Basic polyacrylamide gel electrophoresis of solubilized apoLDL (a), fraction I from Sephadex G-100 chromatography of solubilized apoLDL (b), and purified polypeptides corresponding to band I (c), band II (d), band III (e), and band IV (f).

ApoLDL was heterogeneous as judged from electrophoresis on basic polyacrylamide gels (Figure 1a). Several protein staining bands could be detected (bands I–VI). Band II corresponded to apolipoprotein E. Band III was found in LDL_2 and also in a narrow density cut (d 1.030–1.050 g/mL) of LDL_2. By immunochemical methods band III could be shown to be different from band I. Band IV was primarily found in the VLDL region. It was not possible to isolate this polypeptide in substantial amounts from LDL_2 or a narrow density cut (d 1.030–1.050 g/mL) of LDL_2. This polypeptide could be demonstrated to be different from apoB by immunochemical methods. The isolation and characterization of bands III and IV will be reported separately. Bands V and VI represented trace amounts of the C-II and C-III polypeptides.

This paper deals with the isolation and partial characterization of a polypeptide corresponding to band I. This polypeptide will be referred to as band I in the following presentation. Band I was present and could be isolated from LDL, LDL_2, and a narrow density cut (d 1.030–1.050 g/mL) of LDL_2.

No proteolytic activity in LDL (before and after dialysis against doubly distilled water) or in solubilized apoLDL could be demonstrated during incubation with azocoll. Amino- and carboxy-terminal amino acids of $apoLDL_2$ were analyzed after 10 min, 10 h, and 20 h of exposure to 2 M acetic acid. No changes with time of amino or carboxy terminals could be detected under these conditions. Lowering of the temperature from +20 to 0 °C during solubilization did not influence the electrophoretic behavior of apoLDL (Figure 1a) or the isolation and properties of band I.

Isolation of Band I. ApoLDL was chromatographed on Sephadex G-100 equilibrated with 2 M acetic acid. A major fraction (fraction I, Figure 2) was eluted after the void volume of the column. This fraction contained 81 ± 16% (mean ± SD; n = 4) of the applied protein. Electrophoresis on basic polyacrylamide gels (Figure 1b) revealed the presence of bands I–III. A minor fraction eluted after fraction I (fraction II, Figure 2) contained mainly band IV and the C polypeptides (bands V–VI).

Fraction I from the Sephadex G-100 chromatography was dialyzed against 0.01 M Tris buffer, pH 7.5, in 8 M urea and applied to a DEAE-cellulose column. We obtained an almost total recovery of the protein during the DEAE-cellulose ion-exchange chromatography. The major amount of band I was obtained as an unretained fraction from the DEAE-cellulose column. This fraction contained 55 ± 7% (mean ± SD; n = 4) of the applied amount of protein. Band I was also found as a minor fraction eluted with the first one-third of the gradient. This fraction corresponded to approximately 9% of

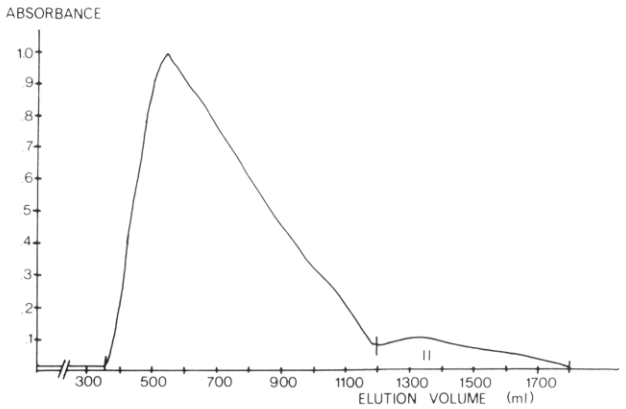

FIGURE 2: Chromatography of solubilized apoLDL on Sephadex G-100.

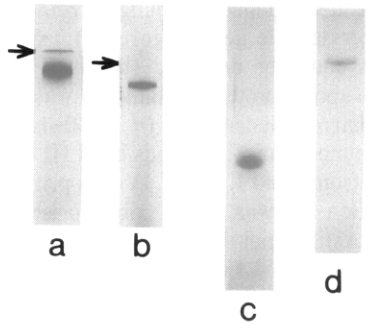

FIGURE 3: Electrophoresis of purified band I on basic (a), acidic (b), and $NaDodSO_4$–polyacrylamide gels with 5% (c) and 10% (d) acrylamide concentration. Arrows indicate the interface between the stacking gel and separating gel.

Table I: Amino Acid Composition of ApoLDL and Band I

	mol/10^3 mol of amino acid	
	apoLDL[a]	band I[b]
Lys	76.9 ± 1.0	69.3 ± 1.9
His	25.1 ± 0.5	19.5 ± 1.7
Arg	35.4 ± 3.3	37.0 ± 6.8
Asp	83.6 ± 1.6	94.5 ± 7.4
Thr	58.1 ± 2.1	64.8 ± 3.1
Ser	72.3 ± 1.8	82.0 ± 4.0
Glu	122.4 ± 2.2	130.1 ± 3.1
Pro	43.7 ± 3.5	46.2 ± 3.1
Gly	54.0 ± 2.4	65.3 ± 7.9
Ala	67.4 ± 1.3	72.6 ± 2.7
Val	59.7 ± 1.3	57.0 ± 2.7
1/2-cystine[c]	8.0 ± 1.4	14.5 ± 2.6
Met	19.7 ± 0.5	16.7 ± 1.4
Ile	65.4 ± 1.9	51.7 ± 1.9
Leu	130.7 ± 2.5	121.2 ± 4.4
Tyr	34.8 ± 1.4	31.0 ± 1.6
Phe	55.3 ± 2.3	38.4 ± 2.6
Trp	nd[d]	nd

[a] Mean ± SD; n = four different preparations. [b] Mean ± SD; n = seven different preparations. [c] Determined as cysteic acid. [d] nd = not determined.

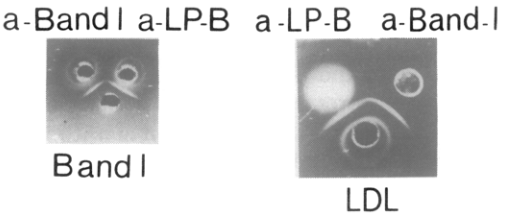

FIGURE 4: Immuno double diffusion of band I and LDL against antiserum to band I and antiserum to Lp-B. a = anti.

the applied amount of protein. The material eluted with the last two-thirds of the sodium chloride gradient (29% of the applied amount of protein) contained a mixture of bands II and III. The fraction eluted with high sodium chloride concentration (7% of the applied amount of protein) contained a mixture of bands I–III. The unretained fraction was finally chromatographed twice on Sepharose 6B CL to further purify band I.

Polyacrylamide gel electrophoresis of band I at acidic pH revealed only one protein staining band (Figure 3b). On $NaDodSO_4$–polyacrylamide gels band I migrated between the standards of 94 000 (phosphorylase *b*) and 67 000 (human serum albumin) (parts c and d of Figure 3). On basic polyacrylamide gel electrophoresis (Figure 3a), one protein staining band was obtained in the separating gel. In addition, there was a faint protein staining band in the interface between the stacking and the separating gel. Acidic and $NaDodSO_4$–polyacrylamide gel electrophoresis indicates that this is not due to small amounts of a polypeptide of higher molecular weight or a more basic polypeptide. The following experiment was performed to study the possibility that this protein staining band represented polymers of band I. After extraction of the minor protein staining band and adjustment to equal protein concentrations as the major band, total identity of immunoprecipitin arcs at immuno double diffusion against monospecific antiserum to band I was obtained. This indicates that the stainable material in the interface between stacking gel and separating gel represented a tendency of band I to polymerize or precipitate under the conditions used for the electrophoresis.

Molecular weight estimation from electrophoresis on $NaDodSO_4$-containing polyacrylamide gels utilizing an acrylamide concentration of 5 or 10% indicated a molecular weight of 72 000 or 76 000, respectively.

Molecular weight estimation from gel chromatography under reduced conditions on Sepharose 6B CL in 8 M urea indicated a molecular weight of 63 000.

Amino acid analysis (Table I) revealed the presence of all commonly found amino acids (tryptophan has not been determined).

Amino- and Carboxy-Terminal Amino Acid Analysis. Only one Dns-labeled amino acid (except for ϵ-lysine) was obtained, corresponding to phenylalanine. Phenylalanine was also detected as a major amino-terminal amino acid in whole apoLDL as well as $apoLDL_2$ and the apolipoprotein from a narrow density cut (d 1.030–1.050 g/mL) of LDL_2.

Glutamic acid was obtained as the carboxy-terminal amino acid after hydrazinolysis of band I. Glutamic acid was also obtained as a major carboxy-terminal amino acid in $apoLDL_2$ and a narrow density cut (d 1.030–1.050 g/mL) of LDL_2.

Immunological Studies. Band I did react with antiserum to Lp-B and antiserum to band I. The precipitin arcs formed showed total identity (Figure 4). No immunoprecipitin arcs were obtained between band I and antiserum to apoA-I, AII, apoC, apoD, apoE, apoF, Lp(a), or albumin.

Band I showed one precipitin arc with cathodal migration on immunoelectrophoresis with antiserum to band I (Figure 5). In its lipoprotein form, however, band I migrated toward the anode (Figure 5). The precipitin arcs formed between LDL or whole serum and antiserum to Lp-B and antiserum to band I formed total identity. Total identity of the immunoprecipitin arcs was also obtained on immuno double diffusion of LDL or whole serum against antiserum to Lp-B and antiserum to band I (Figure 4).

Antiserum to band I did react with both Lp(a) positive and Lp(a) negative serum. The precipitin arcs formed between

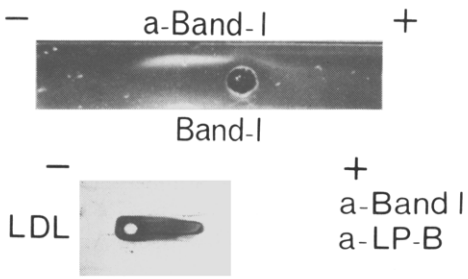

FIGURE 5: Immunoelectrophoresis of band I and LDL against antiserum to band I and antiserum to Lp-B. The immunoelectrophoresis of LDL has been stained with Oil red O. a = anti.

Lp(a) positive serum and antiserum to band I and antiserum to Lp(a) formed partial identity.

The precipitin arcs formed between LDL (or whole serum) and antiserum to band I took up Oil red O stain, indicating that band I is an apolipoprotein (Figure 5).

Presence of Band I in a Population. The presence of band I in serum from 76 men, a representative sample of all men aged 41–48 years in Göteborg, Sweden, was studied with immuno double diffusion. Band I could be detected in all samples.

Discussion

A method to solubilize apoLDL has been developed. Our data indicate that 95% of apoLDL can be solubilized by this method. As less than 10% of the protein moiety of LDL was lost during delipidization and solubilization procedures, our results indicate that the solubilized apoLDL represents a major portion of apoLDL. However, a selective loss of minor constituents of apoLDL could not be ruled out from our data.

Our results suggest that apoLDL is heterogeneous. It has earlier been shown that apoLDL contains minor amounts of apoA-I, A-II, apoC, apoE, and apoF (Curry et al., 1976a,b; Olofsson et al., 1978).

The major component of apoLDL, referred to as apoB, is still poorly characterized. Some data in the literature indicate that apoB consists of a high molecular weight protein (Simons & Helenius, 1970; Smith et al., 1972). Others, however, indicate that apoB consists of subunits (Shore & Shore, 1969; Albers et al., 1972; Chen & Aladjem, 1974; Huang & Lee, 1979; Socorro & Camejo, 1979). The conflicting results have been attributed to several factors such as delipidization procedures (Chen & Aladjem, 1974), proteolytic activity (Krisnaiah & Wiegandt, 1974), reaction between protein and autoxidizing lipids (Schuh et al., 1978), and a high tendency for subunits to aggregate (Bradley et al., 1978). One cause for conflicting results may be that the relation between the polypeptides studied and Lp-B, the major lipoprotein family of LDL, has not unequivocally been studied. This is of course of greatest importance since it cannot be ruled out that there exist so far unrecognized polypeptides within LDL that are not related to apoB. Our preliminary data suggest the presence of such polypeptides in LDL. The presence of minor polypeptides has also been reported for other density classes (McConathy & Alaupovic, 1973; Olofsson et al., 1977, 1978). The use of immunochemical methods for studies on the relation between an isolated apolipoprotein and plasma lipoproteins has been suggested by Alaupovic (1978).

The isolation and partial characterization of a polypeptide from apoLDL are reported in the present study. By immunochemical methods the polypeptide could be shown to be a part of Lp-B and thus by definition belong to apoB. The polypeptide, referred to as band I, is homogeneous on polyacrylamide gel electrophoresis. On the basis of the electrophoretic behavior, the molecular weight, amino acid composition, and immunochemical properties, band I seems to differ from apoA-I, A-II, C-I, C-II, C-III, apoD, apoE, and apoF. It also differs from the protein moiety of Lp(a) (Berg, 1963, 1964).

Earlier reports on the molecular weight of apoB range from 10 000 to 450 000. The presence of a polypeptide with a molecular weight similar to band I has been reported by Chen & Aladjem [cf. band III of Chen & Aladjem (1978)] and recently by Socorro & Camejo (1979). They, however, interpreted the protein staining band on $NaDodSO_4$–polyacrylamide gel electrophoresis, corresponding approximately to a molecular weight of 70 000, as a polymer of smaller polypeptides. By indirect methods Bradley et al. (1978) have estimated the molecular weight to be 30 000. It is of course hard to rule out that band I of the present study does not represent a stable polymer of a polypeptide with lower molecular weight. However, one would expect to find at least traces of the monomer on electrophoresis of the purified protein on basic, acidic or $NaDodSO_4$-containing polyacrylamide gels or on gel chromatography in the presence of urea and β-mercaptoethanol. Thus, we must conclude that band I either is a very stable polymer or, more likely, has a molecular weight of approximately 70 000. Our data do not, however, exclude the presence of polypeptides within apoLDL with lower molecular weights than band I. In fact, preliminary data on the nature of bands III and IV indicate that these polypeptides both have a lower molecular weight than band I. However, these polypeptides differ from band I as could be demonstrated by immunochemical methods.

High molecular weights for apoB have been reported by Smith et al. (1972) and by Schuh et al. (1978). Formation of low molecular weight polypeptides by the action of proteases, associated with LDL, on apoB (Krishnaiah & Wiegandt, 1974) has been suggested. However, the failure in this study to detect proteolytic activity in LDL or apoLDL supports previous suggestions that such a process is unlikely (Chapman & Kane, 1975; Chen & Aladjem, 1978). An effect of autoxidizing of lipids on apoB has also been discussed as a source for heterogeneity of apoB (Schuh et al., 1978). Therefore, the present method has been designed to minimize the influence of oxygen and sodium azide on apoLDL. We also considered the possibility of hydrolysis of apoB in the presence of the 2 M acetic acid. The failure to detect any change with time of the amino- and carboxy-terminal amino acids during solubilization of apoLDL does not support the possibility that band I is formed by such a process. Neither does the fact that the solubilization procedure could be carried out at 0 °C without any influence on the isolation and properties of band I support this possibility. Taken together these results indicate that apoB is not hydrolyzed during the solubilization in 2 M acetic acid. In conclusion, our results indicate that band I neither represents a polymer of polypeptides of lower molecular weight nor is formed by hydrolysis of apoB during the solubilization procedure.

Acknowledgments

We thank Anita Magnusson and Margit Wettesten for skillful technical assistance and Inger Olofsson and Agneta Ladström for typing the manuscript. We are grateful to Dr. K. Berg for antiserum to Lp(a).

References

Alaupovic, P. (1972) *Protides Biol. Fluids, Proc. Colloq. 19*, 9.

laupovic, P. (1978) *Test of the Month* (*Am. Assoc. Bioanalysts*) *4*, 1.
lbers, J. J., Chen, C.-H., & Aladjem, F. (1972) *Biochemistry 11*, 178.
lbers, J. J., Cabana, V. G., & Hazzard, W. R. (1975) *Metab., Clin. Exp. 24*, 1339.
autovich, G. J., Simons, L. A., Williams, P. F., & Turtle, J. R. (1975) *Atherosclerosis 21*, 217.
edford, D. K., Shephard, J., & Morgan, H. G. (1976) *Clin. Chim. Acta 70*, 267.
erg, K. (1963) *Acta Pathol. Microbiol. Scand. 59*, 369.
erg, K. (1964) *Acta Pathol. Microbiol. Scand. 62*, 613.
illheimer, D. W., Eisenberg, S., & Levy, R. I. (1972) *Biochim. Biophys. Acta 260*, 212.
radley, W. A., Rohde, M. F., Gotto, A. M., Jr., & Jackson, R. L. (1978) *Biochem. Biophys. Res. Commun. 81*, 928.
raun, V., & Schroeder, W. A. (1967) *Arch. Biochem. Biophys. 118*, 241.
rown, W. V., Levy, R. I., & Fredrickson, D. S. (1969) *J. Biol. Chem. 244*, 5687.
rown, W. V., Levy, R. I., & Fredrickson, D. S. (1970) *J. Biol. Chem. 245*, 6588.
hapman, M. J., & Kane, J. P. (1975) *Biochem. Biophys. Res. Commun. 66*, 1063.
hen, C.-H., & Aladjem, F. (1974) *Biochem. Biophys. Res. Commun. 60*, 549.
hen, C.-H., & Aladjem, F. (1978) *Biochem. Med. 19*, 178.
urry, M. D. (1976) Dissertation, University of Oklahoma.
urry, M. D., Alaupovic, P., & Suenram, C. A. (1976a) *Clin. Chem.* (*Winston-Salem, N.C.*) *22*, 315.
urry, M. D., McConathy, W. J., & Alaupovic, P. (1976b) *Biochim. Biophys. Acta 326*, 406.
urry, M. D., Gustafson, A., Alaupovic, P., & McConathy, W. J. (1978) *Clin. Chem.* (*Winston-Salem, N.C.*) *24*, 280.
avis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404.
elotte, B. J. (1960) *J. Chromatogr. 3*, 330.
elenius, A., & Simons, K. (1971) *Biochemistry 10*, 1971.
uang, S. S., & Lee, D. M. (1979) *Biochim. Biophys. Acta 577*, 424.
ane, J. P. (1973) *Anal. Biochem. 53*, 350.
ane, J. P., Richards, E. G., & Havel, R. J. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 1075.
rishnaiah, K. V., & Wiegandt, H. (1974) *FEBS Lett. 40*, 265.
owry, O. H., Rosenbrough, N. J., & Farr, A. L., & Randall, R. I. (1951) *J. Biol. Chem. 20*, 150.
McConathy, W. J., & Alaupovic, P. (1973) *FEBS Lett. 37*, 178.
McConathy, W. J., & Alaupovic, P. (1974) *FEBS Lett. 41*, 174.
McFarlane, A. S. (1958) *Nature* (*London*) *182*, 53.
Olofsson, S.-O., Gustafson, A., & Svanberg, U. (1975) *Scand. J. Clin. Lab. Invest. 35*, 363.
Olofsson, S.-O., Fager, G., & Gustafson, A. (1977) *Scand. J. Clin. Lab. Invest. 37*, 749.
Olofsson, S.-O., McConathy, W. J., & Alaupovic, P. (1978) *Biochemistry 17*, 1032.
Oncley, J. L. (1963) in *Brain Lipid and Lipoproteins and Leucodystrophies* (Folchi-Pi, J., & Brown, H., Eds.) pp 1–17, Elsevier, Amsterdam.
Rapp, W., & Kahlke, W. (1968) *Clin. Chim. Acta 19*, 493.
Reisfeldt, R. A., Lewis, U. J., & Williams, D. E. (1962) *Nature* (*London*) *195*, 281.
Reisne, A. H., Nemes, P., & Bucholtz, C. (1975) *Anal. Biochem. 64*, 509.
Scanu, A., Pollard, H., & Reader, W. (1968) *J. Lipid Res. 9*, 342.
Schonfeld, G., Lees, R. S., George, P. K., & Pfleger, B. (1974) *J. Clin. Invest. 53*, 1458.
Schuh, J., Fairclough, G. F., Jr., & Haschemeyer, R. H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3173.
Shelburne, F. A., & Quarfordt, S. H. (1974) *J. Biol. Chem. 249*, 1428.
Shore, V., & Shore, B. (1968) *Biochemistry 7*, 3396.
Shore, B., & Shore, V. (1969) *Biochemistry 8*, 4510.
Simons, K., & Helenius, A. (1970) *FEBS Lett. 7*, 59.
Skipski, V. P., Barclay, M., Barclay, R. K., Fetzer, V. A., Good, J. J., & Archibald, F. M. (1967) *Biochem. J. 104*, 340.
Smith, R., Dawson, J. R., & Tanford, C. (1972) *J. Biol. Chem. 247*, 3376.
Socorro, L., & Camejo, G. (1979) *J. Lipid Res. 20*, 631.
Thompson, G. R., Birnbaumer, M. E., Levy, R. I., & Gotto, A. M., Jr. (1976) *Atherosclerosis 24*, 107.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.
Wiklund, O., Fager, G., Craig, I. H., Wilhelmsson C.-E., Vedin, A., Olofsson, S.-O., Bondjers, G., & Wilhelmsen, L. (1979) *Scand. J. Clin. Lab. Invest.* (in press).
Woods, K. R., & Wang, K.-T. (1967) *Biochim. Biophys. Acta 133*, 369.