Biochem. J. (1991) 278, 677–682 (Printed in Great Britain)

Deletion of two growth-factor repeats from the low-density-lipoprotein receptor accelerates its degradation

Deneys R. VAN DER WESTHUYZEN,* Michael L. STEIN,* Howard E. HENDERSON,† A. David MARAIS,* Anne M. FOURIE* and Gerhard A. COETZEE*

*UCT/MRC Unit for Cell Biology of Atherosclerosis, Department of Medical Biochemistry, University of Cape Town Medical School, Observatory, Cape Town 7925, and †Department of Chemical Pathology, Red Cross Children's Hospital, Rondebosch, Cape Town 7700, South Africa

The region of the low-density-lipoprotein (LDL) receptor showing sequence similarity to the epidermal-growth-factor (EGF) precursor is required for LDL binding and the acid-induced dissociation of ligand and receptor. We describe here a naturally occurring mutant LDL receptor, found in a patient with homozygous familial hypercholesterolaemia, which lacks the first two growth-factor-like repeats of the EGF-precursor-like ('homology') domain. The mutation in the receptor gene is a 2.5 kb deletion including exons 7 and 8. The molecular mass of the mutant receptor (145 kDa) was approx. 15 kDa smaller than the normal LDL receptor. The mutant receptors were derived from precursors (105 kDa) that apparently underwent normal processing. Fibroblasts from the patient had high-affinity binding sites for the the apolipoprotein E-containing ligand, βVLDL, but did not bind LDL. In the presence of βVLDL, receptors were rapidly degraded. The mutant receptors also displayed an abnormally rapid turnover, about four times faster than that of normal receptors, in the absence of ligand; this accelerated degradation accounted for the low level of expression of mutant receptors in up-regulated cells. These data support a role for the growth-factor-like repeats in the binding of LDL (but not βVLDL) and in receptor recycling, and indicate that a normal rate of turnover of unoccupied receptors is dependent on the integrity of these segments of the protein.

INTRODUCTION

The low-density-lipoprotein (LDL) receptor mediates the endocytosis of LDL and certain other lipoproteins by the LDL-receptor pathway [1]. Mutations in the LDL receptor cause the relatively common autosomal dominant disease familial hypercholesterolaemia (FH). Analysis of naturally occurring and artificial mutations has yielded considerable insight into the different functional domains of the molecule [1–3].

The LDL receptor is synthesized in the endoplasmic reticulum as a 120 kDa (apparent molecular mass) precursor that is processed by further glycosylation to a 160 kDa glycoprotein during its transport through the Golgi apparatus. The *N*-terminal domain of the receptor, corresponding to exons 2–6 of the LDL-receptor gene, consists of seven closely related cysteine-rich repeats and is required for the binding of two different apolipoproteins, apolipoprotein B-100 (apoB-100) and apolipoprotein E (apoE) [2]. Alterations in this region can disrupt ligand binding [2,3], as well as the intracellular transport and processing of the receptor precursor [4,5]. Exons 7–14 of the receptor code for another region that shows similarity to part of the EGF precursor. This domain includes three cysteine-rich, growth-factor-like repeats, repeats A, B and C [6,7]. Studies of transfected receptor constructs in CHO (Chinese-hamster ovary) cells revealed that deletion of this domain prevents the acid-dependent dissociation of ligand from the receptor and consequently receptor recycling [8]. In addition, this domain, together with the *N*-terminal 'binding' domain, is required for the binding of apoB-100, the sole polypeptide of LDL, although not for the binding of apoE [2,8]. The domain's role in apoB-100 binding may be to act as a spacer region that extends the receptor-binding domain away from the cell surface, thereby allowing the interaction of the binding domain with the large LDL particles each containing only a single apoB-100 molecule [2].

We have recently detected a 2.5 kb deletion mutation (FH Cape Town-2) in the LDL-receptor gene of some South African FH subjects [9]. This mutation is distinct from two main founder-gene defects that have been identified in the Afrikaner population [10,11]. Here we provide sequence data showing that the mutation results in the deletion of two of the growth-factor-like repeats from the LDL receptor. We further show that deletion of these two repeats abolishes LDL binding, but not the binding of the apoE-containing ligand, βVLDL. In the absence of ligand, the mutant receptors have an abnormally rapid turnover rate, which is further accelerated in the presence of an apoE-containing ligand. This rapid turnover accounts for the low level of mutant LDL receptors in cells homozygous for the mutation, and indicates that normal turnover of LDL receptors is dependent on the integrity of the EGF-precursor-like ('homology') domain.

EXPERIMENTAL

Patient RI

Patient RI was a 17-year-old female diagnosed as having homozygous FH. Her plasma levels of total cholesterol, LDL cholesterol, triacylglycerol and HDL cholesterol were 16.1, 14.4, 0.47 and 1.02 mmol/litre respectively. She had tendenous and severe cutaneous xanthomas, no evidence of coronary heart disease and had a family history of hypercholesterolaemia [9].

Materials

Human LDL (density 1.019–1.063 g/ml) and lipoprotein-deficient serum (LPDS, density > 1.25 g/ml) were prepared as

Abbreviations used: LDL, low-density lipoprotein; βVLDL, β-migrating very-low-density lipoprotein; LPDS, lipoprotein-deficient serum; apoB-100, apolipoprotein B-100; apoE, apolipoprotein E; EGF, epidermal growth factor; FH, familial hypercholesterolaemia; *Taq*, *Thermus aquaticus*; (D)MEM, (Dulbecco's) modified Eagle's medium.

described [12]. βVLDL (density < 1.006 g/ml) was prepared from rabbits that had been maintained for at least 14 days on laboratory chow supplemented with 2% (w/w) cholesterol and 10% (w/w) corn oil [13]. LDL and βVLDL were iodinated with ^{125}I by the ICl method [12]. Tran^{35}S-label (70% [^{35}S]methionine) was obtained from ICN Radiochemicals. IgG-C7, a mouse monoclonal antibody that recognizes an epitope in the first cysteine-rich repeat of the binding domain of the human LDL receptor [14], was prepared and radiolabelled with ^{125}I as described [15]. The human LDL-receptor cDNA probe pLDLR-3 was kindly provided by Dr. D. W. Russell, University of Texas Southwestern Medical Center at Dallas, TX, U.S.A. Oligonucleotides, synthesized on an Applied Biosystems (Foster City, CA) DNA synthesizer, were generously given by Dr. M. C. D. Boyd (Rutgers Medical School). [^{32}P]ATP was purchased from ICN. *Thermus aquaticus* (*Taq*) DNA polymerase and Msp 1 fragments of pBR322 DNA were from New England Biolabs and reverse transcriptase from Seikagaku (St. Petersburg, FL, U.S.A.).

DNA analysis

Genomic DNA isolation from leukocytes and Southern blotting were carried out by standard procedures, as previously described [9].

cDNA amplification and sequencing

RNA was isolated from cultured fibroblasts that had been grown in LPDS-containing medium for 24 h to induce LDL-receptor mRNA synthesis. Cells from twelve 100 mm-diam. dishes were harvested for total RNA purification by the guanidinium thiocyanate/CsCl centrifugation procedure [16]. Total RNA (5 μg) was reverse-transcribed (1.5 h at 42 °C) using reverse transcriptase (20 units) and a synthetic oligonucleotide primer (G2, 1 μM) complementary to a portion of exon 10 of the LDL receptor [17]. One-fifth of the resulting cDNA was amplified by the PCR with *Taq* polymerase and the oligonucleotide primers G1 (exon 6) and G2 (exon 10; see above) for 34 cycles after the method of Saiki *et al.* [18]. Amplification of cDNA was performed in 50 μl reaction mixtures containing 10 mM-Tris, pH 8.4, 50 mM-KCl, 2.5 mM-$MgCl_2$, primers G1 and G2 at 1 μM each, deoxyribonucleotides dATP, dCTP, dTTP and dGTP at 200 μM each, BSA at 200 μg/ml and 2.5 units of *Taq* polymerase. Each cycle included a denaturation step at 93 °C for 1 min, followed by a single annealing/primer-extension step at 68 °C for 5.5 min as previously described [5]. Amplification products were size-fractionated on a 6%-(w/v)-polyacrylamide gel and revealed by ethidium bromide staining. For sequencing, the amplified band was excised from the gel, extracted and re-amplified. Re-amplification and isolation of the amplified DNA was carried out as described above, except that the G1 primer was end-labelled [19]. The amplified DNA was subjected to DNA sequence analysis using the technique of Maxam & Gilbert [20].

LDL-receptor assays

Fibroblasts were obtained from skin biopsy specimens and grown in monolayer culture [21]. Cells were seeded at 3×10^4/60 mm-diameter Petri dish and grown for 5 days in (Dulbecco's) modified Eagle medium [(D)MEM] containing 10% (v/v) fetal-calf serum with a change of medium on day 3. Cells were then maximally up-regulated for LDL receptor content by a further 2-day incubation in LPDS medium (2.5 mg of protein/ml). The binding, internalization and degradation of ^{125}I-LDL at 37 °C and the binding of ^{125}I-LDL at 4 °C were assessed as previously described [22]. Cell-surface binding at 4 °C of ^{125}I-βVLDL [8] and ^{125}I-IgG-C7 [21] were also determined.

Immunochemical analysis of [^{35}S]methionine-labelled LDL receptors

Fibroblasts were incubated with [^{35}S]methionine in methionine-free MEM/LPDS and chased in DMEM/LPDS containing 200 μM unlabelled methionine, as indicated in the Figure legends.

Cells were washed, solubilized and immunoprecipitated using preformed immune complexes containing the monoclonal anti-(LDL receptor) antibody IgG-C7 [23]. Immunoprecipitates were subjected to SDS/PAGE (5–20% gradient acrylamide slab gels). Gels were treated with salicylate, dried, and exposed to preflashed X-ray film, all as previously described [24]. The ^{35}S radioactivity in the precursor and mature receptor bands was quantified by densitometric scanning of the fluorograms. Myosin (heavy chain) and low-molecular-mass markers (Pharmacia, Uppsala, Sweden) were used as standards.

RESULTS

Restriction-fragment-size analysis showed that the FH patient (RI) was homozygous for a 2.5 kb deletion in the LDL-receptor gene [9]. The deletion was clearly detectable after digestion with *Bgl*II, when a 12 kb invariant restriction fragment of the normal receptor allele was replaced with a 9.5 kb fragment of the mutant allele (Fig. 1). The deletion was within the EGF-precursor sequence-identity region of the receptor and included much or all of exons 7 and 8. To define the exact deletion in the LDL-receptor mRNA in RI, we sequenced a cDNA fragment, prepared by the PCR technique, which included the putative deletion. cDNA was prepared from total cellular RNA isolated from cultured skin fibroblasts from RI and a normal subject, using an oligonucleotide complementary to exon 10 as primer. The cDNA sequence between exon 6 and exon 10 was then amplified by PCR. Analysis of the amplified cDNA fragments revealed a deletion of about 250 bp in the receptor mRNA of RI (Fig. 2). The amplified cDNA was sequenced by the Maxam & Gilbert [20] technique and showed the direct coupling of exon 6 to exon 9 in receptor mRNA of RI with the complete deletion of exons 7 and 8 (Fig. 3). Since exons 7 and 8 comprise 120 and 126 bp respectively, their deletion should result in the synthesis of a protein that is 82 amino acids shorter than normal. Furthermore, because the deletion of these two exons is in-frame and conserves the glycine-293 residue normally interrupted by intron 6, the amino acid sequence of the rest of the mutant protein should be normal.

The synthesis and processing of the mutant LDL receptor in cultured fibroblasts of RI were studied by [^{35}S]methionine

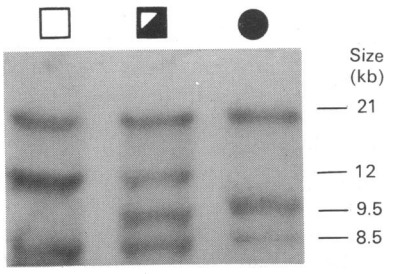

Fig. 1. Southern-blot-hybridization patterns of genomic DNA from patient RI (●, homozygous 2.5 kb deletion), her father (◪, heterozygous 2.5 kb deletion) and a control subject (□, homozygous normal) after digestion with *Bgl*11

The DNA probe used was the *Eco*R1–*Bgl*11 fragment of pLDLR-3 that includes exons 7–11 of the receptor gene.

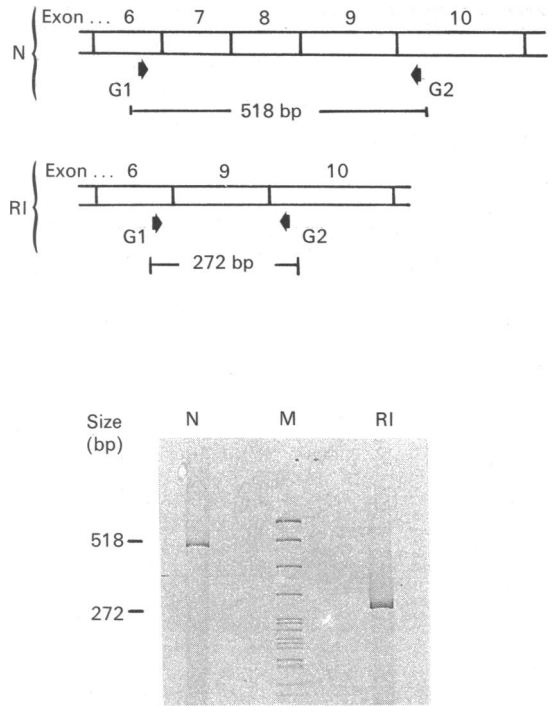

Fig. 2. Amplification by PCR of an LDL-receptor cDNA sequence from patient RI (RI) and a normal subject (N)

Total RNA was isolated from fibroblasts and reverse-transcribed by using the oligonucleotide G2 (5′-TGATGACGGTGTCAT-AGGAAGAGAC-3′), which was complementary to nucleotides 1384–1408 in exon 10 as primer. The resulting cDNA corresponding to the sequence between exons 6 and 10 was amplified by PCR using as primers an upstream oligonucleotide G1 (5′-CATGGCTAGAGACTGCCGGGACTGG-3′), which was complementary to nucleotides 891–915 in exon 6, and oligonucleotide G2. After amplification the DNA was size-fractionated on a 6%-(w/v)-polyacrylamide gel and revealed by ethidium bromide staining. *Msp*1 fragments of pBR322 DNA were used as size markers (M).

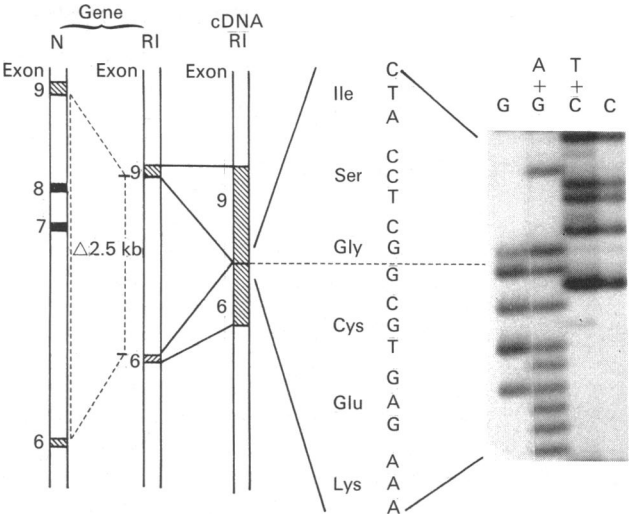

Fig. 3. Partial nucleotide sequence of cDNA from patient RI

The PCR-amplified sequence (prepared as described in Fig. 2) was re-amplified by PCR using end-labelled G1 and unlabelled G2 oligonucleotides as primers. Sequence analysis was performed as described by Maxam & Gilbert [20]. The 25 3′-nucleotides of exon 6 and 80 nucleotides of exon 9 were sequenced. The 2.5 kb deletion in the LDL-receptor gene in RI resulted in the in-frame deletion of exons 7 and 8 from the mature protein with the direct coupling of exons 6 and 9. Abbreviations: RI, patient RI; N, normal subject.

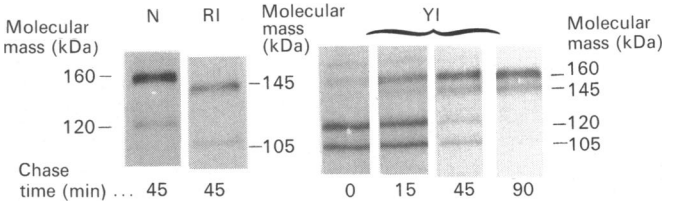

Fig. 4. Processing of LDL receptors in fibroblasts from patient RI, her mother, YI, and a normal subject

After incubation at 37 °C for 30 h in LPDS medium, fibroblasts were preincubated in methionine-free MEM for 30 min at 37 °C, after which the cells were pulse-labelled at 37 °C with [^{35}S]methionine (62 μCi/ml) for 30 min. Cells were then chased at 37 °C in DMEM/LPDS medium containing 200 μM-methionine for the indicated times. Immunoprecipitation, SDS/PAGE and fluorography of labelled receptors were carried out as described in the Experimental section. The 160 and 145 kDa bands correspond to the mature normal (N) and mutant receptors respectively. The 120 and 105 kDa bands correspond to the immature normal and mutant receptors respectively.

pulse–chase experiments. The mature LDL receptor in RI (apparent molecular mass 145 kDa) was, as expected, smaller than the mature normal receptor (apparent molecular mass 160 kDa) as judged by SDS/PAGE (Fig. 4). The RI receptor was initially synthesized as a precursor (105 kDa) that, like the normal receptor, underwent an apparent 40 kDa increase in size as a result of glycosylation. This was clearly shown in fibroblasts from the mother (YI) of RI, who was heterozygous for the LDL-receptor mutation. The precursor and mature forms of both the normal and mutant receptors were present in YI cells (Fig. 4). The rate of processing of mutant precursors was only slightly lower than normal, with the processing of both normal and mutant receptors virtually complete after a 45 min chase period. Similar processing rates were observed in cells homozygous for either the normal or the mutant receptors (results not shown).

The functional activity of the mutant receptor was analysed in cultured fibroblasts preincubated in lipoprotein-deficient medium to maximally up-regulate the number of LDL receptors. Cells from RI showed negligible (< 2%) high-affinity binding of LDL at 4 °C compared with normal cells (Fig. 5). The number of LDL receptors on the surface of RI cells was assessed by high-affinity binding at 4 °C of the IgG-C7 monoclonal antibody to the receptor. This antibody recognizes the first cysteine-rich repeat of the binding domain at the *N*-terminus of the LDL receptor [14]. The binding of IgG-C7 to cells from RI was normal with respect to affinity, but the maximal binding values were approx. 15% of the value in normal cells (Fig. 5). The lipoprotein βVLDL, which binds to LDL receptors via multiple copies of apoE, also bound to cells from RI in low, but significant, amounts (approx. 20% of normal binding) with normal affinity (Fig. 5). These results indicate that cells from RI expressed low numbers of mutant receptor molecules at the cell surface, but that these receptors were unable to bind LDL.

Since the rates of receptor synthesis and processing were relatively normal in cells from RI, we considered the possibility that a higher degradation rate of the mutant receptor could account for the low levels of receptor protein expressed in maximally up-regulated RI cells. Accelerated degradation of the mutant receptor was suggested by the results in Fig. 4; in fibroblasts from a subject heterozygous for the mutation, the loss of ^{35}S label from the mature mutant receptor band during a

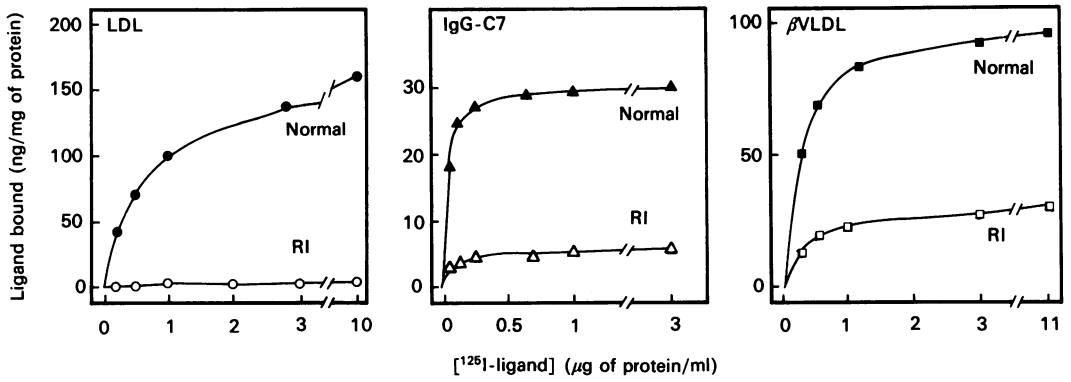

Fig. 5. Surface binding at 4 °C of ^{125}I-LDL, ^{125}I-IgG-C7 and ^{125}I-βVLDL to normal and RI fibroblasts

After incubation for 48 h in LPDS medium, each dish received 1.5 ml of medium containing the indicated concentration of ^{125}I-LDL (882 c.p.m./ng of protein), ^{125}I-IgG-C7 (1551 c.p.m./ng of protein) or ^{125}I-βVLDL (502 c.p.m./ng of protein). After 2 h at 4 °C the total radioactivity bound to the cells was determined in duplicate for each point. The curves represent the specific high-affinity binding calculated using the computer program ENZ-FITTER (Biosoft, Elsevier), assuming binding to one site plus non-specific binding. Non-specific (low-affinity) values for normal cells in the case of ^{125}I-LDL (at 1 μg/ml), ^{125}I-IgG-C7 (at 0.25 μg/ml) and ^{125}I-βVLDL (at 1 μg/ml) comprised < 3%, < 5% and < 14% of the total values respectively. Maximum binding in RI cells of ^{125}I-LDL, ^{125}I-IgG-C7, and ^{125}I-βVLDL were < 5%, 14±2%, and 21±8% respectively of the binding in normal cells in five separate experiments.

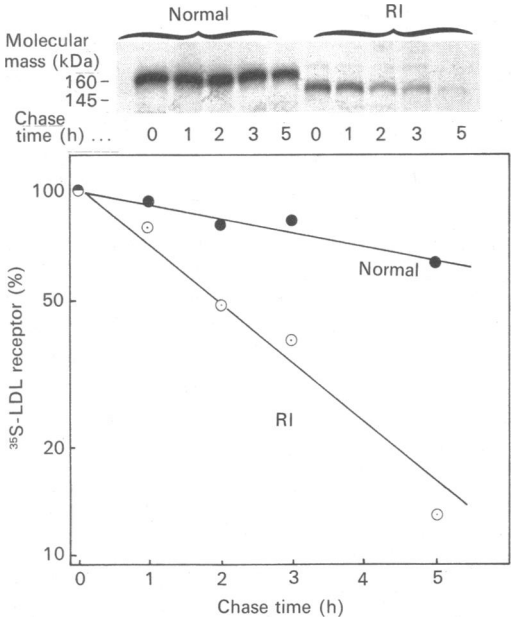

Fig. 6. Degradation of ^{35}S-labelled mature receptors in fibroblasts from patient RI and a normal subject

After incubation for 48 h in LPDS medium, fibroblasts were preincubated in methionine-free Eagle's MEM for 30 min at 37 °C, after which the cells were pulse-labelled at 37 °C with [^{35}S]methionine (100 μCi/ml) for 2 h. Cells were then incubated at 37 °C in DMEM/LPDS medium containing 200 μM-methionine for 1 h (prechase), before incubation at 37 °C in the same medium for the indicated chase times. Immunoprecipitation, SDS/PAGE and fluorography were performed as described in the Experimental section. Each point represents the mean value of duplicate determinations. The half-life ($t_{\frac{1}{2}}$) values for LDL-receptor degradation were: normal, 7.8 h; RI, 2.0 h. Similar differences in the $t_{\frac{1}{2}}$ values for normal and RI receptors were observed in two other experiments.

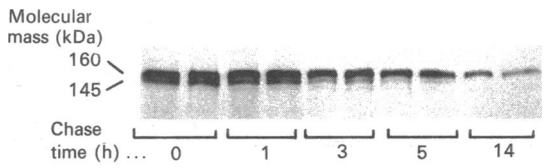

Fig. 7. Degradation of [^{35}S]methionine-labelled receptors in fibroblasts from the heterozygous mother of RI

Up-regulated cells were pulse-labelled with [^{35}S]methionine (70 μCi/ml) for 2 h at 37 °C, and then chased at 37 °C for the indicated times as described in Fig. 6. Immunoprecipitation, SDS/PAGE and fluorography of labelled receptors were performed as described in the Experimental section.

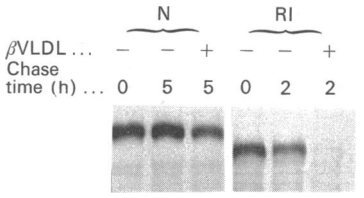

Fig. 8. Degradation of ^{35}S-labelled LDL receptors in fibroblasts from patient RI (RI) and a normal subject (N) after incubation with βVLDL

Up-regulated fibroblasts were pulsed with [^{35}S]methionine (125 μCi/ml) for 2 h at 37 °C as described in Fig. 4. The medium was changed to LPDS medium containing 200 μM-methionine and the cells were incubated at 37 °C for 1 h. βVLDL (10 μg/ml) was then added, as indicated, to some dishes, and the cells were then chased at 37 °C for the times shown. After the chase period, immunoprecipitation, SDS/PAGE and fluorography of labelled receptors were performed as described in the Experimental section.

90 min chase period was much greater than that from the receptors produced by the normal allele. Experiments employing longer chase periods confirmed that the half-life of the mutant LDL receptors in cells from RI was about 2 h, four times shorter than that of normal receptors (Fig. 6). Consistent with these results, relatively higher degradation rates of mutant versus normal LDL receptors were also clearly observed in cells from the heterozygous mother of RI (Fig. 7). The shorter half-life of the mutant receptors therefore accounted for their diminished number in up-regulated RI cells.

Since the EGF-precursor-like domain including exons 7 and 8 is necessary for the acid-induced dissociation of ligand from receptor that occurs during LDL-receptor recycling [8], we measured the rate of receptor degradation at 37 °C in the presence and absence of βVLDL, the ligand shown (Fig. 5) to

bind to the mutant receptor. The rate of breakdown of the mutant receptors of RI was markedly enhanced by the presence of βVLDL, such that the vast majority of labelled receptors had disappeared after a 2 h chase period (Fig. 8). Although the presence of βVLDL also enhanced the rate of receptor breakdown in normal cells, the degradation of mutant receptors in the presence of the ligand was significantly faster than the corresponding degradation of normal receptors. This result is consistent with the behaviour of normal and certain engineered abnormal constructs studied in transfected CHO cells and the interpretation that the growth-factor-like repeats are necessary for ligand dissociation and recycling [8].

DISCUSSION

Here we show that the deletion of growth-factor-like repeats A and B from the EGF-precursor-like domain of a naturally occurring mutant LDL receptor altered its binding specificity and disrupted its ability to recycle in the presence of ligand. An additional distinguishing feature of this mutant receptor was its abnormally rapid turnover, even when unoccupied by ligand.

DNA analysis showed the deletion of exons 7 and 8 from the receptor gene in RI and the direct splicing of exon 6 to exon 9 in the receptor mRNA. The deletion breakpoints of the new mutation were localized to introns 6 and 8. No data are currently available on their exact positions in these introns, but they are likely to lie within Alu-like repeat units which are relatively abundant in the intronic regions of the LDL-receptor gene, including introns 6 and 8 [25,26]. Numerous deletions and an insertion mutation, altogether involving at least nine different Alu sequences, have been detected in the LDL receptor gene (reviewed in [3]). It has been suggested that recombination between Alu 'hotspots' accounts for the high population frequency of FH [6].

The mutant receptor in RI showed an altered binding specificity such that it could not bind LDL but retained the ability to bind the apoE-containing ligand, βVLDL. In the presence of βVLDL, the mutant receptors were rapidly degraded, probably owing to their inability to dissociate from ligand and to recycle back to the cell surface. These properties are similar to those of receptors in transfected cells expressing engineered receptor constructs that lack either the entire EGF-precursor sequence-identity region or only the same two growth-factor-like repeats deleted in RI [8]. A naturally occurring mutant receptor also lacking repeats A and B was previously reported to exhibit a similarly altered binding specificity [27]. No other properties of that receptor were described, and the mutation, which corresponded to a 4 kb deletion in the receptor gene, was different from the mutation found in RI (2.5 kb deletion). In another FH patient a mutant receptor was found that lacked the entire EGF-precursor sequence-identity domain [26]. This receptor displayed essentially the same characteristics as its artificial counterpart expressed in transfected CHO cells [8].

The mutant receptors of RI were degraded, in the absence of ligand, at a rate that was four times higher than that for normal receptors. Abnormal degradation has previously been identified in the case of precursor LDL-receptor molecules that fail to undergo normal processing [4,27,28]. Retarded transfer of receptor precursors from the endoplasmic reticulum to the Golgi apparatus is accompanied by rapid degradation of the mutant proteins, possibly by the non-lysosomal proteolytic system reportedly associated with the endoplasmic reticulum [29]. However, in the case of RI, it was clearly the mature receptors that displayed accelerated degradation. They represent, therefore, another example of a new class of mutation recently described giving rise to rapidly degraded receptors [10]. The rapidly degraded mutant receptor previously described also contains an altered EGF-precursor-like domain resulting from a single base change in exon 9, and the substitution of methionine for valine at position 408. Funahashi *et al.* [30] have described a mutation which produces LDL receptors, with apparent molecular mass 5 kDa less than normal, which are also rapidly degraded with a half-life of about 0.5–1 h. The molecular defect in these mutant receptors is not known.

Kozarsky *et al.* [31] have shown in transfected CHO cells that *O*-linked sugar-deficient LDL receptors reach the cell surface but are unstable, the *N*-terminal extracellular domain being released into the medium as a result of proteolytic cleavage. In the case of RI, we were not able to detect any degradation fragments of receptor released into the medium. We assume, from the apparently normal 40 kDa increase in molecular mass associated with processing of the 105 kDa precursor to the 145 kDa mature form, believed to be the result of maturation of *O*-linked sugars, that *O*-linked glycosylation in RI is normal. No information is available on *N*-linked glycosylation of the mutant receptors. *N*-Linked sugars in the LDL receptor are normally attached to a part of the EGF-precursor-like region not removed by the deletion in RI. Nevertheless, the deletion may indirectly interfere with *N*-linked glycosylation of these sites. However, in this regard, inhibition of *N*-linked glycosylation by tunicamycin was reported not to have any effect on LDL-receptor stability [32].

A faster-than-normal turnover of unoccupied receptors was not observed in the case of the transfected receptor construct lacking the entire EGF-precursor sequence-identity region, although this aspect was not studied in detail [8]. The reason for this difference is not clear. Perhaps there are basic differences in receptor metabolism in human fibroblasts and CHO cells. The turnover of the naturally occurring mutant lacking the entire EGF-precursor-like region was not studied directly [26], although the levels of receptor protein expressed in fibroblasts (50–100% of normal) were not suggestive of a markedly accelerated turnover. It is possible that the effects of different deletions in this region might vary significantly. Deletion of the first two growth-factor-like repeats may disrupt the conformation of the remaining portion of the domain and consequently decrease receptor stability. Deletion of the entire EGF domain, in contrast, may leave the conformation of the remaining domains undisturbed and the receptors subject to the normal rate of degradation. One possible explanation for the rapid turnover of unoccupied mutant receptors of RI is that their recycling might be impaired in such a way that they are misdirected to lysosomes after internalization. The EGF-precursor-like domain plays a role in ligand dissociation, possibly by mediating acid-dependent changes in conformation or protein–protein interactions [8]. Alterations in this functionally important domain may disrupt the structure of receptors in such a way that their intracellular targeting is altered. Alternatively, the rapid degradation may result from either the exposure or generation of sites sensitive to proteolytic degradation. Such proteolysis might occur extralysosomally, as we have proposed for the degradation of normal LDL receptors [33] (possibly mediated by proteinases in the endocytic compartment [34]) or at the cell surface [35]. Whether the mutant receptors are degraded unusually rapidly via the same pathway as normal LDL receptors or by a distinct proteolytic mechanism remains to be determined.

It is interesting to reflect on the likely status *in vivo* of LDL receptors in this patient. Our results indicate that there would be very few molecules at cellular surfaces because of their rapid basal turnover augmented by their even more rapid trapping and destruction in the presence of ligands. This may preclude any

potential physiological significance for these mutant receptors, despite their intrinsic ability to bind apoE-containing lipoproteins important in intravascular lipid transport.

We thank Kathy Lyner and Rolene Liebenberg for their excellent technical assistance and Wieland Gevers for his critical appraisal of the manuscript. This work was supported by the South African Medical Research Council, the University of Cape Town and the Cape Provincial Administration.

REFERENCES

1. Brown, M. S. & Goldstein, J. L. (1986) Science **232**, 34–47
2. Russell, D. W., Brown, M. S. & Goldstein, J. L. (1989) J. Biol. Chem. **264**, 21682–21688
3. Russell, D. W., Esser, V. & Hobbs, H. H. (1989) Arteriosclerosis Suppl. **9**, 8–13
4. Yamamoto, T., Bishop, R. W., Brown, M. S., Goldstein, J. L. & Russell, D. W. (1986) Science **232**, 1230–1237
5. Leitersdorf, E., Hobbs, H. H., Fourie, A. M., Jacobs, M., van der Westhuyzen, D. R. & Coetzee, G. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7912–7916
6. Yamamoto, T., Davis, C. G., Brown, M. S., Schneider, W. J., Casey, M. L., Goldstein, J. L. & Russell, D. W. (1984) Cell (Cambridge, Mass.) **39**, 27–38
7. Sudhof, T. C., Goldstein, J. L., Brown, M. S. & Russell, D. W. (1985) Science **228**, 815–822
8. Davis, C. G., Goldstein, J. L., Sudhof, T. C., Anderson, R. G. W., Russell, D. W. & Brown, M. S. (1987) Nature (London) **326**, 760–765
9. Henderson, H. E., Berger, G. M. B. & Marais, A. D. (1988) Hum. Genet. **80**, 371–374
10. Fourie, A. M., Coetzee, G. A., Gevers, W. & van der Westhuyzen, D. R. (1988) Biochem. J. **255**, 411–415
11. Leitersdorf, E., van der Westhuyzen, D. R., Coetzee, G. A. & Hobbs, H. H. (1989) J. Clin. Invest. **84**, 954–961
12. Aulinskas, T. H., van der Westhuyzen, D. R. & Coetzee, G. A. (1984) Atherosclerosis **47**, 159–171
13. Kovanen, P. T., Brown, M. S., Basu, S. K., Bilheimer, D. W. & Goldstein, J. L. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 1396–1400
14. van Driel, I. R., Goldstein, J. L., Sudhof, T. C. & Brown, M. S. (1987) J. Biol. Chem. **262**, 17443–17449
15. Beisiegel, U., Schneider, W. J., Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1981) J. Biol. Chem. **256**, 11923–11931
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 5294–5299
17. Sudhof, T. C., van der Westhuyzen, D. R., Goldstein, J. L., Brown, M. S. & Russell, D. W. (1987) J. Biol. Chem. **262**, 10773–10779
18. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) Science **239**, 487–491
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in Molecular Cloning: A Laboratory Manual, pp. 122–123, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
20. Maxam, A. M. & Gilbert, W. (1980) Methods Enzymol. **65**, 499–560
21. Goldstein, J. L., Basu, S. K. & Brown, M. S. (1983) Methods Enzymol. **98**, 241–260
22. van der Westhuyzen, D. R., Coetzee, G. A., Demasius, I. P. C., Harley, E. H., Gevers, W., Baker, S. G. & Seftel, H. C. (1984) Arteriosclerosis **4**, 238–247
23. Tolleshaug, H., Hobgood, K. K., Brown, M. S. & Goldstein, J. L. (1983) Cell (Cambridge, Mass.) **32**, 941–951
24. Casciola, L. A. F., van der Westhuyzen, D. R., Gevers, W. & Coetzee, G. A. (1988) J. Lipid Res. **29**, 1481–1488
25. Lehrman, M. A., Goldstein, J. L., Russell, D. W. & Brown, M. S. (1987) Cell (Cambridge, Mass.) **48**, 827–835
26. Miyake, Y., Tajima, S., Funahashi, T. & Yamamoto, A. (1989) J. Biol. Chem. **264**, 16584–16590
27. Russell, D. W., Lehrman, M. A., Sudhof, T. C., Yamamoto, T., Davis, C. G., Hobbs, H. H., Brown, M. S. & Goldstein, J. L. (1986) Cold Spring Harbor Symp. Quant. Biol. **51**, 811–819
28. Esser, V. & Russell, D. W. (1988) J. Biol. Chem. **263**, 13276–13281
29. Lippincott-Schwartz, J., Bonofacino, J. S., Yuan, L. C. & Klausner, R. D. (1988) Cell (Cambridge, Mass.) **54**, 209–220
30. Funahashi, T., Miyake, Y., Yamamoto, A., Matsuzawa, Y. & Kishino, B. (1988) Hum. Genet. **79**, 103–108
31. Kozarsky, K., Kingsley, D. & Krieger, M. (1988) Proc. Natl. Acad. Sci. **85**, 4335–4339
32. Filipovic, I. (1989) J. Biol. Chem. **264**, 8815–8820
33. Casciola, L. A. F., Grant, K. I., Gevers, W., Coetzee, G. A. & van der Westhuyzen, D. R. (1989) Biochem. J. **262**, 681–683
34. Diment, S., Leech, M. S. & Stahl, P. D. (1988) J. Biol. Chem. **263**, 6901–6907
35. Hammons, G. T., Smith, R. M. & Jarett, L. (1982) J. Biol. Chem. **257**, 11563–11570

Received 24 October 1990/28 January 1991; accepted 15 February 1991