Evidence that Familial Hypercholesterolemia Mutations of the LDL Receptor Cause Limited Local Misfolding in an LDL-A Module Pair†

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Received June 30, 2000; Revised Manuscript Received August 11, 2000

ABSTRACT: Mutations at conserved sites within the ligand-binding LDL-A modules of the LDL receptor cause the genetic disease familial hypercholesterolemia (FH), and several of these FH mutations in modules five and six prevent the isolated single modules from folding properly to a nativelike three-dimensional structure. Because LDL-A modules occur as a series of contiguous repeats in the LDLR and related proteins, we investigated the impact of two FH mutations in LDL-A module five (D203G and D206E) and two mutations in module six (E219K and D245E) in the context of the covalently connected module five−six pair. HPLC chromatography of the products formed under conditions that efficiently refold the native module five−six pair demonstrate that, for each mutation, a folding defect persists in the module pair. NMR spectroscopy and calcium affinity measurements of the ensemble of misfolded products demonstrate that the unaltered module of each pair can fold to its native structure regardless of the range of misfolded conformations adopted by its mutated neighbor. These findings lend additional support to a model in which individual LDL-A modules of the LDL receptor act as independent structural elements.

The low-density lipoprotein receptor (LDLR),[1] a modular type-1 transmembrane protein of 839 amino acids (*1*−*4*), is the principal mechanism for uptake of plasma cholesterol into cells. Low-density lipoprotein particles (LDLs), which contain a single copy of apolipoprotein B-100, are the primary ligands for the receptor (*5*, *6*) and carry approximately 65−70% of plasma cholesterol in humans. Other ligands for the LDLR include lipoprotein particles that contain multiple copies of apolipoprotein E (*7*), such as intermediate density lipoproteins and β-migrating forms of very low-density lipoproteins.

Familial hypercholesterolemia (FH), an autosomal dominant genetic disorder affecting one in 500 persons worldwide, results from any of a number of different mutations in the gene encoding the LDLR (*8*, *9*). Clinically, the major consequences of this molecular defect are elevated levels of plasma low-density lipoprotein (LDL) and cholesterol. Heterozygotes, who inherit one inactive copy of the LDLR gene, have a substantially increased risk for coronary artery disease. Phenotypic homozygotes, who harbor two defective copies of the LDLR gene, develop severe premature atherosclerosis which leads to death from coronary artery disease at a very early age (*8*).

Lipoprotein binding is mediated primarily by seven contiguous LDLR type-A (LDL-A) modules that lie at the amino-terminal end of the receptor (*10*, *11*). Each LDL-A module spans about 40 amino acids and contains six cysteine residues engaged in three disulfide bonds (Figure 1). These LDL-A modules, first identified in the LDL receptor and now predicted to exist in numerous proteins (*12*−*16*), are also responsible for the ligand-binding activity of many other different cell-surface receptors that recognize a wide variety of ligands (see ref *17* for a review).

Calcium-binding motifs play an important structural role in LDL-A (*18*−*23*), Notch/LIN12 (*24*), and EGF-like (e.g., refs *25* and *26*) cysteine-rich modules. Calcium is needed to guide formation of native disulfide bonds in refolding reactions performed in vitro with LDL-A modules and is required to maintain their structural integrity (*18*, *21*). Most importantly, binding of lipoprotein particles by the LDLR requires calcium (*27*). The high-resolution crystal structure of the fifth LDL-A module of the LDLR (*19*), which reveals a bound calcium ion tightly coordinated by two backbone carbonyl groups and a cluster of four highly conserved acidic residues, provides an explanation for the observed calcium dependence of folding, structure, and function.

Mutations that cause FH frequently alter residues in the conserved calcium-binding motif or in other crucial scaffolding positions of the LDL-A modules of the LDLR (*8*, *9*). In experiments performed with isolated LDL-A modules in vitro, FH mutations prevent the altered modules from folding properly to a nativelike three-dimensional structure (*18*, *28*, *29*).

† Supported by NIH Grant HL61001 (to S.C.B.) and by the Armenise Center for Structural Biology at Harvard Medical School. S.C.B. is a Pew Scholar in the Biomedical Sciences.

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[1] Abbreviations: amu, atomic mass unit; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid; EGF, epidermal growth factor; FH, familial hypercholesterolemia; GSH, reduced glutathione; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; HSQC, heteronuclear single quantum coherence; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LR5, LDL-A module five of the low-density lipoprotein receptor; LR6, LDL-A module six of the LDL receptor with the M243L substitution; LR5−6, the LR5−LR6 module pair; MALDI-TOF, matrix-assisted laser-desorption ionization time-of-flight; NMR, nuclear magenetic resonance.

10.1021/bi0015156 CCC: $19.00
Published on Web 10/04/2000

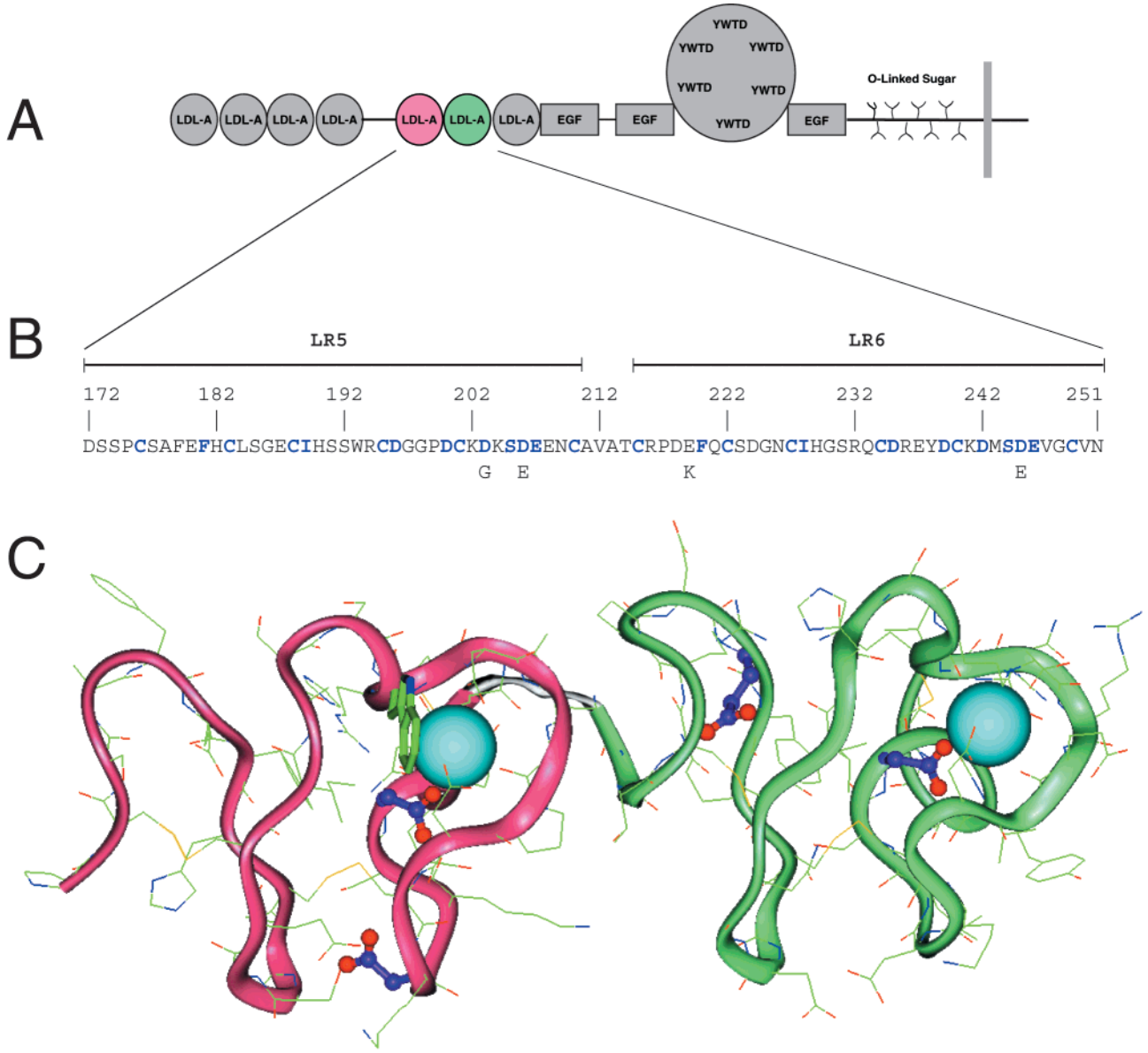

FIGURE 1: (A) Schematic of the modular organization of the LDL receptor, illustrating the positions of LDL-A modules, EGF-like modules, and repeats containing the YWTD motif. Adapted from Springer (*44*). (B) Amino acid sequence of LDL-A modules five (LR5) and six (LR6). Residue numbers (above) correspond to the sequence position in the mature receptor. Conserved residues are highlighted in blue. Point mutations investigated in these studies are listed on the bottom row. (C) Model illustrating the X-ray structure of LR5 (*19*) and the NMR structure of LR6 (*29*). A blue sphere represents the bound calcium ion. Side chains are superimposed on a ribbon trace of the module backbone. FH mutations studied here are highlighted by coloring side-chain carbon atoms purple and side-chain oxygen atoms red. Figure generated using the program Insight II, using the pdb entries 1ajj (*19*) and 1d2j (*29*).

Because LDL-A modules occur as a series of contiguous repeats in the LDLR and related proteins, these studies were designed to investigate the impact of an FH mutation located in one LDL-A module on one of its neighbors. Thus, the structural consequences of four FH mutations (D203G, D206E, E219K, and D245E), two from each module, were evaluated in the context of the covalently connected module five−six pair (see Figure 1).

Each of the four mutations has been previously shown (*18*, *29*) to result in misfolding of isolated module five (LR5) or module six (LR6). The D203G and D206E mutations alter conserved acidic residues at the C-terminus of module five and indirectly (D203G) or directly (D206E) disrupt the calcium-binding site required for proper folding. The E219K mutation lies near the N-terminus of module six, potentially within contact distance of module five, and the D245E mutation is the homologue in LR6 of the D206E mutation in LR5.

In the work reported here, we demonstrate that, for each mutation, a folding defect persists in the module pair, but remains restricted to the module in which the mutation lies. In each of the four mutated module pairs, minimal structural perturbation is imparted to the unmutated module, which retains its native conformation regardless of the precise misfolded conformation of its mutated neighbor.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification. The D203G, D206E, E219K, and D245E proteins were generated by Kunkel mutagenesis of the vector pMM-LR5−6 (*23*). In the resulting plasmids, the LDL-A modules are expressed as a fusion with

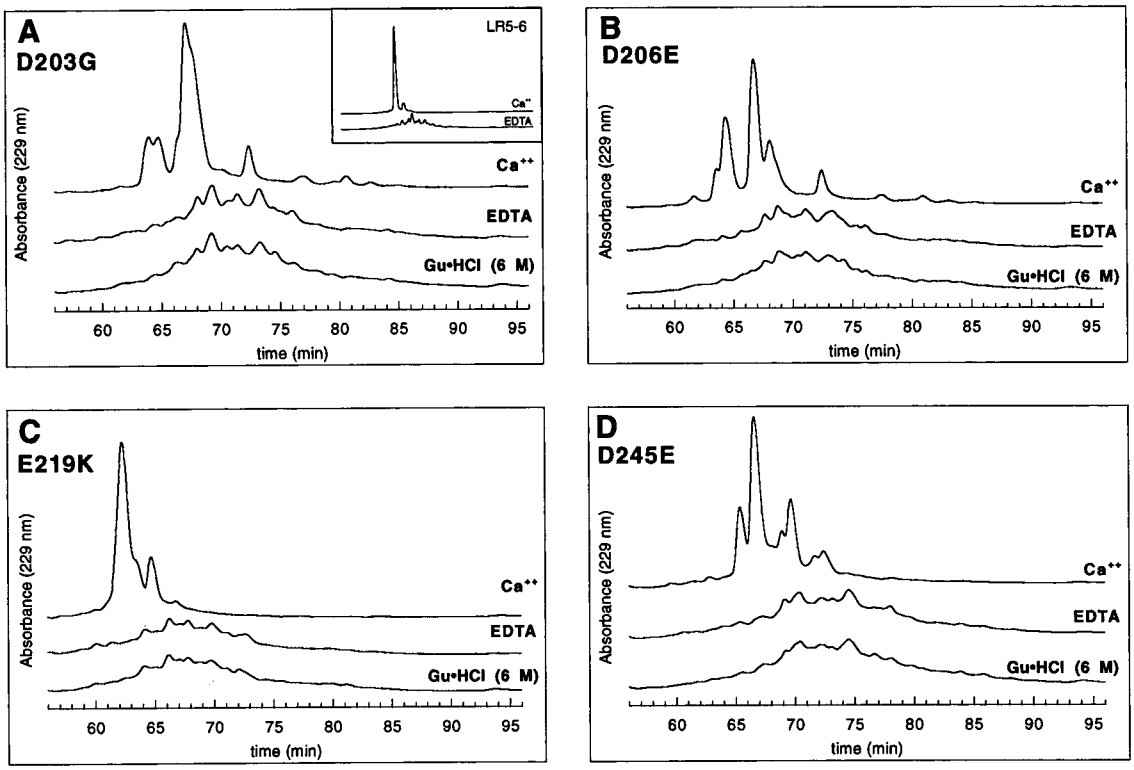

FIGURE 2: HPLC chromatograms of products formed after folding of the D203G mutant of LR5−6 (A), the D206E mutant of LR5−6 (B), the E219K mutant of LR5−6 (C) and the D245E mutant of LR5−6 (D) for 24 h. Folding of each mutant in the denaturant guanidine hydrochloride (6.0 M; panels A−D, bottom trace), or in the absence of calcium (panels A−D, middle trace) produces a broad distribution of nonnative disulfide-bonded species. In contrast, folding of each mutant in the presence of calcium (panels A−D, top trace) yields a highly restricted population of distinct disulfide-bonded species. (Inset in panel A: Folding of native LR5−6 in the presence of calcium (top) for 24 h results in the formation of a single predominant disulfide isomer, which contrasts with the distribution of products observed when the four mutants are allowed to refold under the same conditions. When native LR5−6 is allowed to refold in the absence of calcium (bottom), a broad distribution of non-native disulfide bonded species, similar to the population of species seen upon refolding of the four mutants under the same conditions, results. The data used to prepare the inset trace are from ref *23*.)

a modified form of the trpLE sequence (*18*), creating a $(His)_9$−TrpLE−Met−LDLR chimeric protein. The identity of each construct was verified by DNA sequencing. Unlabeled proteins were expressed in Luria broth (DIFCO); uniformly ^{15}N-labeled proteins were expressed in M9 minimal media using $^{15}NH_4Cl$ as the sole nitrogen source (*30*).

Each chimeric protein was expressed in *Escherichia coli* strain BL21(DE3) pLys(S). Synthesis of recombinant protein was induced by addition of IPTG at a measured cell density of 0.8 OD_{600}, and cells were harvested 4 h after induction. Inclusion bodies containing the recombinant protein were isolated and purified from the cell pellet as described (*18*, *31*) and then dissolved in 10 mM tris buffer, pH 8.0, containing 6.0 M guanidine HCl and 10 mM oxidized 2-mercaptoethanol. After oxidation overnight at room temperature, the sample was diluted 10-fold to precipitate the fusion protein, which was recovered by centrifugation at 4000*g* for 15 min.

Each fusion protein was cleaved with cyanogen bromide, and the cleavage products were dialyzed overnight against water acidified with 5% acetic acid. The hydrophobic TrpLE leader sequence was then selectively precipitated from each cleaved sample by adjustment to neutral pH with a stock solution of sodium hydroxide. The precipitate was removed from each sample by centrifugation at 4000*g* for 15 min. After treatment of the desired LDLR mutant protein (remaining in the supernatant) with DTT (50 mM) for 30 min at 37 °C, the reduced protein was purified by reversed-phase HPLC on a Vydac C-18 column. Solvent reservoirs contained water with 0.1% trifluoroacetic acid (A), and 90% acetonitrile with 0.1% trifluoroacetic acid (B); the protein was eluted with a linear gradient of 0.1% per min of solvent B. The observed mass of each purified protein was checked by MALDI-TOF mass spectrometry. Purified peptides were stored in reduced and lyophilized form at 4 °C.

Disulfide Exchange Experiments. The calcium dependence of folding was investigated for each protein (D203G, D206E, E219K, or D245E) under conditions permitting disulfide exchange. After purification in reduced form, each peptide (10 μM) was equilibrated in an anaerobic chamber (Coy laboratory products). Each reaction was performed at room temperature in a redox buffer of 50 mM tris, pH 8.5, containing GSH (0.5 mM) and GSSG (0.25 mM) and either $CaCl_2$ (2 mM), EDTA (2 mM), or guanidinium hydrochloride (6.0 M). Aliquots were removed from each sample at 24 and 48 h; disulfide exchange was stopped by addition of acetic acid to a final concentration of 5% (v/v). Samples were analyzed by reversed-phase HPLC on an analytical (4.6 mm) Vydac C-18 column. A linear gradient, starting at 22% solvent B and increasing by 0.1% per min, was used to separate refolded products. This gradient differs slightly from the conditions used to analyze products from refolding of native LR5−6 (23). Native LR5−6, which elutes from the column at about 86 min using the previous conditions (see Figure 2A and ref *23*), comes off of the column after

approximately62 min using the new gradient. Comparison of 24 and 48 h time points suggests that an equilibrium distribution of disulfide-bonded isomers is reached within 24 h (*18*).

HSQC Experiments. For NMR spectroscopy, large-scale preparations of each protein were first purified by HPLC in reduced form. After lyophilization, each ^{15}N labeled mutant protein (~50 μM, 5 mL total) was refolded by exhaustive dialysis against tris buffer (10 mM, pH 8.0) containing 10 mM $CaCl_2$, 0.5 mM GSH, and 0.25 mM GSSG at 4 °C. The resulting distributions of refolded protein were purified by preparative HPLC on a C18 Vydac column using a 0.1% per min linear gradient of solvent B. The HPLC profiles of these mixtures were similar to those shown in the top traces of Figure 2. For detailed analysis of the D245E mutant, three separate fractions were resolved by preparative HPLC, and the second of these fractions was resolved further into two separate peaks by multiple injections onto the analytical HPLC column. The four fractionated samples correspond approximately to the 64−67 min, 67−69 min, 69−71 min, and 71−74 min periods of the top chromatographic trace in Figure 2D.

Two-dimensional ^1H-^{15}N HSQC spectra (*32*) were obtained on uniformly ^{15}N-labeled protein in 10% D_2O at pH 6.5 and 25 °C. Samples of the four fractions of D245E were buffered with 2 mM PIPES at pH 6.5; all other samples were prepared in the absence of added buffer or salt. The wild-type HSQC spectrum was acquired using a 750 MHz Varian Unity Plus spectrometer. 1024 × 256 complex points were acquired with sweep widths of 12 000 Hz in D1 (^1H) and 2000 Hz in D2 (^{15}N). The HSQC spectrum of each mutant protein was acquired on a 500 MHz Varian Unity spectrometer. 1024 × 64 complex points were acquired with sweep widths of 7000 Hz in D1 (^1H) and 1800 Hz in D2 (^{15}N). A 90° shifted sine function was applied to the data in D1, and a 90° shifted sine function was applied in D2. All spectra were processed using Felix 97.0 software (MSI Inc.) and/or NMRpipe (*33*).

Fluorescence Assay. The Ca^{2+} affinity constants of E219K and of the four fractions of the D245E mutant were determined by observing the change in the relative fluorescence emission intensity of tryptophan 193 in LR5 (*18*). Spectra were acquired on an Aminco model AB2 fluorescence spectrometer by excitation at 280 nm, monitoring relative emission at 350 nm. Protein samples (0.2 μM each) were prepared after refolding from HPLC-purified, lyophilized material. Aliquots of an atomic absorption standard calcium solution (incrementing total calcium concentration by 5 μM in each step) were added to a stirred cuvette containing a solution of native protein (2.0 mL of total volume) in 10 mM PIPES buffer, pH 7.0, containing 100 mM NaCl and 0.1 mM EGTA. The EGTA-buffered concentration of free Ca^{2+} in the solution (0−2.1 μM) was calculated using the program MaxChelator [version 6.50 (*34*)]. The normalized relative emission intensities less the relative intensity in the absence of Ca^{2+} were fit to eq 1 using the Kaleidagraph software program (version 3.08).

$$Em_{350nm}([Ca^{++}]) = \frac{Em_{350nm}(\infty) \times [Ca^{++}]}{K_d + [Ca^{++}]} \qquad (1)$$

RESULTS

We have previously shown by HPLC analysis of the distribution of refolded products and by NMR spectroscopy that the native LR5−6 module pair folds with high efficiency to its native structure (*23*). With calcium present, the native LR5−6 module pair forms a single predominant isomer when allowed to fold in a buffer permitting disulfide exchange (Figure 2A, inset, and ref *23*). However, in the absence of calcium under otherwise identical conditions, a broad distribution of disulfide-bonded species is formed (*23*).

HPLC Analysis of the Products of Refolding Is Consistent with One Folded Module, and One Misfolded Module. Here, we have evaluated how four different FH mutations affect the structure and folding properties of the LR5−6 module pair. First, we investigated the distribution of products formed after allowing the reduced peptides to refold both with and without calcium in a buffer allowing disulfide exchange. When calcium is omitted from the folding buffer or oxidation is allowed to proceed in the presence of the denaturant guanidine hydrochloride (6.0 M), a broad distribution of products is formed, as judged by reversed-phase HPLC (Figure 2). In contrast, when folding of each mutant is performed in the presence of calcium, a much smaller number of products predominate (Figure 2). These findings indicate that certain disulfide bond patterns are preferred when oxidation is allowed to occur under native folding conditions, consistent with the hypothesis that only the module harboring the mutation misfolds, and that the unaltered module is capable of folding properly despite being covalently attached to a misfolded module.

NMR Demonstrates a Native Fold for the Unmutated Module. We used NMR spectroscopy to test the hypothesis that the disruption in folding observed for each mutated module pair is indeed restricted to the module harboring the mutation. The correlated ^1H-^{15}N chemical shifts of HSQC NMR spectra provide a sensitive probe of changes in backbone protein conformation. ^{15}N-Labeled proteins were therefore prepared for NMR spectroscopy, and the HSQC spectrum of wild-type LR5−6 (Figure 3) was compared with the spectrum of each LR5−6 mutant (Figure 4) in a chemical shift perturbation experiment. Each mutant protein was first purified in reduced form, and then allowed to fold in calcium-containing buffer. After a "refolded" protein was repurified by HPLC, an HSQC spectrum of the protein was acquired using the ensemble of its refolded products.

When the HSQC spectra of D203G or D206E (both mutations which lie in LR5) are compared with the spectrum of the native protein, almost every resonance of module six appears at its native location (compare Figure 3 with Figure 4, panels A and B). In contrast, the native resonances of most of the module five residues are missing or shifted to new positions, with new broad, poorly resolved signals present in the middle of the spectrum. Although slight perturbations (~0.1 ppm) in the chemical shifts of G230 and E237 of module six are observed in each spectrum, all other chemical shifts in module six are essentially unaffected.

Conversely, when the HSQC spectra of E219K and D245E (both mutations in LR6) are compared with the spectrum of the native protein, LR5 resonances generally appear at their native positions, whereas the LR6 resonances disappear or are shifted, with a broad unresolved signal present in the

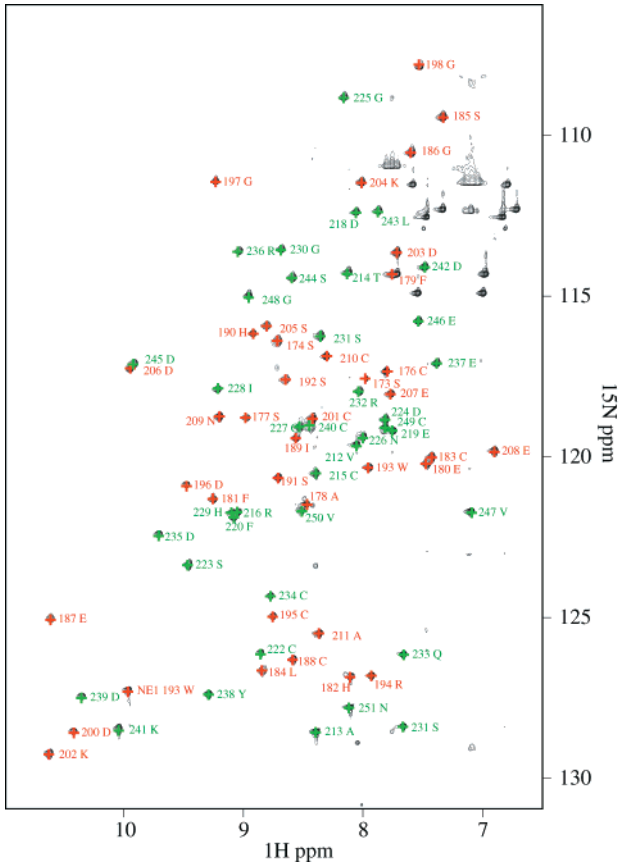

FIGURE 3: Assigned ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled native LR5−6 in 10 mM calcium chloride at pH 6.5, 25 °C. Residues in LR5 (172−211) are identified in red, and residues in LR6 are marked in green.

middle of the spectrum. Together, these NMR observations provide compelling evidence that, for each mutant, the unaltered module of the pair can fold to its native structure despite being covalently attached to a misfolded module.

Native LR5 Is Not Affected by Different Attached Misfolded Forms of LR6. The HPLC chromatograms of the analytical refolding reactions (Figure 2) reveal that a complex mixture of products is formed when folding of any of the four mutated LR5−6 module pairs is attempted. However, the NMR spectra taken with the entire ensemble of products (Figure 4) does not establish whether folding has been essentially quantitative. To determine whether an overwhelming majority of the molecules in the ensemble have one nativelike module attached to a misfolded module, the mixture of products from refolding of the D245E mutant was separated into four fractions that could be resolved by HPLC (see Experimental Procedures). The HSQC spectrum of each individual fraction shows that LR5 acquires nativelike structure in all four fractions and that the LR5 conformation is essentially unperturbed regardless of the nature of the LR6 misfolded species attached (Figure 5). These data demonstrate that minimal structural perturbation is imparted to the unmutated module, which retains its native conformation regardless of the range of misfolded conformations adopted by its mutated neighbor.

Minimal Variation in LR5 Ca^{++} Affinity Even When Attached to Different Misfolded Forms of LR6. The fluorescence emission signal of tryptophan 193 (W193) of LR5 increases in response to calcium binding (*18*). Because LR6 contains no tryptophan residues, W193 also provides a probe for the binding of calcium to LR5 in the LR5−6 pair. Therefore, the affinity of module five for Ca^{2+} in the presence of different misfolded forms of LR6 was determined by measuring the change in fluorescence emission of tryptophan 193 as a function of free Ca^{2+} in an EGTA buffered solution.

The K_d for the binding of Ca^{2+} by module five of native LR5−6 under these conditions is 170 ± 8 nM. The K_d values for each of the four D245E fractions used for NMR spectroscopy and the apparent K_d value for the ensemble of E219K species all lie within 20% of the value measured for module five in native LR5−6 (Figure 6). Thus, the calcium-binding affinity of LR5 does not change when attached to a misfolded form of LR6. Because the fluorescence signal from the lone tyrosine residue of LR6 is overwhelmed by W193 of LR5, it is not possible to determine by fluorescence whether the calcium affinity of LR6 (∼200 nM, see ref *23*) is altered when covalently connected to a misfolded form of LR5.

DISCUSSION

Many cell surface receptors have contiguous cysteine-rich repeats that comprise modular structural elements. These receptors are frequently targets of point mutations implicated in human disease. Well-studied examples include the protein fibrillin-1, which is mutated in Marfan's syndrome (*35*) and other connective tissue disorders, and the LDLR, which is mutated in familial hypercholesterolemia (*36*).

Disease-causing point mutations in these two proteins result in protein folding defects when examined in the context of isolated modules in vitro (e.g., refs *18*, *28*, *37*, and *38*). However, relatively little is known about whether a folding defect that results from a point mutation in a multimodular cell surface receptor is restricted to the module in which the mutation lies or is propagated to adjacent modules. Conversely, it is not known whether a properly folded module can partially or completely rescue a protein folding defect observed in a neighboring module.

FH mutations in modules five or six of the LDLR studied in the context of the LR5−6 module pair still fail to refold to a single native disulfide isomer under conditions that allow disulfide interchange (Figure 2). However, detailed analysis of the products of refolding indicates that the presence of a covalently attached, misfolded form of module five does not prevent proper folding of module six (Figure 4, panels A and B), nor does a misfolded form of module six interfere with proper folding of covalently connected module five (Figure 4, panels C and D). In fact, in the D245E LR5−6 mutant, LR5 retains its native conformation regardless of the precise misfolded conformation of its mutated LR6 neighbor, supporting the proposal that individual LDL-A modules of the LDLR fold independently and that misfolding from an FH mutation in LR5 or LR6 is a local event (see also ref *39*). Our findings also suggest that the structure of an LDL-A module is robustly encoded by its native sequence, because even an extreme folding defect in an adjacent module presenting a high local concentration of nonnative surface is unable to prevent folding of the covalently attached native module.

Previous structural, biophysical and functional studies of the LDLR are consistent with these studies of FH mutations

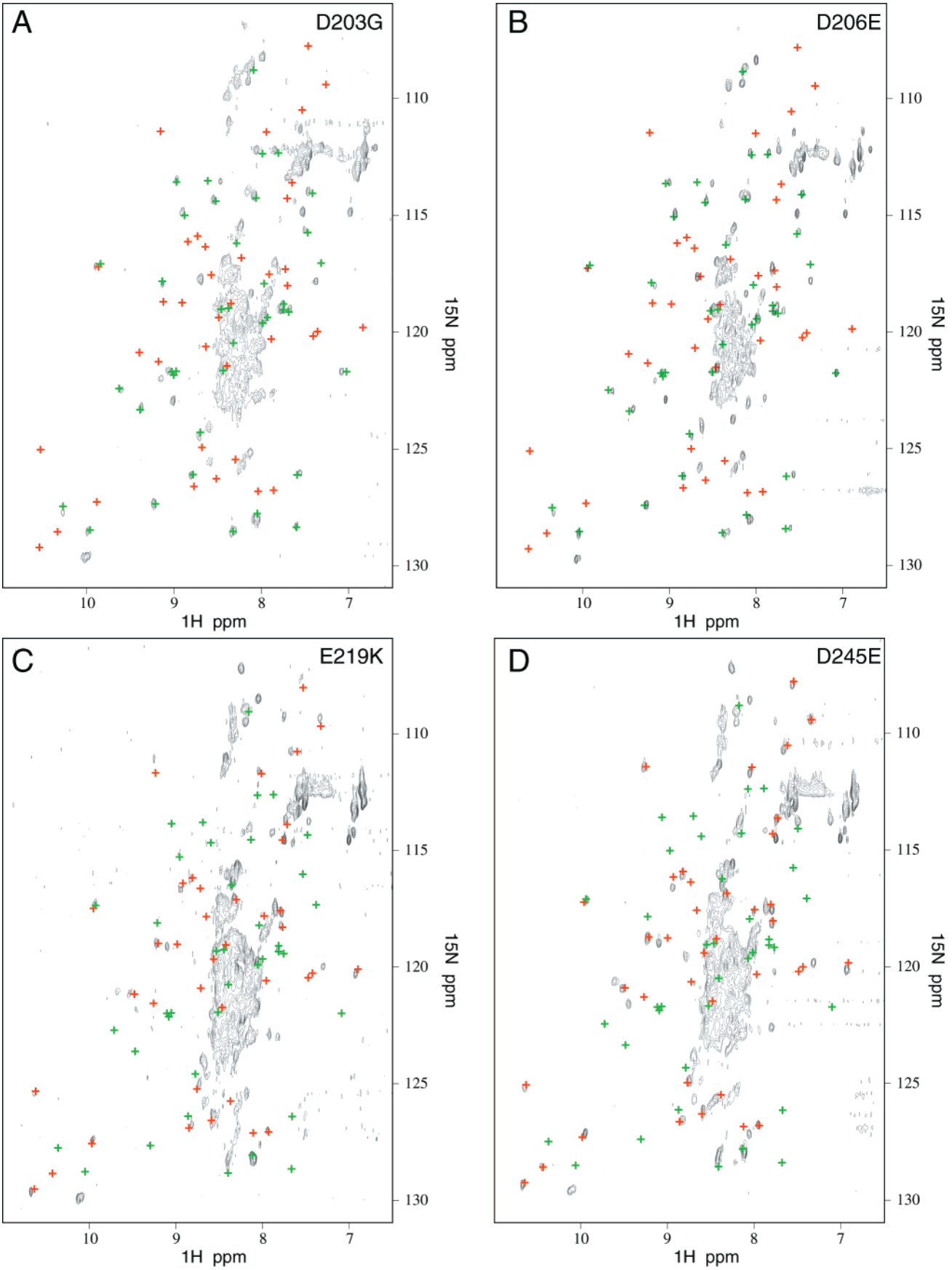

FIGURE 4: HSQC spectra of the ensemble of products after refolding of the D203G (A), D206E (B), E219K (C), and D245E (D) mutants of LR5−6. Positions of native LR5 resonances are indicated with red crosses; green crosses mark the positions of native LR6 resonances.

on folding of the LR5−6 module pair. Solution NMR studies of the LR1−2 and LR5−6 module pairs indicate that covalent connection of the individual modules results in little structural interaction between the two modules in the pair,

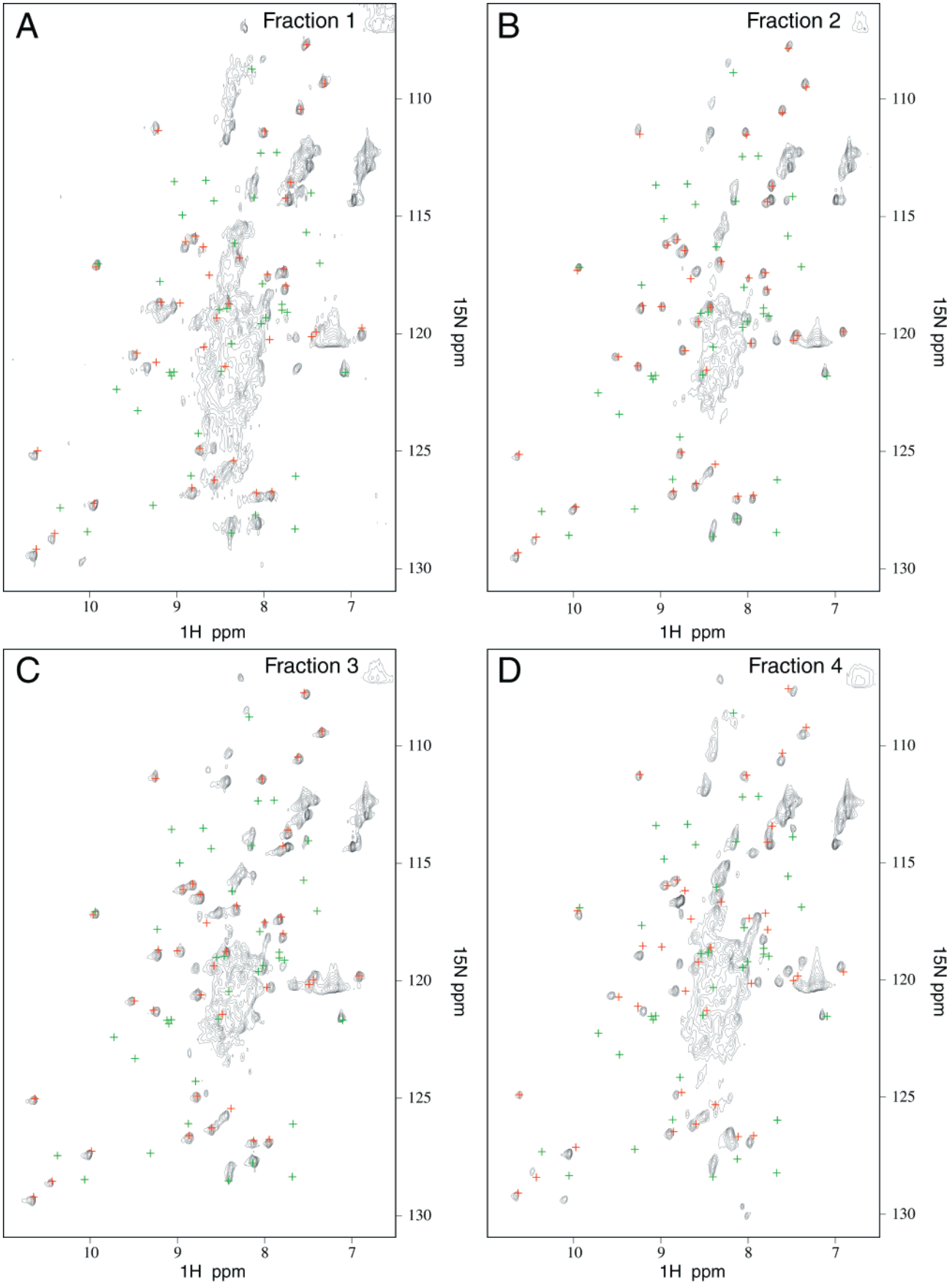

FIGURE 5: HSQC spectra of four different HPLC fractions of the products of refolding of D245E. Positions of native LR5 resonances are indicated with red crosses; green crosses mark the positions of native LR6 resonances.

as detected by chemical shift perturbation of Cα-H resonances (*20*) or of backbone NH resonances in HSQC spectra (*23*). In addition, studies of the backbone dynamics of the module 5−6 pair by NMR show that the linker region

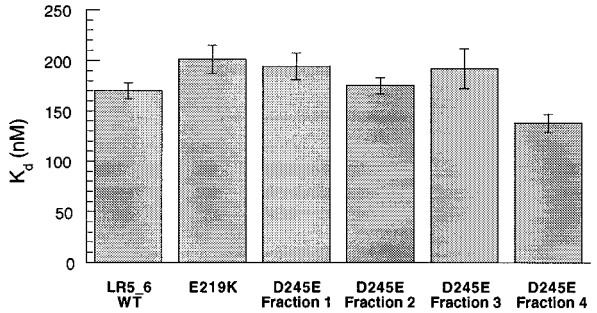

FIGURE 6: Bar graph comparing the calcium affinity of the LR5 module in native LR5−6 with the affinity of the LR5 module in the E219K LR5−6 mutant and in each of the four HPLC-purified fractions of the D245E mutant.

between the two repeats is flexible and undergoes fast internal motion. Furthermore, no changes in the pattern of internal dynamics are detected in the individual modules of the 5−6 repeat pair as a consequence of being covalently connected (Natalia Beglova, CLN, and SCB, unpublished results).

Relationship to Previous Functional Studies. To investigate the functional role of individual LDL-A modules in lipoprotein binding, Brown and Goldstein and their co-workers constructed a series of deletion and site-directed mutants of the LDL-A modules of the LDLR (*10*, *11*). Point mutation of a conserved aspartate or isoleucine residue (nonconservative I→D or D→Y substitutions) mimics the effect of deletion of the entire repeat (*11*). Each of the two point mutations (I189D or D206Y) causes misfolding (*23*) of a prototype module (LR5), but neither one appears to affect processing of full-length receptors, which still arrive at the cell surface with near normal efficiency (*11*).

Their observations led to a model for LDLR function in which individual modules provide independent, additive contributions to ligand binding (*10*, *11*). Our observations regarding module independence in structure, folding, and flexibility, anticipated by the functional studies of Brown and Goldstein's group, lend complementary structural and biophysical support for such a model.

Comparison with Other Calcium-Binding Cysteine-Rich Modules of Cell Surface Receptors. The other types of calcium-coordinating cysteine-rich modules that have been studied as contiguous pairs by NMR are epidermal growth factor (EGF) modules. These modules occur as contiguous repeats in numerous proteins, including the LDLR and human fibrillin-1, which is mutated in Marfan's syndrome. Though EGF-like modules with calcium-binding motifs are capable of folding independently (*25*), adjacent modules exhibit cooperativity in their calcium binding properties (*40*, *41*), and the domain interface between contiguous EGF modules is stabilized by local contacts between modules induced upon calcium binding (*42*).

The G1127S fibrillin-1 mutation, which lies in module 13, causes misfolding of both the isolated module and of module 13 in the 13−14 pair (*38*). However, the 2D-NOESY spectrum of the product ensemble from attempted refolding of the G1127S module 13−14 pair has cross-peaks characteristic of natively folded module 14, demonstrating that module 14 is nativelike in at least some refolded molecules, despite being attached to a misfolded module 13.

Correlation with FH Phenotype. The LDL uptake activities of fibroblasts from patients homozygous for the D206E and D245E mutations have been reported to be 5−15% and 15−30% of normal, respectively (*9*). (The uptake activities of fibroblasts from patients with D203G and E219K mutations have only been measured in compound heterozygotes.) Although D206E and D245E lead to comparable folding defects in vitro (*18*, *29*), the severity of the D206E functional defect is greater in vivo, probably because module five plays a more important role in lipoprotein binding, rather than because of differences in receptor processing or turnover.

Implications for Small Molecule Rescue of Folding Defects in FH and Other Folding Diseases. A small molecule that rescues a folding defect in the DNA binding domain of the tumor suppressor p53 has been identified from screening of a compound library (*43*). By analogy, a ligand that binds preferentially to the native conformation of an LDL-A module with high enough affinity should rescue a folding defect resulting from a mutation in that module. If the stability of an individual folded LDL-A module is indeed highly dominated by local interactions, a small molecule identified in a screen or selection performed on an isolated LDL-A module should also rescue folding of the LDLR in vivo if it penetrates efficiently into the endoplasmic reticulum where folding takes place. Because of the independence of individual modules, the effective concentration of the small molecule could be raised in the screen by tethering it to a ligand that binds to an adjacent module on the native receptor, or even via a short linker to the mutant module itself.

ACKNOWLEDGMENT

We thank Lynn Kim for excellent technical assistance in preparing and refolding unlabeled LR5−6 mutants. We are also indebted to Susan Pochapsky of the MIT/Harvard Center for Magnetic Resonance and Gregory Heffron of Dr. Gerhard Wagner's laboratory for technical assistance with NMR spectrometers, Dr. Gerhard Wagner for use of NMR spectrometers at Harvard Medical School, and the MIT/Harvard Center for Magnetic Resonance (supported by Grant RR00995 from the NIH) for use of NMR spectrometers housed at MIT.

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BI0015156