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SYNTHESIS OF TWO FORMS OF APOLIPOPROTEIN B BY CULTURED RAT HEPATOCYTES

Julia Bell-Quint, Trudy Forte and Paul Graham

Donner Laboratory, Lawrence Berkeley Laboratory
University of California, Berkeley, CA 94720

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SUMMARY

Cultured rat hepatocytes were used to demonstrate that the liver can synthesize two forms of apolipoprotein B. Separation of apolipoprotein B by disc gel electrophoresis indicated that hepatocyte low density lipoprotein contains predominantly apolipoprotein B with an apparent molecular weight of 345,000 ± 5,055. In contrast, the major apolipoprotein B component of hepatocyte very low density lipoprotein is a variant form with a molecular weight of 242,000 ± 2,720. Hepatocyte high density lipoprotein, unlike plasma HDL, also contains apolipoprotein B with an apparent molecular weight of 244,000 ± 2,742. Incorporation of [^{3}H] leucine into hepatocyte apolipoprotein B components suggested de novo synthesis.

The liver and small intestine are the major sites of apolipoprotein B synthesis. Recently, Krishnaiah et al. (1) found a variant of apolipoprotein B with an apparent molecular weight of 240,000 in rat lymph lipoproteins, plasma VLDL of normally fed rats and in VLDL and LDL of rats fed a high-fat, high-cholesterol diet. This variant protein is thought to be of intestinal origin and differs in its immunological properties from the 335,000 molecular weight apolipoprotein B of normal plasma LDL. To understand the mechanisms involved in regulation of the synthesis and metabolism of the variant 240,000 molecular weight protein it is important to determine whether it is produced solely by the intestine or whether it is also synthesized by the liver.

In a recent report (2), we demonstrated the synthesis and secretion of VLDL, LDL and HDL by rat hepatocytes cultured as monolayers. This system provides a unique opportunity to study the synthesis of B proteins by liver parenchymal cells and to determine their distribution in all the major lipoprotein classes derived from the liver. In the present study, the apolipoprotein B content of the hepatocyte lipo-

Abbreviations: VLDL, very low density lipoprotein (d < 1.006 g/ml); LDL, low density lipoprotein (d 1.006-1.063 g/ml); HDL, high density lipoprotein (d 1.063-1.21 g/ml).

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proteins has been examined by electrophoresis on 3.5% sodium dodecyl sulfate polyacrylamide gels.

MATERIALS AND METHODS

Preparation of monolayer cultures of primary rat hepatocytes. Monolayer cultures of hepatocytes were prepared from 200-250 g male WAG/rij rats either fed normally or fasted for 48 h as described previously (2). The cells were incubated at 37°C in humidified air in Leibovitz's L-15 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 8.3 mM glucose, 32 mU/ml insulin, 100 U-0.1 mg/ml penicillin/streptomycin and 10 µg/ml gentamicin sulfate, pH 7.6 (standard medium) containing 20% fetal calf serum (20% w/v). After 24 h, the standard medium containing 20% fetal calf serum was removed and 2.5 ml of fresh serum-free standard medium was added to the rinsed monolayers. Lipoproteins were isolated from the culture medium after 6.5 h incubation. The hepatocytes were 85-90% viable as determined by trypan blue exclusion during the course of study.

Isolation of rat plasma and hepatocyte culture media lipoproteins. Blood from fed and fasted rats was separated by centrifugation at 700 x g at 5°C for 30 min. The culture media were concentrated at 30-40 ml by ultrafiltration under N_2 in an Amicon stirred cell using PM-30 membranes after centrifugation at 700 x g at 5°C to remove cell debris. The concentrated media were then dialyzed extensively at 5°C against a 154 mM NaCl, 0.24 mM EDTA solution containing 3 mM NaN_3. Lipoproteins were isolated sequentially from rat plasma and hepatocyte culture media by preparative centrifugation according to the procedure of Lindgren (3). Very low density lipoprotein ($d < 1.006$ g/ml) was isolated by ultracentrifugation for 18-20 h, LDL (d 1.006-1.063 g/ml) for 24 h and HDL (d 1.063-1.21 g/ml) for 48 h. Culture media VLDL, LDL and HDL were concentrated by recentrifugation at d=1.006, d=1.20 and d=1.217 g/ml, respectively, for an additional 24 h at 40,000 rpm.

Analyses. Apolipoproteins were analyzed by disc-gel-electrophoresis in 3.5% polyacrylamide containing 0.10% sodium dodecyl sulfate according to the method of Weber and Osborn (4). All gels were stained with 0.2% Coomassie blue G-250 and destained in 10% acetic acid. The relative intensities of various bands were estimated by densitometry. Apolipoprotein bands were sliced from stained gels, digested and counted in liquid scintillation solution according to the method of Mahin and Lofberg (5).

RESULTS

Figure 1 shows the separation of two high molecular weight proteins tentatively identified as apolipoprotein B components from rat hepatocyte culture media VLDL, LDL and HDL; the apolipoprotein B content of rat plasma VLDL and LDL are also shown. Hepatocyte VLDL contains two apolipoprotein B forms; band II, the faster migrating band is the major component (83% of the total). This is similar to plasma VLDL where 71% of the total stained apolipoprotein B is band II. Hepatocyte LDL, however, contains predominantly band I, the slower migrating component, which is also the major form in plasma LDL (80% of the total). Hepatocyte HDL consistently contains band II

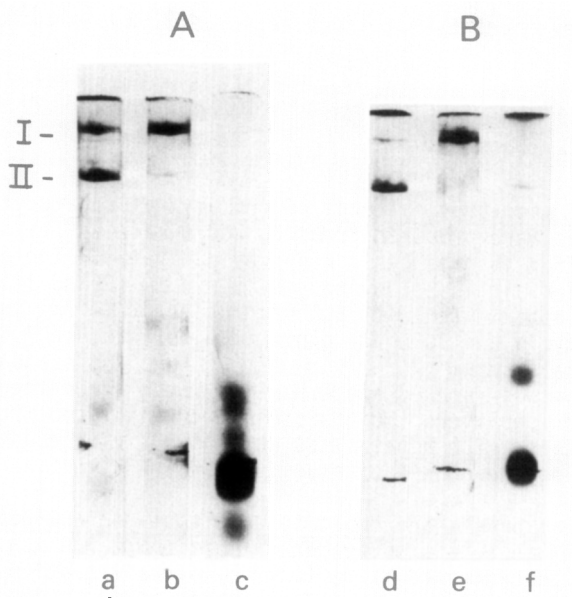

Figure 1. Apolipoprotein B components of (A) plasma and (B) hepatocyte culture (6.5 hr) lipoproteins from fed rats. The B protein components are identified on 3.5% sodium dodecyl sulfate polyacrylamide gels; band I designates the slow migrating component and band II the fast migrating component. Samples in each set are: a, b, c, plasma VLDL, LDL and HDL; d, e, f, hepatocyte culture medium VLDL, LDL and HDL. Fifty to one hundred ug of protein were loaded on each gel.

protein in contrast to plasma HDL which contains no detectable apolipoprotein B. This latter result confirms our earlier finding (2) of high molecular weight (> 100,000) proteins in hepatocyte HDL.

The apparent molecular weights of the apolipoprotein B components in hepatocyte and plasma lipoproteins were determined by electrophoretic calibration in 3.5% sodium dodecyl sulfate polyacrylamide gels using polymers of bovine serum albumin and hemocyanin as standard proteins, according to the procedure described previously by others (1,6). The apparent molecular weights of band I protein in hepatocyte VLDL and LDL are 351,000 ± 5,319 (N=5) and 345,000 ± 5,055 (N=4), respectively, while band II protein is 242,000 ± 2,720 (N=5) for hepatocyte VLDL and 244,000 ± 2,472 (N=6) for hepatocyte HDL. The apparent molecular weights of apolipoprotein B components of plasma lipoproteins were not substantially different; band I protein in VLDL and LDL are 342,000 ± 7,363 (N=4) and 337,000 ± 3,794 (N=3), respectively. Band II protein

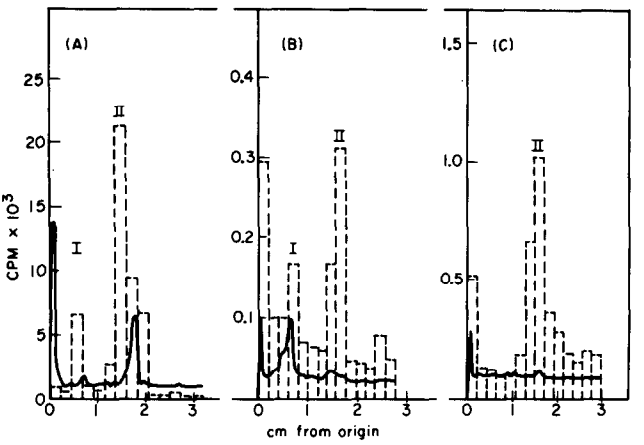

Figure 2. Incorporation of [^{3}H] leucine into hepatocyte apolipoproteins. Labeled lipoproteins isolated from the culture medium after 6.5 h of incubation were separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gels. Protein was stained with Coomassie blue G-250 and scanned with a densitometer (———). Radioactivity in the gel slices is indicated by the bars. Region I identifies the slow migrating apolipoprotein B component while II identifies the fast migrating component.

has an apparent molecular weight of 242,000 ± 5,951 for plasma VLDL and 244,000 ± 5,629 for plasma LDL. Based on these assigned molecular weight values, it appears that hepatocyte lipoproteins examined in this study possess apolipoprotein B components comparable to those described for plasma and lymph lipoproteins of rats subjected to a variety of dietary regimens (1).

To establish de novo synthesis of the apolipoprotein B proteins, hepatocytes were incubated with [^{3}H] leucine and radioactivity in individual slices of stained polyacrylamide gels was assessed after scanning by densitometry. As shown in Figure 2, [^{3}H] leucine is incorporated into both band I and band II proteins.

Figure 3 shows the apolipoprotein B components of hepatocyte and plasma lipoproteins isolated from rats fasted for 48 h. The relative distribution of band I and II protein in hepatocyte lipoproteins from fasted rats was not significantly different from that of fed rats. The band II component predominates in the VLDL and HDL, while hepatocyte LDL contains primarily band I. Both proteins are also present in plasma VLDL and LDL. However, in contrast to fed rats, band I protein now predominates in the VLDL (60% of total apolipoprotein B) while band II is decreased from 71% to

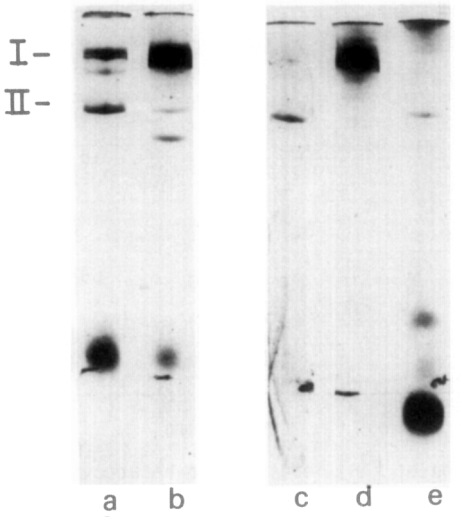

Figure 3. Plasma and hepatocyte culture media (6.5 h) apolipoproteins from 48 h fasted rats determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Samples in each set are: a, b, plasma VLDL and LDL; c, d, e, hepatocyte culture medium VLDL, LDL and HDL. Bands I and II identify the slow and fast migrating components, respectively.

40% of the total. Plasma LDL from fasted rats also contain a decreased amount of band II protein (3% of total apolipoprotein B) compared to fed rats (20%).

DISCUSSION

This study demonstrates for the first time that the rat liver synthesizes two apolipoprotein B components with distinct molecular weights. The evidence is based on the incorporation of [^{3}H] leucine into both forms of hepatocyte apolipoprotein B. The predominance of the ca 243,000 molecular weight protein of apolipoprotein B in hepatocyte VLDL and HDL suggests that the liver, as well as the intestine, is able to synthesize a variant, or non-LDL, form of apolipoprotein B. The lower molecular weight protein was also the major apolipoprotein B component of hepatocyte VLDL from fasted rats even though the total VLDL secreted under these conditions is substantially decreased as reported previously (2). The parallel decrease in hepatocyte VLDL triacylglycerol and apolipoprotein B concentrations suggests that synthesis of the 242,000 molecular weight protein by the liver may be regulated by hepatic triacyl

glycerol synthesis just as synthesis of the lower molecular weight form by the intestine appears to be linked to fat absorption (1).

The presence of the 244,000 molecular weight (band II) apolipoprotein B in hepatocyte HDL may be due to the significantly higher triacylglycerol content of this fraction compared to plasma HDL (2). High molecular weight (> 100,000) proteins and high triacylglycerol concentrations have also been found in HDL isolated from rat liver perfusates (7,8). The variant (band II) protein in hepatocyte HDL may be part of a separate apolipoprotein B-containing particle similar to the one isolated from liver perfusate HDL by Fainaru et al. (9).

The finding of apparently two distinct B apolipoproteins as major components of hepatocyte VLDL and LDL is consistent with the differences in estimated specific activities of the B protein in these particles reported earlier (2), and suggests that the LDL may be synthesized directly by the hepatocytes from a separate hepatic pool of apolipoprotein B.

Further studies will be required to determine the relationship, if any, between the variant apolipoprotein B apparently synthesized by rat liver and that reported by Krishnaiah et al. (1) to be synthesized by rat intestine.

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