NMR Solution Structure of Complement-like Repeat CR8 from the Low Density Lipoprotein Receptor-related Protein*

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The low density lipoprotein receptor-related protein is a member of the low density lipoprotein receptor family and contains clusters of cysteine-rich complement-like repeats of about 42 residues that are present in all members of this family of receptors. These clusters are thought to be the principal binding sites for protein ligands. We have expressed one complement-like repeat, CR8, from the cluster in lipoprotein receptor-related protein that binds certain proteinase inhibitor-proteinase complexes and used three-dimensional NMR on the ¹³C/¹⁵N-labeled protein to determine the structure in solution of the calcium-bound form. The structure is very similar in overall fold to repeat 5 from the low density lipoprotein receptor (LB5), with backbone root mean square deviation of 1.5 Å. The calcium-binding site also appears to be homologous, with four carboxyl and two backbone carbonyl ligands. However, differences in primary structure are such that equivalent surfaces that might represent the binding interfaces are very different from one another, indicating that different domains will have very different ligand specificities.

The low density lipoprotein receptor-related protein (LRP) is a member of the low density lipoprotein receptor family (1). Members of this family of receptors contain a small cytoplasmic domain, a single membrane-spanning helix, and clusters of two types of small cysteine-rich repeats on the extracellular side, interspersed with regions that contain a YWTD motif. The two types of cysteine-rich repeat are an epidermal growth factor-like repeat of about 50 residues and a complement-like repeat, so named for its presence in complement components C8 and C9, of about 42 residues. Each of these types of repeat contains 6 cysteines that are involved in three intradomain disulfide bonds. The limited evidence on the location of protein ligand-binding sites indicates that such sites are restricted largely to the clusters of complement-like repeats. The LDL receptor contains one cluster of seven such repeats and binds various apolipoproteins, including apoE and apoB100 (2). LRP contains a total of 31 such repeats organized into four clusters of 2, 8, 10, and 11 repeats (3). These clusters have been designated clusters I, II, III, and IV, respectively. LRP binds a much wider range of protein ligands including apolipoproteins, serpin-proteinase complexes, and α₂-macroglobulin-proteinase complexes (1). Studies on truncated LRP species suggest that cluster II is a major locus for protein ligand binding (4).

Although structures of three complement-like repeats have been reported from the LDL receptor, one by x-ray crystallography (5) and two by NMR spectroscopy (6, 7), no structure of such a repeat has previously been reported from LRP. Both because of the sequence differences between repeats in regions that might form the ligand-binding sites and also because of the more extensive range of protein ligands that LRP can bind compared with the LDL receptor, it is important to make structural comparisons between repeats from different receptors and between repeats from the same receptor to understand differences in ligand and calcium specificity (Fig. 1). We report here the first structure of a complement-like repeat from LRP, CR8. CR8 is the eighth complement-like repeat from the N terminus and is the sixth repeat in cluster II. In overall fold CR8 is very similar to LB5, which in turn is similar to the folds of two other domains from the LDL receptor for which NMR structures are known, LB1 (6) and LB2 (7). However, because only 4 of 25 residues are identical between these four domains (excluding cysteines and calcium coordinating residues), 1) each domain presents very different charge densities and hydrophobic patches on its surface, suggesting that each domain will be capable of very different interactions with protein ligands and thus have its own distinct ligand specificity.

EXPERIMENTAL PROCEDURES

Cloning, Expression, and Folding of CR8—CR8 was cloned as described previously (8). ¹³C/¹⁵N-labeled CR8 was expressed as a GST fusion protein in minimal medium containing 0.6% basal medium Eagle vitamin solution (Life Technologies, Inc.), 1 g liter⁻¹ (¹⁵NH₄)₂SO₄, and 2 g liter⁻¹ ¹³C-labeled glucose. Optimal yield was achieved by harvesting the cells 6 h after induction. GST-complement repeat fusion protein was purified according to Ref. 9, cleaved with thrombin (1/1000 w/w, 5–10 min at 20 °C) and rechromatographed on GSH-Sepharose. Refolding was performed as described. Correct folding was indicated by the ability of the protein to bind calcium tightly, as shown by tryptophan fluorescence change, and to give sensitized terbium fluorescence emission when terbium was bound (8). Protein concentrations were determined spectrophotometrically using an extinction coefficient of 5500 M⁻¹ cm⁻¹ estimated from the amino acid composition (10).

NMR Spectroscopy—NMR spectra were recorded at the University of Illinois at Chicago on a four channel Bruker DRX600 equipped with a pulsed field gradient accessory and operating at 600.13 MHz for ¹H. NMR data were processed and analyzed using Triad 6.3 software (Tripos, Inc., St. Louis, MO). Lyophilized CR8 was dissolved in 20 mM 2-macroglobulin-proteinase complexes and used three-dimensional NMR on the ¹³C/¹⁵N-labeled protein to determine the structure in solution of the calcium-bound form. The structure is very similar in overall fold to repeat 5 from the low density lipoprotein receptor (LB5), with backbone root mean square deviation of 1.5 Å. The calcium-binding site also appears to be homologous, with four carboxyl and two backbone carbonyl ligands. However, differences in primary structure are such that equivalent surfaces that might represent the binding interfaces are very different from one another, indicating that different domains will have very different ligand specificities.

The atomic coordinates and structure factors (code 1cr8) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

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The abbreviations used are: LRP, low density lipoprotein receptor-related protein; LDL, low density lipoprotein; GST, glutathione S-transferase; HSQC, heteronuclear single quantum coherence; rmsd, root mean square deviation; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy.
**CD3COOD, 10% D2O, pH 5.5, and the pH was maintained at 5.5 by addition of NaOH. The final concentration of protein was approximately 2 mg/mL. Spectra were recorded at 298 K. Resonance assignments and distance constraints were determined using HHB/CO/NH (11), CB-CA(CO/NH) (12), TOCSY-HSQC (13), 15N-NOESY-HSQC (13), HCCH-TOCSY (14), and 13C-NOESY-HSQC and HNHA (15) experiments. HNCA (16) was used for the backbone sequential assignment through α-carbon. Some ambiguities in sequential connectivities of backbone Cα atoms were resolved on the basis of Hα and Hβ chemical shift comparisons between HHB/CO/NH and TOCSY-HSQC. From these three triple resonance experiments, it was possible to complete the sequential assignments. TOCSY-HSQC and HCCH-TOCSY were used for side chain proton chemical shift assignment. HHB/CO/NH was used to distinguish β-protons and γ-protons when their proton chemical shift frequencies were very close. The ambiguities in Hα and Hβ assignment (threonine and serine) were resolved by their connected carbon using HCCH-TOCSY. HCCH-TOCSY and 15N-NOESY-HSQC were also used for side chain carbon assignments from known proton assignment. NOE constraints were obtained from 15N-NOESY-HSQC and 13C-NOESY-HSQC. Dihedral angle constraints were obtained from an HNHA experiment. HNQC spectra of a sample of CR8 freshly prepared in D2O were recorded to identify slowly exchanging amide protons and hydrogen bonds. Identification of backbone carbonyls involved in coordination to the calcium was made using a two-dimensional HACACO to permit assignment of backbone and side chain carbonyl chemical shifts. A two-dimensional [15N-H1] HSQC experiment was also carried out on the doubly labeled sample, with 13C decoupling at CO and Cα frequencies. Sensitivity enhancement gradient pulse sequences were employed for all experiments in which magnetization was detected on the amide proton. Center frequencies were 4.70 ppm for 1H, 118 ppm for 13C, and 55 ppm for 15N. 55 ppm for 13C was used for 13C decoupling at CO and Cα frequencies. The sequential connectivities from the C-terminal valine through to Pro21 were determined by HNCA and, where Cα assignments were not available. These distance constraints, as well as the sequential assignments, were used as additional constraints before annealing. Six hydrogen bonds derived from proton exchange HSQC experiment were also included in the calculation. Energy minimization used the package AMBER (20) and was carried out for steps up to a maximum of 0.01 ps. Force constants of 32 kcal mol⁻¹ Å⁻² for distance constraints and 50 kcal mol⁻¹ radian⁻² for dihedral angle constraints were employed. The constraint energy given in Table I is a measure of the mean energy violation, based on these force constants and using the formula: constraint energy = force constant • (current interatom separation - target separation)². Comparisons of the structures of CR8 and LB5 and graphics representations of structures of CR8, LB5, LB1, and CR8, the eighth complement-like repeat from LRP, only adopts a well defined structure in the presence of calcium (8), which is consistent with the requirement for calcium for tight binding of protein ligands by both LRP and the closely related LDL receptor. The assignments and structure determination reported here are therefore for the calcium complex, obtained in the presence of a saturating level of calcium. All NMR assignments of CR8 were carried out on a uniformly 13C-,15N-labeled and 13N-labeled 44-residue construct prepared as described above. This molecule contains 42 residues (3–44) from LRP (residues 1040–1081) with an additional N-terminal Gly-Ser that resulted from the restriction site present in the vector used to create the GST-CR8 fusion protein DNA construct. The calcium complex was well behaved and gave a single set of resonances, consistent with a single well defined conformation. Despite the small size of the molecule, there was good spectral dispersion, which is illustrated by a 1H decoupled [15N-H1]-HSQC NMR spectrum (Fig. 2). Backbone amides had a 1H chemical shift range of 3.5 ppm, (from 6.73 ppm for Lys19 to 10.23 ppm for Asp33) and a 13N chemical shift range of 21ppm (from 107.7 ppm for Gly2 to 128.8 ppm for Asp33). Assignment of backbone amides (amide 15N and 1H, Cα, 13C, and 1H) was made with the aid of triple resonance experiments. Starting from distinctive residues such as glycine, small sequence elements were determined through sequential connectivities using triple resonance experiments. Experiments used in this work include HNCA, HHB/CO/NH and N-15 edited TOCSY-HSQC. From these experiments, the identities of individual amino acids were tentatively determined from their Cα, Hα, and Hβ chemical shifts, and these small sequence elements were then further assigned to amino acids in the protein by comparison with the known sequence. The C-terminal amino acid triplet Glu-Gly-Val Cα sequential assignment was easily made from an HNCA experiment, because Gly-Val is a unique pair in the CR8 sequence. This assignment was independently confirmed by comparison of Hα and Hβ between HHB/CO/NH and TOCSY-HSQC experiments. The sequential connectivities from the C-terminal valine through to Pro21 were determined by HNCA and, where Cα...**

**Table 1.** NMR Structure of CR8 from LRP

| Repeat | CR8 | LB5 |
|--------|-----|-----|
| #      |     |     |
| 1      | G   | P   |
| 2      | S   | C   |
| 3      | P   | S   |
| 4      | G   | F   |
| 5      | G   | F   |
| 6      | G   | H   |
| 7      | C   | G   |
| 8      | H   | D   |
| 9      | T   | E   |
| 10     | E   | D   |
| 11     | D   | E   |
| 12     | R   | D   |
| 13     | L   | C   |
| 14     | D   | A   |
| 15     | E   | C   |
| 16     | P   | S   |
| 17     | T   | H   |
| 18     | L   | S   |
| 19     | T   | E   |
| 20     | L   | D   |
| 21     | H   | A   |
| 22     | I   | S   |
| 23     | H   | S   |
| 24     | I   | S   |
| 25     | H   | S   |
| 26     | R   | W   |

**Fig. 1.** Primary structure of CR8 and alignment with primary structure of LB5 from the LDL receptor, for which an x-ray structure of the calcium complex has been determined (5). The first two residues of the CR8 construct (GS) are not present in LRP and were introduced as part of the restriction site in the GST-CR8 fusion protein. Alignment was made at the six conserved cysteines. The 6 residues shown in bold are those that coordinate to the calcium in LB5 either through backbone carbonyls (W and G) or through carboxyl side chains. The numbering for LB5 is that used by Fass et al. (5).

**RESULTS AND DISCUSSION**

**Assignments**—We have previously shown qualitatively that CR8, the eighth complement-like repeat from LRP, only adopts a well defined structure in the presence of calcium (8), which is consistent with the requirement for calcium for tight binding of protein ligands by both LRP and the closely related LDL receptor. The assignments and structure determination reported here are therefore for the calcium complex, obtained in the presence of a saturating level of calcium. All NMR assignments of CR8 were carried out on a uniformly 13C-,15N-labeled and 13N-labeled 44-residue construct prepared as described above. This molecule contains 42 residues (3–44) from LRP (residues 1040–1081) with an additional N-terminal Gly-Ser that resulted from the restriction site present in the vector used to create the GST-CR8 fusion protein DNA construct. The calcium complex was well behaved and gave a single set of resonances, consistent with a single well defined conformation. Despite the small size of the molecule, there was good spectral dispersion, which is illustrated by a 1H decoupled [15N-H1]-HSQC NMR spectrum (Fig. 2). Backbone amides had a 1H chemical shift range of 3.5 ppm, (from 6.73 ppm for Lys19 to 10.23 ppm for Asp33) and a 13N chemical shift range of 21ppm (from 107.7 ppm for Gly2 to 128.8 ppm for Asp33). Assignment of backbone amides (amide 15N and 1H, Cα, 13C, and 1H) was made with the aid of triple resonance experiments. Starting from distinctive residues such as glycine, small sequence elements were determined through sequential connectivities using triple resonance experiments. Experiments used in this work include HNCA, HHB/CO/NH and N-15 edited TOCSY-HSQC. From these experiments, the identities of individual amino acids were tentatively determined from their Cα, Hα, and Hβ chemical shifts, and these small sequence elements were then further assigned to amino acids in the protein by comparison with the known sequence. The C-terminal amino acid triplet Glu-Gly-Val Cα sequential assignment was easily made from an HNCA experiment, because Gly-Val is a unique pair in the CR8 sequence. This assignment was independently confirmed by comparison of Hα and Hβ between HHB/CO/NH and TOCSY-HSQC experiments. The sequential connectivities from the C-terminal valine through to Pro21 were determined by HNCA and, where Cα...
Three-dimensional 15N-edited TOCSY-HSQC and three-dimensional 13C-edited HCCH-TOCSY were used for other protons, 73 side chain carbons, and 158 side chain hydrogen atoms, but no amide-amide and side chain-amide NOE cross-peaks. The molecule was used for the sequential assignment from Ile20 to the amide of Gln12. The cross-peak for Ser2 is weak and below the contour level used. Its location, at 8.46 ppm for 1H, is indicated by an empty box.

was degenerate, by HBHA(CO)NH and TOCSY-HSQC. Cys19 has the highest 1H chemical shift (5.29 ppm). This was also used as a starting point for sequential assignment by comparison of HBHA(CO)NH and TOCSY-HSQC spectra. The same strategy as described above for the C-terminal half of the molecule was used for the sequential assignment from Ile20 to Pro3. Sequential assignments were checked against both amide-amide and side chain-amide NOE cross-peaks. Three-dimensional 15N-edited TOCSY-HSQC and three-dimensional 13C-edited HCCH-TOCSY were used for other proton and carbon side chain assignments. Some ambiguities of proton assignment were resolved by HBHA(CO)NH connectivities or by their attached carbon in HCCH-TOCSY (e.g. Hα and Hβ of threonine). Carbon side chain assignment ambiguities were resolved by 13C-HCC-HN-NOESY. Some aromatic proton assignment were made from two NOESY experiments. A two-dimensional experiment, Hα/Cα/CO, was used for side chain and backbone carbonyl assignments to unambiguously assign seven backbone carbonyls, including the backbone carbonyls of residues 24 (Trp in both CR8 and LB5) and 29 (Asp in CR8 but Gly in LB5), which are the two residues that have been shown by x-ray crystallography to contribute carbonyl ligands to the bound calcium in a repeat from the LDL receptor (5). A total of 419 assignments were made, consisting of all backbone amide and Cα atoms, except for the Cα 13C for residues Gly1 and Ser2 and the two proline 15N resonances, 7 backbone carbonyl carbons, 73 side chain carbons, and 158 side chain hydrogen atoms.

NOE constraints (Fig. 3) were obtained from the following two experiments: three-dimensional 15N-edited NOESY-HSQC with a mixing time of 120 ms and a three-dimensional 1H-13C-correlated NOESY-HSQC in D2O with a mixing time of 120 ms.

Only three i, i+3 dNOE were observed, between Pro21 and Trp24, between Leu22 and Arg25, and between Ser26 and Lys30. Dihedral angle constraints were obtained from HHNA measurements of 3JH-Hα coupling constants and chemical shift index-based assignment of secondary structure. Six slowly exchanging amide protons were identified from HSQC spectra of a freshly prepared sample in D2O (Fig. 3).

Structure Calculation—Structural calculations for Ca2+-CR8 were performed using the torsion angle dynamics annealing simulation program DYANA, with a total of 545 NOE upper distance constraints, 31 torsion angle constraints, three disulfide bonds, and 6 hydrogen bonds as input (Table I). The NOEs consisted of 208 intraresidue, 160 sequential, 74 medium range (1<i,j<5), and 103 long range interactions. The composition of the three disulfides was based on the previously determined pattern of disulfides in the homologous LB1 (19) and LB5 (5) domains from the LDL receptor. Disulfides were accordingly specified for the cysteine pairs 6–19, 13–32, and 26–41.

The final 40 best conformers produced by DYANA from an input of 100 initial structures were subject to energy minimization using the all-atom potential program AMBER 5.0 (20, 21). All NMR constraints were employed in the energy minimization. Energy minimization calculations were carried out both without and with calcium specifically coordinated. Because the four carboxylates and two backbone carbonyls that coordinate to calcium in LBS are all present in CR8 in equivalent positions (carboxylates of Asp27, Asp31, Asp37, and Glu38 and carboxyls of Trp24 and Asp29 using CR8 numbering), similar ligation was used for CR8. Evidence concerning the coordination of the two backbone carbonyls was provided from 13C carbonyl chemical shifts of the tryptophan and amide 15N chemical shifts of the residues immediately C-terminal to Trp24 and Asp29. Thus the tryptophan carbonyl was strongly downfield shifted relative to the random coil value (177.9 ppm compared with 173.6 ppm, respectively) (22), and the amide nitrogens of Arg25 and Thr30 were also strongly downfield shifted about 7 ppm relative to random coil values (observed values of 127.3 and 121.0 ppm compared with random coil values of 120.8 and 114.2 ppm respectively (22)). The chemical shift of the carboxyl of Asp29 was less definitive, being close to the expected random coil value. However, the large perturbation of the amide nitrogen of the adjacent threonine makes it likely that calcium does coordinate to the Asp29 carboxyl.

Although the number of distance and angle violations did not differ very much between calculations carried out in the absence or presence of calcium coordination (Table I), the overall energy of the final structures obtained in the presence of calcium (mean of $-362$ kcal mol$^{-1}$) was very much lower than in the absence of calcium (mean of $-262$ kcal mol$^{-1}$) (Table I). The best 20 structures reported (Fig. 4) and the mean structure (Fig. 5) are therefore for structures calculated with calcium coordinated. For the 20 best structures only 1.3 distance violations >0.2 Å were found per structure, with no angle violations >10° (Table I).

Final Structures—The final set of 20 best structures (Fig. 4) and mean structure (Fig. 5) can be roughly described as formed from a Greek Ω in which the top of the loop has been folded backwards and down. This gives two loops, one on the left and one on the right. Each loop is held together by a disulfide between front and back, with a third disulfide holding the two loops together at the top. The left-hand loop contains a small two-stranded anti-parallel β-sheet, and the left- and right-hand loops are linked at the bottom through a short α-helix.

Most of the structure is very well defined, with rmsd for the backbone of 0.52 Å for residues 6 to 41 (Table I). This increases to 1.34 Å when all heavy atoms are included for this region. The
N-terminal 5 residues and C termini 3 residues are only poorly defined and so cause large increases in rmsd for both backbone (2.10 Å) and heavy atoms (2.44 Å). This is not surprising because both ends act as linkers to the next domains in intact LRP and presumably must show some conformational flexibility. In addition residues 1 and 2 (Gly and Ser, respectively) are not part of CR8, being present as a consequence of the means of expressing and purifying the domain.

Detailed inspection of the best low energy structures (Figs. 4 and 5) shows that residues His7–Glu10 form a β-turn structure. The amide proton of residue Glu10 exchanges slowly and thus appears to be involved in a hydrogen bond. This residue is followed by a short two-strand anti-parallel β-sheet formed by residues Phe11–Cys13 and residues Leu18–Ile20. Strong sequential dNN and cross-strand dNN, dNN, and dnn NOEs could be observed in this β-sheet. The amide protons of Phe11 and Ile20 also exchange slowly and thus appear to form hydrogen bonds with each other. This mini-sheet is followed by a one and a quarter turn α-helix from residues Pro21–Arg25. NOE cross-peaks were observed in 15N NOESY-HSQC between the Pro21 α-proton and the Trp24 amide proton and between the Leu22 α-proton and the Arg25 amide proton. The amide protons of residues Trp24 and Arg25 exchanged slowly, consistent with a hydrogen bond formation to the carbonyls of Pro21 and Leu22, respectively. Residues Phe51 and Ile60, which are strongly conserved in LRP repeats and also present in LB5 from LDL expressing and purifying the domain. Without calcium present

| Distance restraint violations of 0.2–0.3 Å | 1.2 ± 0.6 |
| Distance restraint violations of 0.3–0.4 Å | 0.2 ± 0.3 |
| Distance restraint violations of 0.4–0.5 Å | 0 |
| Distance restraint violations >0.5 Å | 0 |
| Angle constraint violation >10° | 0 |
| Six-coordinate calcium | 1.1 ± 0.5 |
| Distance restraint violations of 0.2–0.3 Å | 0.2 ± 0.3 |
| Distance restraint violations of 0.4–0.5 Å | 0 |
| Distance restraint violations >0.5 Å | 0 |
| Angle constraint violation >10° | 0 |
| AMBER energies (mean ± S.D.) | kcal mol⁻¹ |
| Without calcium present | Total energy | −262 ± 28 |
| Constraint energy | 7.6 ± 0.9 |
| Six-coordinate calcium | Total energy | −362 ± 38 |
| Constraint energy | 7.4 ± 0.8 |

The calcium-binding site is composed entirely of ligands from the right-hand loop of the structure (Fig. 6). Because the front and back parts of the loop do not form a β-sheet with one another, which might stabilize the structure (in contrast to the situation in the left-hand loop of the domain (Fig. 5)), it is understandable why calcium binding serves to rigidify the conformation of this and other complement-like domains (5, 8, 24). Thus, the calcium bridges the front and back parts of the loop (Asp27 and Glu48 in front and Asp27, Asp29, and Asp31 in the back) and holds them together and, at the same time, tethers the loop to the n-helix that links the left and right loops, through the carbonyl of Trp24. The simultaneous neutralization by the positively charged calcium of multiple negatively charged residues, which would otherwise repel one another, would further help to stabilize this single structure. A potentially significant difference between LB5 and CR8 is that the glycine that coordinates the calcium in LB5, through its carbonyl, is an aspartate in CR8. Although chemical shift evidence strongly suggests that Asp28 also coordinates calcium through its carbonyl, it is also possible that the aspartate carbonyl could act as a calcium ligand, either in addition to the other four carboxyls or in place of one of them. Energy minimizations carried out with this side chain as a seventh ligand resulted in no significant improvement in the energy of the structure (data not shown), so this must remain speculative.

![Diagram](Image 255x553 to 554x729)
Comparison of the Structures of CR8 and LB5—In the region between the first and last of the six cysteines, LB5 is one residue shorter than CR8 (Fig. 1): an arginine inserted at position 14. Despite this insertion, there is overall a very high degree of structural similarity between the two proteins (Fig. 7 and Table II). Thus the rmsd for the backbone of the whole structure, excluding residues beyond the end cysteines (residues 6–41) is 1.45 or 1.34 Å, depending on whether comparison is made with the lowest energy CR8 structure or the mean structure, respectively. If the left- and right-hand loops are considered separately, these comparisons further improve substantially. The left-hand "β-sheet loop" (residues 6–23) shows 1.20 Å rmsd between the two structures, and the calcium-binding loop (residues 24–41) shows even smaller rmsd of 0.90 Å. This high degree of similarity is despite very high variability of the primary structure, when cysteines and calcium coordinating ligands are excluded (Fig. 1). It is therefore likely that in overall fold, all of the complement-like domains in LRP and the LDL receptor will be very similar. Given the major differences in ligand specificity of LRP and the LDL receptor, the basis for these differences must therefore lie in the different residues that are presented on the surface.

Comparison of the Surfaces of CR8, LB5, LB1, and LB2—Two different proposals have been made to explain the binding of protein ligands to complement-like repeats of proteins of the
LDL receptor family. One is that clusters of basic residues on the ligand interact with acidic residues on the receptor (2), recognizing the four acidic residues that are almost completely conserved in all of these repeats and that a cluster of basic residues has been shown to be required for binding of apoE to the LDL receptor (2). The second is that hydrophobic interactions play a more important role (5), because most of the conserved acidic residues present in the C-terminal parts of these domains are involved in calcium binding and would therefore not be available to bind basic residues on the protein ligands.

Both of these proposals are perhaps too one-sided and assume a common binding mode for all ligands to receptors from this family. Instead, it should be recognized that because most residues in these repeats that are neither cysteines nor putative calcium ligands will be on the surface of these small domains, the high variability of sequence between different domains will result in unique surfaces for each domain and corresponding unique ligand specificities. This is well illustrated by a comparison of the face of LB5 proposed to be a ligand-binding face (5) with the equivalent face of CR8 (Fig. 8).

Very different charge densities are found in these two domains. These arise from substantial differences in residues that occur on this face. The most notable differences are as follows, using the numbering for each domain given in Fig. 1: Phe in LB5 is Asp in CR8; Arg in CR8 is an insertion; Ser in LB5 is Arg in CR8; Glu of LB5 is Lys in CR8; and Ala in LB5 is Glu in CR8.

These represent changes from acidic to basic, from polar to positively charged, from hydrophobic to negatively charged, and, for Arg of CR8, an addition of a positive residue. CR8 is not atypical in regard to these differences between pairs of complement-like domains. There are thus major differences in composition of equivalent residues in two other domains of known structure from the LDL receptor, LB1 and LB2 (Table III), and differences in the resulting contour surface and charge density (Fig. 8), again despite very similar backbone folds. This further emphasizes that from the point of view of understanding receptor-ligand interactions involving this family, the structure of each interacting domain must be specifically considered and therefore be available by x-ray and/or NMR means.

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**TABLE II**

| Comparison of the structures of CR8 and LB5 |
|------------------------------------------|
| Comparisons were made using MOLMOL (18). |
|------------------------------------------|

| Domain | rmsd of mean CR8 structure compared with structure of LB5 (Å) |
|--------|-------------------------------------------------------------|
| Whole structure (5–42) | 1.46 |
| Whole structure (6–41) | 1.34 |
| β-Sheet domain (5–23) | 1.45 |
| β-Sheet domain (6–23) | 1.20 |
| Calcium-binding domain (24–42) | 0.95 |
| Calcium-binding domain (24–41) | 0.90 |

| Domain | rmsd of best CR8 structure compared with structure of LB5 (Å) |
|--------|-------------------------------------------------------------|
| Whole structure (5–42) | 1.57 |
| Whole structure (6–41) | 1.45 |
| β-Sheet domain (5–23) | 1.46 |
| β-Sheet domain (6–23) | 1.24 |
| Calcium-binding domain (24–42) | 1.05 |
| Calcium-binding domain (24–41) | 0.97 |

* Residues 5–42 represent the entire region of LB5 for which coordinates were deposited. Residues 6–41 represent the region from the first to the last cysteine.
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