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Carboxyl-terminal truncation of apolipoproteinB-100 inhibits lipoprotein(a) particle formation

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Abstract

Recombinant expression systems for both apo(a) and apoB were used to identify sequences in apoB which are required for Lp(a) formation. Incubation of a [^{35}S]Cys-labelled 17-kringle form of apo(a) with supernatants from rat hepatoma (McA-RH7777) cells expressing apoB-88, apoB-94 and apoB-100 resulted in covalent r-Lp(a) formation only with apoB-100. Additionally, apoB-86 present in the LDL of a hypobetalipoproteinemic subject did not associate with a 12-kringle form of recombinant apo(a) to form r-Lp(a) complexes. Our data suggest that sequences within the C-terminal 6% of apoB-100 are essential for Lp(a) assembly.

Key words: Lipoprotein(a); Apolipoprotein(a); ApolipoproteinB-100; Assembly

1. Introduction

Numerous studies have identified lipoprotein(a) (Lp(a)) as an independent risk factor for the development of coronary heart disease [1]. Marked inherited variability has been observed with respect to plasma Lp(a) levels, which vary over 1000-fold in the population. Roughly 25% of the human population possesses Lp(a) levels above 20 mg/dl, which more than doubles their risk of developing coronary heart disease. Lp(a) closely resembles a low density lipoprotein (LDL) particle with respect to lipid composition and the presence of a single molecule of apolipoprotein B-100 (apoB-100). However, Lp(a) is distinguishable from LDL by the presence of the protein moiety apolipoprotein(a) (apo(a)), which is covalently attached to apoB-100. Human apo(a) consists of multiple tandem repeats of a sequence that closely resembles plasminogen kringle IV, followed by sequences exhibiting a high degree of sequence similarity to the kringle V and protease regions of plasminogen [2]. Apo(a) is covalently linked to apoB-100 by a single disulfide bond, involving Cys^{4057} in the penultimate kringle IV repeat of apo(a) [3,4]. Fluorescence labelling studies [5,6] in addition to predictions based on computer modeling [6] suggest that Cys^{3734} in apoB-100 participates in disulfide bond formation with apo(a). In addition to covalent bond formation, there is evidence that strong hydrophobic interactions between apo(a) and apoB-100 may also contribute to the formation of Lp(a) particles in vivo [7].

It has recently been demonstrated that Lp(a) particle assembly occurs extracellularly in human plasma [3]. Assembly of Lp(a) in plasma has also been observed upon infusion of human LDL into transgenic mice expressing human apo(a) [8]. Extracellular assembly of Lp(a) also appears to occur in baboons based on studies in which Lp(a) could not be detected intracellularly in cultured baboon hepatocytes, but could be detected in culture supernatants [9]. However, the structural feature(s) which underlie the specific interaction of apo(a) and apoB-100 to form Lp(a) particles remains to be determined.

Using an in vitro Lp(a) association assay, we now report that sequences in the carboxyl-terminal region, independent of Cys^{3734}, are required for Lp(a) particle assembly.

2. Experimental

2.1. Construction and expression of the 12-kringle form of apo(a)

An apo(a) construct containing 12 kringle IV repeats was derived from partial *Hha*I digestion of the 17-kringle recombinant apo(a) (r-apo(a)) construct pRK5ha17 [10]. A 385 bp *Eco*RI–*Hha*I fragment, containing 291 bp of the first kringle IV repeat of apo(a), was fused to a 4828 bp *Hha*I–*Eco*RI fragment containing 51 bp of the 26th kringle repeat, followed by kringles 27–37 and the kringle V and protease domains [2]. The DNA fragments were ligated into pRK5ha17 digested with *Eco*RI, and the resultant construct (pRK5ha12) was verified by DNA sequence analysis. This construct was used to stably transfect 293 (human embryonic kidney [11]) cells as previously detailed for the 293/apo(a).24 cell line [10]. Briefly, cells were cultured in 100-mm dishes using minimal essential medium (MEM) (Gibco/BRL) supplemented with 10% fetal calf serum (FCS) (ICN). Cells were transfected by the calcium-phosphate co-precipitation method [12] using 10 μg of pRK5ha12 expression plasmid and 1 μg of a plasmid containing the

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Abbreviations: Lp(a), lipoprotein(a); apoB-100, apolipoproteinB-100; LDL, low density lipoprotein; r-apo(a), recombinant apo(a); r-Lp(a), recombinant lipoprotein(a).

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neomycin gene (pRSVneo [13]) per 100 mm dish. The selective agent G418 (800 μg/ml of medium) (Gibco/BRL) was added to the plates 48 h post-transfection. Individual G418-resistant foci were subsequently transferred to 24-well dishes and positive clones were identified by immunoperoxidase staining using an affinity purified anti-apo(a) polyclonal antibody raised in sheep. The isolate containing the greatest number of immunoreactive cells was subsequently cloned by limiting dilution; the resultant stable line was designated 293/apo(a).12.

Stably transfected 293 cells expressing the 17-kringle r-apo(a) (293/apo(a).24) or the 12-kringle form of r-apo(a) (293/apo(a).12; see above) were routinely cultured in MEM supplemented with 10% FCS and G418 (400 μg/ml of media). The generation and characterization of McA-RH7777 cells stably transfected with plasmids expressing human apoB-100 as well as carboxyl-terminal truncations of apoB (apoB-88 and apoB-94) has been previously described [14]. ApoB-88 contains the amino-terminal 4,002 amino acids of apoB-100 whereas apoB-94 contains the amino-terminal 4,270 amino acids of apoB-100. These cell lines were routinely cultured in MEM supplemented with 20% FCS and 400 μg/ml G418.

2.2. Analysis of recombinant apoB (r-apoB) expression levels

Wild-type McA-RH7777 cells (ATCC# CRL 1601) or those stably expressing C-terminal apoB variants were grown to confluence and incubated for 24 h in serum-free MEM. Cell culture supernatants were harvested, clarified by brief centrifugation at 1815×*g* and concentrated in Centricon microconcentrators (100 kDa size exclusion; Amicon). Concentrated media or purified LDL (see below) was added to Laemmli sample buffer, boiled for 5 min, and subjected to SDS-PAGE using a 5% gel [15]. The gel was blotted onto nitrocellulose (Bio-Rad) overnight at 40 V in 25 mM Tris-HCl, 192 mM glycine containing 20% methanol. Immunoblots were incubated in blocking buffer (1×NET (1.5 M NaCl, 50 mM EDTA, 0.5 M Tris-HCl, 0.5% Triton X-100) containing 3% BSA), followed by incubation with the apoB-specific monoclonal antibody IDI [16] in 1×NET buffer (1D1 was a gift from Drs. R.W. Milne and Y.L. Marcel, Lipoprotein and Atherosclerosis Group, University of Ottawa Heart Institute, Ottawa). Specific immunoreactive complexes were detected using enhanced chemiluminescence as detailed by the manufacturer (Amersham).

2.3. Metabolic labelling of cells

293/apo(a).24 or 293/apo(a).12 cell lines were incubated for 45 min in Met/Cys-depleted MEM (Gibco/BRL), supplemented with 2 mM glutamine, in the absence of FCS. [^{35}S]Cys (ICN) was then added (5 μCi/ml of media) and cell supernatants were harvested 3.5 h post-labelling. Supernatants were clarified by brief centrifugation at 1815 × *g* for use in in vitro association studies (see below).

2.4. Assay for recombinant Lp(a) particle formation

Concentrated supernatants from either wild-type McA-RH7777 cells or those expressing various apoB species were incubated with [^{35}S]Cys-labelled media harvested from the 293/apo(a).24 cell line at 37°C for 1 h. Control samples consisted of either an equal volume of PBS or purified human LDL (see below) equivalent to the amount of r-apoB-100 detected by Western blot analysis. At the end of the incubation period, samples were immunoprecipitated overnight at 4°C with 10–20 μg of a monoclonal antibody directed against apo(a) [17]. Samples were then incubated with protein A–Sepharose (Pharmacia LKB Biotechnology Inc.) for 1 h, after which the Sepharose was pelleted by brief centrifugation at 16,000 × *g*, washed with RIPA buffer [10] and resuspended in Laemmli sample buffer; dithiothreitol (DTT) was added to a final concentration of 10 mM in order to reduce samples where required. Samples were boiled for 5 min and proteins were resolved by SDS-PAGE using either a 5% gel or a 2.5–15% gradient gel. Gels were then treated with Enlightning (Dupont) for 15 min, dried under vacuum and exposed to film at −70°C with intensifying screens.

Human plasma from either a normolipidemic control individual or a human subject with hypobetalipoproteinemia (H.J.B. [18]; reviewed in [19]) was subjected to ultracentrifugation at 436,000 × *g* for 2 h at 15°C. The top one-tenth representing the $d < 1.006$ g/ml fraction was discarded and the density of the infranatant was adjusted to 1.063 g/ml with NaBr and centrifuged using the conditions described above. The top one-tenth of each tube representing the LDL fraction was harvested and the protein levels determined using a Bradford assay (Bio-Rad). Purified LDL isolated from a control individual (0.3 μg) or from H.J.B. (3 μg) was incubated with [^{35}S]Cys-labelled media (950 μl) harvested from the 293/apo(a).12 cell line for 2 h at 37°C. Samples were immunoprecipitated at 4°C overnight with 20 μg of a polyclonal antibody raised against human LDL (Boehringer-Mannheim) and analyzed by SDS-PAGE and fluorography as described above.

3. Results and discussion

Using an in vitro association assay [3], we have studied the effect of carboxyl-terminal deletions of apoB on the ability of apoB to associate with recombinant apo(a) (r-apo(a); [10]) to form Lp(a) particles (r-Lp(a)). We initially assessed the ability of full-length recombinant apoB-100 (r-apoB-100) to form covalent r-Lp(a) complexes with r-apo(a). In this experiment, equivalent amounts of either purified human LDL or r-apoB-100 (present in the cell culture supernatants harvested from the McA-RH7777 B-100 stable line) were incubated with [^{35}S]Cys-labelled r-apo(a); mixtures were immunoprecipitated using an apo(a)-specific monoclonal antibody, and analyzed by SDS-PAGE. The resulting fluorogram is shown in Fig. 1A. Similar amounts of a disulfide-linked species corresponding to r-Lp(a) (which disappears upon sample reduction) was observed when either purified LDL or conditioned media from the McA-RH7777 B-100 cell line were used in the assay. Furthermore, the formation of r-Lp(a) increased (relative to free apo(a)) upon addition of increased amounts of r-apoB-100 (Fig. 1B), as has been previously demonstrated for LDL [3]. The r-Lp(a) formed was specific for human r-apoB-100 as no r-Lp(a) was observed in control samples containing conditioned media from wild-type McA-RH7777 cells, although these cells express rat apoB-48 and apoB-100 [20]. A non-specific band is, however, frequently observed under non-reducing conditions (Fig. 1A); this band corresponds to a dimerized form of r-apo(a), which cannot be immunoprecipitated with antibodies directed against LDL. The results shown in Fig. 1A and B demonstrate that the r-apoB expression system could be utilized to analyze the effect of carboxyl-terminal truncations of apoB-100 on r-Lp(a) formation, and that the interpretation of these studies would not be affected by the presence of rat apoB. It is noteworthy that the absence of covalent association of rat apoB-100 with r-apo(a) is consistent with previous data obtained from a study of transgenic mice expressing human apo(a); in that study, covalent association of apo(a) with murine LDL was not observed [8]. Taken together with our data, these observations indicate that rat and murine apoB-100 have distinct structural features which preclude covalent association with human apo(a).

We have also used the McA-RH7777 apoB expression system [14] to examine the effect of carboxyl-terminal truncation of human apoB on r-Lp(a) formation. Conditioned media collected from wild-type McA-RH7777 cells or those cells stably expressing human apoB-88,

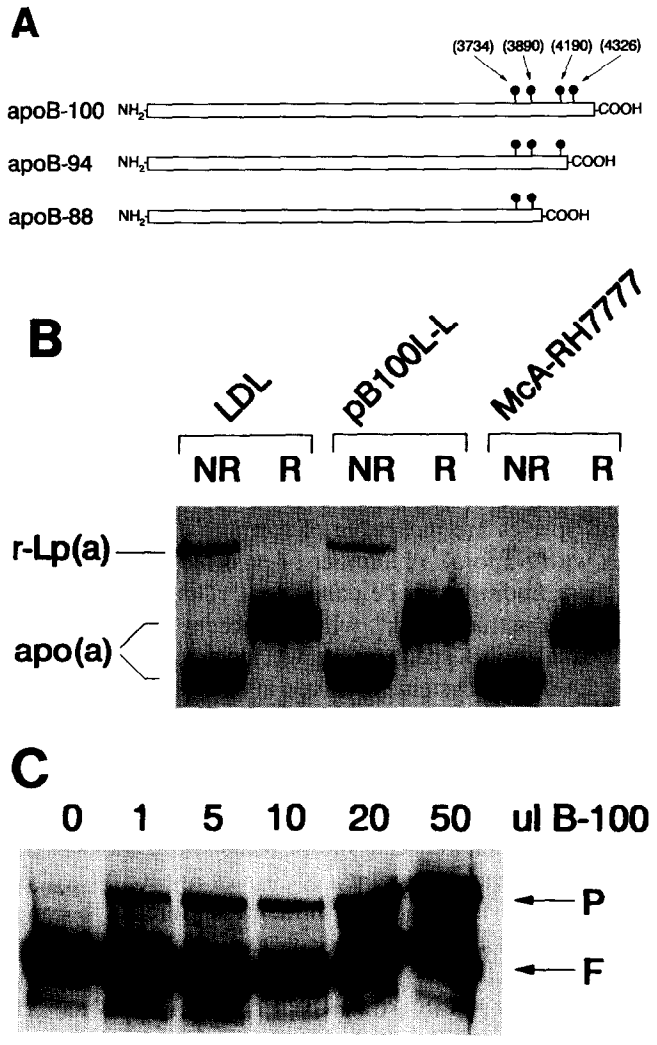

Fig. 1. In vitro formation of r-Lp(a) employing r-apoB-100 and human LDL. (A) The organization of the recombinant proteins corresponding to apoB-100, apoB-94 and apoB-88 are shown. Positions and amino acid numbers of the last four cysteines in apoB-100 are indicated; Cys^{3734} and Cys^{4190} are exposed on the LDL surface as demonstrated by fluorescent labelling studies [6]. (B) Purified human LDL (1.5 μg) or cell culture supernatants (50 μl) from either wild-type McA-RH7777 cells or the stable cell line McA-RH7777 B-100 were incubated with [^{35}S]Cys-labelled media (950 μl) harvested from the 293/apo(a).24 cell line for 1 h at 37°C. Samples were imunoprecipitated with an apo(a)-specific monoclonal antibody and analyzed by SDS-PAGE using a 5% gel. The gel was treated with Enlightning, dried and exposed to film. The positions of free r-apo(a) and the r-Lp(a) complex are indicated. Non-reduced (NR) samples were resuspended in Laemmli sample buffer while reduced samples (R) were resuspended in the same volume of sample buffer containing 10 mM DTT; samples were boiled for 5 min prior to loading. (C) [^{35}S]Cys-labelled media (950 μl) harvested from the 293/apo(a).24 cell line was incubated with increasing volumes of media harvested from the McA-RH7777 B-100 cell line for 1 h at 37°C. Samples were treated as detailed above with the exception that they were resolved on a 2.5–15% gradient gel. The positions of free r-apo(a) (F) and the r-Lp(a) particle (P) are indicated to the right of the gel.

apoB-94, or apoB-100 (Fig. 2A) was analyzed for the production of truncated apoBs by Western blot analysis (Fig. 2B); similar amounts of all of the recombinant apoB species were detected. For in vitro association studies (Fig. 2C), samples of the supernatants (equivalent to ~1.5 μg of each truncated apoB, and ~10 times the amount of apoB used in the Western blot experiment shown in Fig. 2B) was added to [^{35}S]Cys-labelled supernatants from the 293/apo(a).24 cell line. Although both LDL and r-apoB-100 formed significant amounts of covalent r-Lp(a) complexes, no r-Lp(a) was observed when r-apo(a) was incubated with conditioned media from the McA-RH7777 B-94 or McA-RH7777 B-88 cell lines. Reduced levels of expression of r-apoB-88 or r-apoB-94 could not account for this result as virtually identical amounts of the recombinant apoB species and purified LDL were used in these assays, based on the data presented in Fig. 2B. It could be suggested that the presence of r-apoB-48 observed in the different apoB cell lines may have inhibited r-Lp(a) formation. However, this does not appear to be the case since similar amounts of r-Lp(a) were observed using both the purified LDL and r-apoB-100, despite the considerable amount of apoB-48 present in the supernatants from the McA-RH7777 cells (Fig. 2B). Furthermore, less apoB-48 was observed in the conditioned media isolated from the McA-RH7777 B-94 cell line compared to the McA-RH7777-B-100 cell line (Fig. 2B). Thus, neither the expression levels of the carboxyl-terminal truncated apoB species, nor the variable presence of apoB-48, could explain the absence of r-Lp(a) formation.

We have previously demonstrated that r-Lp(a) formation is a time-dependent reaction that is virtually complete within 90 min [3]. It is conceivable that carboxyl-terminal truncations of apoB could alter the kinetics of r-Lp(a) formation. To address this possibility, either purified LDL, or conditioned media from the McA-RH7777 B-94 and McA-RH7777 B-100 cell lines, were incubated with [^{35}S]Cys-labelled r-apo(a) for 4 h. Similar amounts of r-Lp(a) could be observed with LDL and r-apoB-100, but no r-Lp(a) was observed with r-apoB-94; over-exposure of this gel still did not allow detection of any r-Lp(a) containing r-apoB-94 (data not shown).

To verify the data obtained using the apoB expression system, we analyzed r-Lp(a) formation using LDL purified from the plasma of a hypobetalipoproteinemic subject, H.J.B. [18]. H.J.B. is a compound heterozygote for hypobetalipoproteinemia, and his plasma lipoproteins contain apoB-100, apoB-86 (containing the same cysteine residues as apoB-88; see Fig. 2A), apoB-48, and apoB-37 ([19] and references therein). We incubated LDL purified from a control individual and from H.J.B. with [^{35}S]Cys-labelled media harvested from the 293/apo(a).12 cell line, and immunoprecipitated the Lp(a) complexes with a polyclonal antibody specific for human LDL; the results are shown in Fig. 3. r-Lp(a) was observed when purified LDL from either individual was used; however, the r-Lp(a) corresponded in size to that consisting of only the full-length apoB-100. The 293/

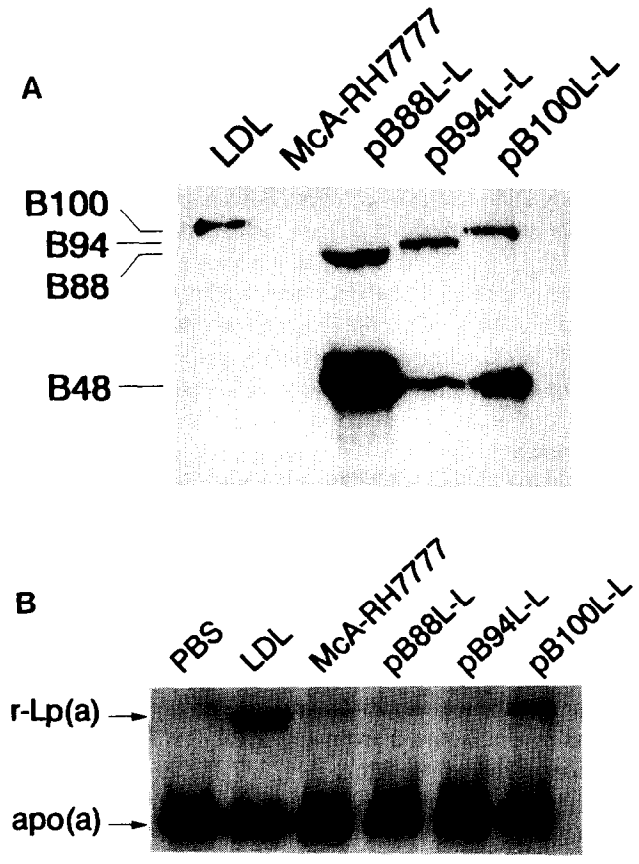

Fig. 2. C-Terminal truncation of apoB-100 eliminates r-Lp(a) formation. (A) Expression levels of cell culture supernatants from McA-RH7777 cells stably transfected with apoB-100, apoB-94 and apoB-88 [14] were analyzed by SDS-PAGE (5% gel) followed by Western blotting. The blot was probed with the 1D1 monoclonal antibody specific for the amino terminal region of apoB-100. The apoB plasmids used to generate the stable rat hepatoma cell lines are indicated above each lane. Conditioned media from wild-type McA-RH7777 cells as well as purified human LDL (150 ng) were included as controls. The positions of the truncated recombinant apoB proteins are indicated to the left of the blot. (B) [^{35}S]Cys-labelled media (1 ml) from the 293/apo(a).24 cell line was incubated for 1 h at 37°C with purified LDL (1.5 μg) or with supernatants harvested from the apoB-expressing cell lines corresponding to 10 times the volume used for the Western blot analysis in A. Samples were immunoprecipitated with an anti-apo(a) monoclonal antibody and resolved by SDS-PAGE using a 5% gel. The gel was treated with Enlightning, dried under vacuum and exposed to film. The positions of free r-apo(a) and r-Lp(a) complexes are indicated to the left of the fluorogram; the apoB plasmids used to generate the cell lines are indicated above each lane.

apo.12 cell line was utilized for this study since its smaller size would easily allow for the discrimination of r-Lp(a) particles containing either apoB-100 or r-Lp(a) particles containing apoB-86. The results of this experiment support those obtained using the recombinant apoB expression system, and confirm that carboxyl-terminal truncations of apoB render it incapable of forming a covalent complex with apo(a). These results are also in agreement with earlier studies in which carboxyl-terminal apoB truncations could not be detected in the Lp(a) from hypobetalipoproteinemic subjects [19]. However, in those studies, alternative explanations such as accelerated clearance of Lp(a) particles containing truncated apoB species could not be ruled out [19]. Our studies, which used in vitro association methodologies, are not subject to these criticisms and strongly indicate that carboxyl-terminal truncation of apoB is not compatible with r-Lp(a) formation.

The results that we have obtained in this study, indicating that apoB species as long as apoB-94 cannot form r-Lp(a), are unexpected since previous work has implicated the involvement of apoB Cys^{3734} [5,6] in disulfide bridge formation with apo(a) Cys^{4057} [3,4]. Cys^{3734} of apoB is present in both r-apoB-88 and r-apoB-94, as well as in apoB-86 from H.J.B.'s LDL, yet these truncated apoB species are incapable of forming a covalent complex with r-apo(a). A number of possible interpretations of these findings can be considered. The simplest interpretation is that Cys^{4326}, which is absent from all of the truncated apoB species that we have investigated (see Fig. 2A), is the cysteine involved in disulfide bond formation with Cys^{4057} in apo(a). However, the work of Coleman et al. [5] and Guevara et al. [6] seems to preclude this possibility since Cys^{4326} appears to be inaccessible for association with apo(a), although the evidence presented to date has been indirect. Site-directed mutagenesis of the carboxyl-terminal cysteine residues of apoB-100 will be necessary for the unequivocal identification of the cysteine residue(s) that is involved in the covalent linkage to apo(a).

It remains possible that Cys^{3734} within apoB partici-

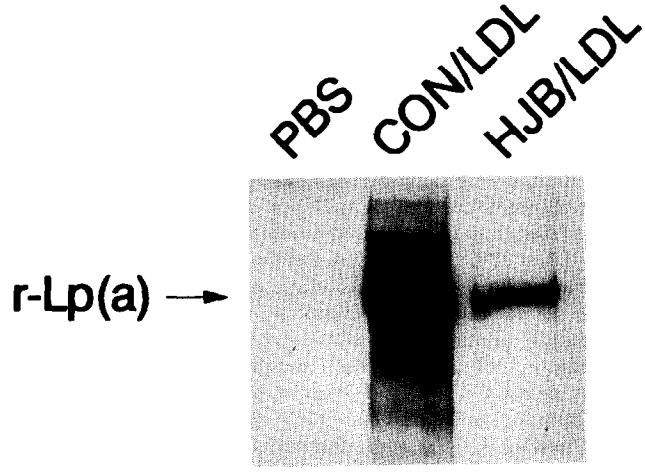

Fig. 3. r-Lp(a) formation employing LDL from a subject with hypobetalipoproteinemia (H.J.B.). [^{35}S]Cys-labelled media (950 μl) from cells stably transfected with pRK5ha12 were incubated for 2 h at 37°C with LDL isolated from a control individual (CON/LDL) or 3 μg of LDL isolated from an individual expressing apoB-100, apoB-86, apoB-48 and apoB-37 (HJB/LDL). Samples were immunoprecipitated with a polyclonal antibody against human LDL and the products were analyzed by SDS-PAGE (5% gel) and fluorography as previously described. The position of r-Lp(a), corresponding to a particle containing apoB-100, is indicated.

pates in covalent linkage with apo(a), but that other factors prevent truncated apoBs containing Cys^{3734} from forming Lp(a) complexes. Carboxyl-terminal truncation of apoB-100 impairs lipid recruitment by apoB [14]. This reduction in lipid associated with the carboxyl-terminal truncated apoB species, such as apoB-94, may affect their ability to associate with apo(a). Indeed, immunochemical studies with monoclonal antibodies have demonstrated that alterations in core lipid content can affect the surface conformation of apoB ([21] and references therein). Moreover, it is possible that deletion of the carboxyl-terminal 6% of apoB results in the removal of sequences which are necessary for mediating the association of apoB and apo(a). Interestingly, no non-covalent association of apo(a) with either apoB-86, apoB-88 or apoB-94 was observed (data not shown). Therefore, sequences within the C-terminal 6% of apoB may be required for the initial non-covalent association of apoB with apo(a) but would not preclude the involvement of a more remote cysteine residue such as Cys^{3734} in disulfide bond formation.

This study represents the first report that sequences present within the carboxyl-terminal 6% of apoB-100 are required for its covalent association with apo(a) to form Lp(a) particles. This region may contain sequences required for covalent or non-covalent interactions with apo(a), or perhaps both. Utilization of the recombinant expression systems for both apo(a) and apoB will be useful in further characterizing the nature of these sequence requirements. This knowledge will be essential to our understanding of the mechanism of Lp(a) particle assembly.

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References

[1] Scanu, A.M. (1992) J. Internal Med. 231, 679–683.
[2] McLean, J.W., Tomlinson, J.E., Kuang, W., Eaton, D.L., Chen, E., Fless, G., Scanu, A. and Lawn, R.M. (1987) Nature 330, 132–137.
[3] Koschinsky, M.L., Côté, G.P., Gabel, B. and van der Hoek, Y.Y. (1993) J. Biol. Chem. 268, 19819–19825.
[4] Brunner, C., Kraft, H.-G., Utermann, G. and Muller, H.-J. (1993) Proc. Natl. Acad. Sci. USA 90, 11643–11647.
[5] Coleman, R.D., Kim, T.W., Gotto Jr., A.M. and Yang, C.-Y. (1990) Biochim. Biophys. Acta 1037, 129–132.
[6] Guevara, J., Jr., Spurlino, J., Jan, A.Y., Yang, C.-Y., Tulinsky, A., Venkataram Prasad, B.V., Gaubatz, J.W. and Morrisett, J.D. (1993) Biophys. J. 64, 686–700.
[7] Trieu, V.N. and McConathy, W.J. (1990) Biochemistry 29, 5919–5924.
[8] Chiesa, G., Hobbs, H.H., Koschinsky, M.L., Lawn, R.M., Maika, S.D. and Hammer, R.E. (1992) J. Biol. Chem. 267, 24369–24374.
[9] White, A.L., Rainwater, D.L. and Lanford, R.E. (1993) J. Lipid Res. 34, 509–517.
[10] Koschinsky, M.L., Tomlinson, J.E., Zioncheck, T.J., Schwartz, K., Eaton, D.L. and Lawn, R.M. (1991) Biochemistry 30, 5044–5051.
[11] Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1977) J. Gen. Virol. 36, 59–77.
[12] Graham, F.L. and van der Eb, A.J. (1973) Virology 52, 456–467.
[13] Gorman, C., Padmanahban, R. and Howard, B. (1983) Science 221, 551–553.
[14] McLeod, R.S., Zhao, Y., Selby, S.L., Westerlund, J. and Yao, Z. (1994) J. Biol. Chem. 269, 2852–2862.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Pease, R.J., Milne, R.W., Jessup, W.K., Law, A., Provost, P., Fruchart, J.-C., Dean, R.T., Marcel, Y.L. and Scott, J. (1990) J. Biol. Chem. 265, 553–568.
[17] Wong, W.L.T., Eaton, D.L., Berloui, A., Fendly, B. and Hass, P.E. (1990) Clin. Chem. 36, 192–197.
[18] Steinberg, D., Grundy, S.M., Mok, H.Y.I., Turner, J.D., Weinstein, D.B., Brown, W.V. and Albers, J.J. (1979) J. Clin. Invest. 64, 292–301.
[19] Linton, M.F., Farese Jr., R.V. and Young, S.G. (1993) J. Lipid Res. 34, 521–541.
[20] Tanabe, S., Sherman, H., Smith, L., Yang, L.-A., Fleming, R. and Hay, R. (1989) In Vitro 25, 1129–1140.
[21] Chan, L. (1992) J. Biol. Chem. 267, 25621–25624.