Human liver RNA-programmed in vitro synthesis of a polypeptide related to human apolipoprotein B

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In an in vitro synthesizing system programmed with RNA from human liver a polypeptide with an estimated M_r of 80000 (80 kDa) ± 1400 (mean ± SD, $n = 5$) was synthesized. This polypeptide could be precipitated with antiserum to a narrow density cut of LDL ($d = 1.030–1.055$) or antiserum against the high-M_r form of apoB (apoB 100 [4]). The synthesized protein is immunologically related to a 75 kDa protein isolated from LDL. We suggest that the 80 kDa protein represents a primary translation product of apoB synthesized in human liver.

Apolipoprotein B *Human liver RNA* *In vitro translation* *Low density lipoprotein*

1. INTRODUCTION

The dominant protein component of LDL is known as apolipoprotein B (apoB). In several investigations apoB has been found to be a high-M_r protein (250 kDa or 549 kDa) when analysed by SDS–PAGE [1–4] or by sedimentation equilibrium centrifugation [5,9]. However, proteins with considerably lower M_r-values have also been reported to be associated with LDL [4,6–10]. These proteins have been shown to be related to apoB by immunological methods [8–10]. The reasons for these conflicting findings are not clear. The possibility that the proteins with lower M_r-values are proteolytic products of the high-M_r form of apoB has been discussed [3,4,11–13]. On the other hand, it has also been suggested that the high-M_r form of apoB represents a polymer of the low-M_r polypeptide(s) [8,14]. One reason for such polymerization may be the different methods used for delipidization of LDL and for solubilization of apoB [18].

To further investigate the structure of apoB, we have studied the formation in an in vitro protein synthesizing system programmed with human liver RNA of a polypeptide that reacted with antiserum against a narrow density cut of LDL ($d = 1.030–1.055$) and antiserum against the high-M_r form of apoB.

Abbreviations: DU, density units; LDL, low density lipoproteins ($d = 1.019–1.063$); LDL ($d = 1.030–1.055$), a narrow density cut of LDL; Lp-B, lipoprotein B; apo, apolipoprotein; apo LDL ($d = 1.030–1.055$), the protein component of a narrow density cut of LDL; SD, standard deviation; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride

2. MATERIALS AND METHODS

2.1. *Materials*

Sepharose CL 4B, CNBr-activated Sepharose 4B, and the Electrophoretic Calibration Kit were from Pharmacia (Uppsala). The reticulocyte lysate translation kit and EN^{3}HANCE were from New England Nuclear (Boston MA). [^{35}S]Methionine (1500 Ci/mmol) was from Amersham (Buckinghamshire).

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2.2. *Isolation of a high-M_r protein from a narrow density cut of LDL*

A narrow density cut of LDL (d = 1.030–1.055) was isolated in the presence of 0.1% EDTA, 1 mM PMSF, 0.1% thiomerasal, 50000 IU/l penicillin and 50 mg/l streptomycin with stepwise ultracentrifugation as in [10]. The delipidization of LDL and solubilization of the remaining protein were carried out as in [10]. The solubilized apoLDL was chromatographed on Sephadex G-100, DEAE-cellulose and Sepharose CL 6B as in [10], with the following exceptions:

(i) All buffer solutions contained 0.1% NaN_3 and 1 mM PMSF;
(ii) Chromatography was carried out at 4°C or 9°C (when buffers containing 8 M urea were used).

The majority of the material recovered from the column (~70%) was eluted with the void volume. This fraction was rechromatographed 3 times on a Sepharose CL 4B column (93 × 2.5 cm) in 0.01 M Tris–HCl (pH 8.0) with 8 M urea, 5% 2-mercaptoethanol, 0.1% NaN_3 and 1 mM PMSF. The purified fraction appeared as one band on electrophoresis in 3 or 7.5% polyacrylamide gels containing SDS. The estimated M_r and the amino acid composition agreed closely with those reported for the liver-derived high-M_r protein of apoLDL, referred to as apoB 100 [4]. The isolated protein will, therefore, be referred to as apoB 100.

2.3. *Isolation of a 75 kDa protein from a narrow density cut of LDL*

Isolation of the 75 kDa protein from LDL (d = 1.030–1.055) was carried out as in [10] with the following exceptions:

(i) The LDL was isolated in the presence of 0.1% EDTA, 1 mM PMSF, 0.1% thiomerasal, 50000 IU/l penicillin and 50 mg/l streptomycin;
(ii) All buffer solutions used contained 0.1% NaN_3 and 1 mM PMSF;
(iii) Chromatography was carried out at 4°C or 9°C (when buffers containing 8 M urea were used). The protein will be referred to as $apoB^{75\ kDa}$.

2.4. *Immunological methods*

Antisera were raised as in [10]. Antiserum to Lp-B was prepared by injecting LDL (d = 1.030–1.055). Four different immunoglobulin preparations were used for the immunoprecipitation of in vitro synthesized apoB:

(i) The total immunoglobulin fraction prepared by ammonium sulphate precipitation [18] of serum from non-immunized rabbits;
(ii) The total immunoglobulin fraction prepared by ammonium sulphate precipitation of serum from rabbits immunized with LDL (d = 1.030–1.055);
(iii) The specific immunoglobulin fraction from rabbits immunized with LDL (d = 1.030–1.055);
(iv) The specific immunoglobulin fraction from rabbits immunized with apoB 100.

The LDL- and apoB 100-specific immunoglobulin fractions (iii,iv) were obtained by separate chromatographies of the corresponding antisera on a column of CNBr-activated Sepharose 4B coupled with LDL (d = 1.030–1.055). The fraction of each of the antisera retained by the column was eluted with 3 M NaSCN and desalted on a Sephadex G-25 column equilibrated with 0.1 M $NaHCO_3$ (pH 8.3) with 0.5 M NaCl.

All immunoglobulin fractions (i–iv) were coupled to CNBr-activated Sepharose 4B. They will be referred to as:

(i) non-immune-IG–Sepharose;
(ii) anti-Lp-B–Sepharose;
(iii) anti-Lp-B (specific IG)–Sepharose;
(iv) anti-apoB 100 (specific IG)–Sepharose.

Coupling of proteins to CNBr-activated Sepharose 4B was carried out as recommended by the manufacturer.

2.5. *Synthesis of protein in a cell-free system*

RNA was prepared [17] from human liver biopsies (1–4 g wet wt) obtained during cholecystectomy. The incubation mixture contained 50 μl reticulocyte lysate, 27.5 μl translation cocktail, 10 μl [^{35}S]methionine (9.7 mCi/ml), 10 μl 1 M potassium acetate, 2.5 μl 32.5 mM magnesium acetate, 15 μl distilled water and 10 μl human liver RNA (100 DU/ml). The reaction mixture was incubated for 60 min at 37°C.

Immunoprecipitation of polypeptides reacting with antibodies against LDL (d = 1.030–1.055) or apoB 100 was done as in [18] using the Sepharose coupled immunoglobulin fractions described above (i.e., non-immune-Ig–Sepharose, anti-Lp-

B–Sepharose, anti-Lp-B (specific IG)–Sepharose and anti-apoB 100 (specific IG)–Sepharose).

Precipitated proteins were analysed with SDS–PAGE (7.5%) [15], using proteins of known M_r-values as standards. Autoradiography was performed by fluorography [19] in the presence of EN^{3}HANCE.

3. RESULTS

The rabbit reticulocyte lysate system, programmed with unfractionated RNA from human liver, synthesized a polypeptide that was specifically bound to the antibodies against LDL (d = 1.030–1.055) (fig.1) as well as to the antibodies against apoB 100 (fig.2). About 0.4% of the total radioactivity incorporated into protein was recovered in this polypeptide fraction. The M_r of the synthesized polypeptide was estimated to be 80000 ± 1400 (mean ± SD, n = 5) as judged from its migration in the 7.5% SDS-slab gels in relation to proteins with known M_r-values (cf. fig.1–3).

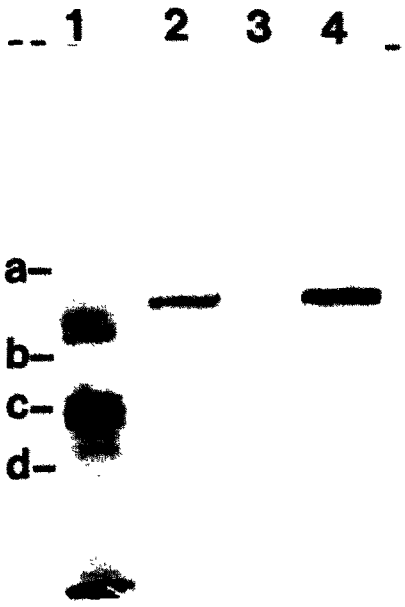

Fig.1. SDS–PAGE (7.5%) [15] of proteins synthesized in the in vitro protein-synthesizing system programmed with human liver RNA. The dried gels were fluorographed for 12 h: (1) total translation mixture; (2) proteins bound to anti-Lp-B–Sepharose; (3) proteins bound to non-immune IG–Sepharose; (4) proteins bound to anti-Lp-B (specific IG)–Sepharose. Dotted lines indicate the start of the separating gel. (a)–(d) Migration of M_r standards: (a) 94 kDa; (b) 67 kDa; (c) 60 kDa; (d) 43 kDa.

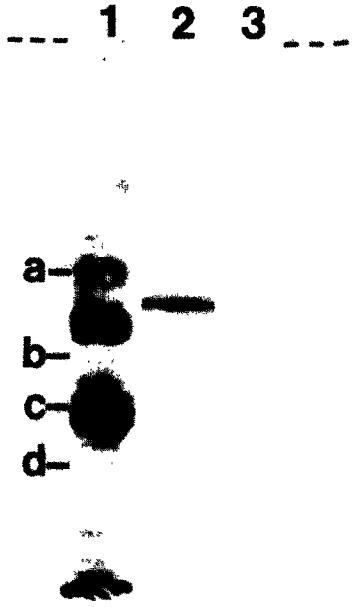

Fig.2. SDS–PAGE (7.5%) [15] of proteins synthesized in the in vitro protein-synthesizing system programmed with human liver RNA. The dried gels were fluorographed for 12 h (1) and 76 h (2): (1) total translation mixture; (2) proteins bound to anti-apoB 100 (specific IG)–Sepharose; (3) proteins bound to non-immune IG–Sepharose. Dotted lines indicate the start of the separating gel. (a)–(d) indicate the migration of M_r standards (cf. fig.1).

Since the M_r-value of the synthesized polypeptide is very similar to that of the 75 kDa protein (apoB$^{75\ kDa}$) isolated from LDL by us [10], we investigated the relationship between the two polypeptides in an experiment where the protein-synthesizing mixture was supplemented with 100 μg non-radioactive apoB$^{75\ kDa}$ before adding anti-Lp-B–Sepharose. This resulted in a significant reduction of the amount of radioactive protein specifically bound to anti-Lp-B–Sepharose (fig.3). Thus, it appears that apoB$^{75\ kDa}$ and the protein of M_r 80000 compete for the same antigen-binding sites.

Under the conditions used we have not been able

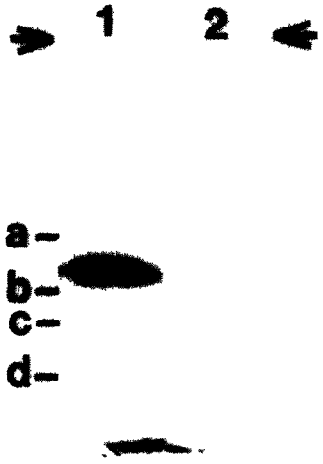

Fig.3. SDS–PAGE (7.5%) [15] of proteins synthesized in the in vitro protein-synthesizing system with human liver RNA. The dried gels were fluorographed 12 h: (1) proteins bound to anti-Lp-B–Sepharose; (2) proteins bound to anti-Lp-B–Sepharose when the in vitro protein-synthesizing has been supplemented with 100 μg non-radioactive apoB$^{75\ kDa}$ before adding the anti-Lp-B–Sepharose. Arrows indicate the start of the separating gel. (a)–(d) Migration of M_r standards (cf. fig.1).

to detect the synthesis of a protein with an M_r corresponding to apoB 100. Obviously, this does not exclude the possibility of its presence.

4. DISCUSSION

The structure of the protein moiety of LDL (apoB) is still unsettled. Several authors have reported that apoB consists of a high-M_r protein (250 kDa or 549 kDa) [1–5]. The possibility that this high-M_r protein is a polymer of polypeptides with a considerably lower M_r has been suggested by other authors [6–8,14]. This hypothesis is compatible with the results presented here showing that an in vitro protein-synthesizing system programmed with RNA from human liver, synthesized a 80 kDa polypeptide that could be precipitated with antibodies against LDL (d = 1.030–1.055) and with antibodies against apoB 100 [4]. Since the synthesized 80 kDa protein is immunologically related to a protein of an estimated 75 kDa present in LDL, our data can also lend support to the finding by several authors that a protein component of 70–80 kDa is present within LDL [7–10].

Based on these data, we suggest that a 80 kDa polypeptide belonging to apoB is synthesized in the human liver. This polypeptide appears to be present as a monomer within LDL. It is also possible that it occurs as one subunit of a high-M_r protein of LDL. The nature of this high-M_r protein and its relationship to the 80 kDa protein synthesized in human liver is under investigation in our laboratory.

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