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Role of an intramolecular contact on lipoprotein uptake by the LDL receptor

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ABSTRACT

The LDL receptor (LDLR) is an endocytic receptor that plays a major role in the clearance of atherogenic lipoproteins from the circulation. During the endocytic process, the LDLR first binds lipoprotein at the cell surface and then traffics to endosomes, where the receptor releases bound lipoprotein. Release is acid-dependent and correlates with the formation of an intramolecular contact within the receptor. Human mutations at residues that form the contact are associated with familial hypercholesterolemia (FH) and the goal of the present study was to determine the role of contact residues on LDLR function. We show that mutations at nine contact residues reduce the ability of the LDLR to support lipoprotein uptake. Unexpectedly, only four of the mutations (W515A, W541A, H562Y and H586Y) impaired acid-dependent lipoprotein release. The remaining mutations decreased the lipoprotein-binding capacity of the LDLR through either reduction in the number of surface receptors (H190Y, K560W, H562Y and K582W) or reduction in the fraction of surface receptors that were competent to bind lipoprotein (W144A and W193A). We also examined three residues, distal to the contact, which were predicted to be necessary for the LDLR to adopt the acidic conformation. Of the three mutations we tested (G293S, F362A and G375S), one mutation (F362A) reduced lipoprotein uptake. Together, these data suggest that the intramolecular interface plays multiple roles in LDLR function.

1. Introduction

The LDL receptor (LDLR) removes all major classes of atherogenic lipoproteins from the circulation including chylomicrons, chylomicron remnants, VLDL, VLDL remnants and LDL. LDLR-mediated uptake involves four principal steps: receptor binding to lipoproteins at the cell surface, internalization of bound lipoproteins through clathrin coated pits, release of lipoproteins in endosomes and recycling of receptors back to the cell surface for further rounds of uptake. Hereditary defects that impair any step in the uptake process result in familial hypercholesterolemia (FH) and heightened risk of coronary artery disease [1].

Different domains of the LDLR are responsible for different steps during lipoprotein uptake. The LDLR is composed of 7 LDLR type A repeats (LA repeats 1–7), two EGF-like repeats (EGF-A and EGF-B), six YWTD repeats that form a six-bladed β-propeller, a third EGF-like repeat (EGF-C), a domain that is heavily glycosylated, a single transmembrane domain and a short cytoplasmic tail (Fig. 1). The LDLR uses LA repeats 4 and 5 (LA4/5) to bind to apolipoprotein E (apoE) on chylomicrons, VLDL and remnant particles [2–4]. LDL lacks apoE, and the LDLR instead binds to apolipoprotein B100 (apoB100) on LDL using LA repeats 3–7 and the EGF-A module of the receptor [2,3]. Both LA repeats and EGF-like repeats bind calcium and calcium is required for lipoprotein binding activity [5–9]. Lipoprotein uptake occurs through clathrin-coated pits and requires interaction of the cytoplasmic domain of the LDLR with adaptor proteins that couple LDLR-lipoprotein complexes to the endocytic machinery [10–12]. Lipoprotein release in endosomes requires acidic pH and may also require efflux of calcium from the endosomal lumen [9,13–17]. How acidic pH drives release is not fully understood; however, acidic pH has been shown to drive a conformational change in the LDLR from an extended conformation at neutral pH to a compact conformation at acidic pH [18,19]. In the acidic state, the β-propeller module makes an intramolecular contact with LA4/5 [19]. LA4/5 is required for all lipoprotein binding, suggesting that the intramolecular contact may participate in acid-dependent release. Consistent with this possibility, deletion of the β-propeller prevents lipoprotein release at endosomal pH (pH 6–6.5) and cripples LDL uptake [17,20].

The intramolecular contact between the β-propeller and LA4/5 can be divided into several groups of interactions (Fig. 1). The largest hydrophobic (van der Waals) contact group is composed of P141 and W144 on LA4 and W515 and W541 on the β-propeller. W193, E581 and K582 form a second, smaller van der Waals contact grouping between LA5 and the β-propeller. H562 and H586 of the β-propeller interact with D149 of LA4 with what are likely ionic contacts at acidic pH. Ionic contacts are also made by K560 and K582 with acidic residues that chelate calcium in LA4 and LA5, respectively. H190 of

Abbreviations: LDLR, LDL receptor; FH, familial hypercholesterolemia; LA repeat, LDLR type A repeat; YWTD repeat, repeat having the tyrosine, tryptophan, threonine, and aspartic acid motif; ApoE, apolipoprotein E; ApoB100, apolipoprotein B100; LRP1, LDLR-related protein 1

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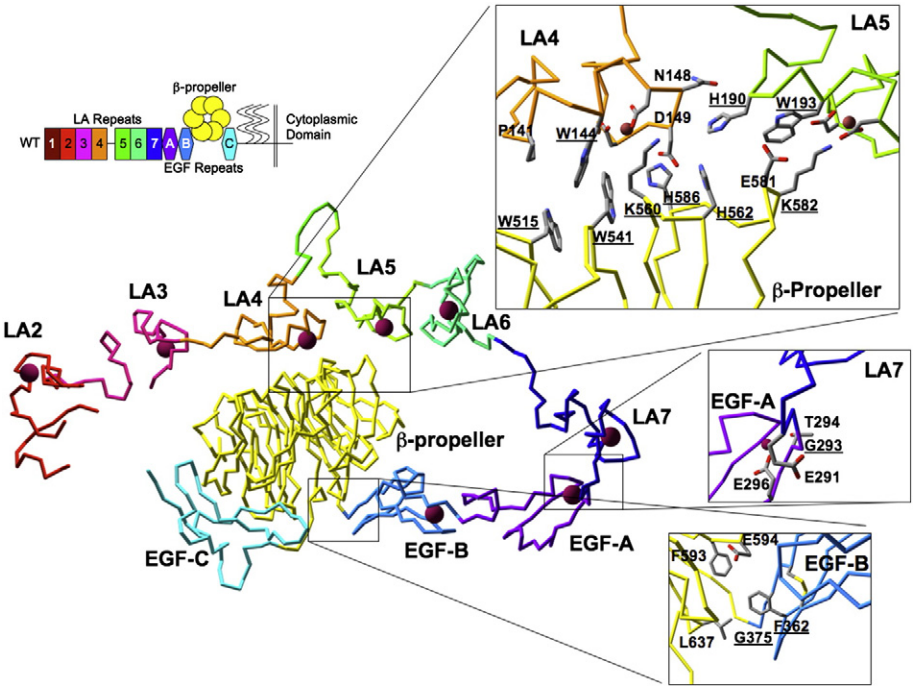

Fig. 1. Structure of the LDLR ectodomain and the location of mutations. In the upper left is a diagram of the domain structure of the LDLR with seven LA repeats (1–7), three EGF-like repeats (A, B, C), the β-propeller, a region of heavy glycosylation (wavy lines), a single transmembrane domain and a short cytoplasmic domain. Centrally located in the figure is the structure of the LDLR ectodomain at acidic pH (PDB ID: 1N7D). The LA repeats, EGF repeats and the β-propeller have the same color as in the diagram in the upper left. The red balls in the structure indicate bound calcium ions. The three insets at the right show the contact residues, the region around G293 and the region around F362 and G375. Residues that have been mutated are underlined.

LA5 packs against the main chain of LA4 between N148 and D149 and the side chain of H562. H190 may make ionic contact with D149 depending upon the rotomer of the D149 side chain.

The role of these contacts in lipoprotein uptake is not clear; however, mutations at contact residues correlate with FH, suggesting that the contact plays an important role in lipoprotein uptake. Known FH mutations at contact residues include P141L, H190Y, H190L and H562Y [21–25]. Prior studies have focused on the role of H190, H562 and H586 on lipoprotein uptake. These histidines are implicated in uptake because the pK_a of histidine is similar to the pH necessary to drive LDL release. Surprisingly, replacement of all three histidines with alanine has no effect on the acid-dependent conformational change [26]; however, replacement of the three histidines with either alanine or tyrosine reduces the acid sensitivity of LDL release [26,27]. Recently, Huang and colleagues made mutations at several additional interface residues and tested for the effect of these mutations on acid-dependent conformational change and LDL release [28]. They chose to examine the W144A, H190Y, W193A, W515A, W541A, K560W, H562Y, K582W and H586Y mutations. None of these mutations had a measurable effect on acid-dependent conformational change; however, several mutations had effects on acid-dependent LDL release in *in vitro* assays using purified ectodomains [28]. Whether these effects correlated with impaired lipoprotein uptake was not examined. Because the mutations did not influence conformational change, Huang and colleagues also examined the G293S, F362A and G375S mutations [28]. G293 and G375 were proposed to function as pivots during conformational change because these residues sit at the boundaries between LA7/EGF-A and EGF-B/β-propeller, respectively. FH mutations have also been identified at these positions [23,29]. F362 provides van der Waals contacts that hold the EGF-B module against the β-propeller and may influence the ability of the β-propeller to sample conformational space. As with the other nine mutations, the G293S, F362A and G375S mutations had no effect on receptor conformational change [28]. Again, the role of these residues in lipoprotein uptake was not tested.

The goal of this study is to characterize how the contact between the β-propeller and LA4/5 participates in lipoprotein uptake. Because of the prior biochemical work done with the W144A, H190Y, W193A, G293S, F362A, G375S, W515A, W541A, K560W, H562Y, K582W and H586Y mutations, we chose to introduce these mutations into full-length LDLRs, express these receptors in LDLR deficient fibroblasts and compare the ability of these cells to support uptake of LDL and the VLDL remnant, β-VLDL. Our results show that all but the G293S and G375S mutations impair lipoprotein uptake. We investigated how each mutation impaired lipoprotein uptake, using assays for receptor surface expression, lipoprotein binding and acid-dependent release. Our results show that different mutations produce different defects in LDLR-dependent lipoprotein uptake, suggesting that contact residues play multiple roles in LDLR function. We present a model for how these residues may function during lipoprotein uptake.

2. Methods

2.1. Materials

Human LDL and rabbit β-VLDL were provided by Michael Brown and Joseph Goldstein. Rabbit polyclonal antibody against the LDLR was provided by Joachim Herz. The C7 mouse monoclonal antibody against the LDLR was purchased from Santa Cruz. The CD44 antibody was purchased from Chemicon. Cell culture media was from Invitrogen. All other chemicals and buffers were purchased from Sigma.

2.2. Cell lines

LDLR$^{-/-}$ fibroblasts were infected with recombinant retroviruses that encode a bicistronic mRNA in which both an LDLR variant and GFP were linked by an internal ribosomal entry site (IRES). Because both the LDLR variant and GFP are translated from the same mRNA, GFP expression is proportional to LDLR synthesis [30]. This feature allows LDLR expressing cells to be selected by fluorescence activated cell