Structure of the LDL Receptor Extracellular Domain at Endosomal pH

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The low-density lipoprotein receptor mediates cholesterol homeostasis through endocytosis of lipoproteins. It discharges its ligand in the endosome at pH < 6. In the crystal structure at pH = 5.3, the ligand-binding domain (modules R2 to R7) folds back as an arc over the epidermal growth factor precursor homology domain (the modules A, B, β propeller, and C). The modules R4 and R5, which are critical for lipoprotein binding, associate with the β propeller via their calcium-binding loop. We propose a mechanism for lipoprotein release in the endosome whereby the β propeller functions as an alternate substrate for the ligand-binding domain, binding in a calcium-dependent way and promoting lipoprotein release.

The low-density lipoprotein receptor (LDL-R) regulates cholesterol homeostasis in mammalian cells. LDL-R removes cholesterol-carrying lipoproteins from plasma circulation in a process known as receptor-mediated endocytosis (1). Ligands bound extracellularly by LDL-R at neutral pH are internalized and then released in the endosomes (pH < 6), leading to their subsequent lysosomal degradation. The receptor then recycles to the cell surface. Mutations in the LDL-R gene cause familial hypercholesterolemia (FH), one of the most common simply inherited genetic diseases (2). FH heterozygotes exhibit a reduced rate of receptor-mediated removal of plasma LDL by the liver, ultimately leading to early onset coronary heart disease and atherosclerosis. More than 920 mutations in LDL-R are known, some of which have been functionally characterized (2, 3).

The extracellular domain of LDL-R is composed of a “ligand-binding domain” (with cysteine-rich repeats R1 to R7) and an “epidermal growth factor (EGF) precursor homology domain” (with the EGF-like repeats A, B, and C, as well as a β propeller between B and C) (4, 5). LDL-R binds LDL via the single protein in LDL, the 550-kD apolipoprotein B (apoB) (6); deleting R3, R4, R5, R6, or R7 reduces LDL binding to <20% of that of the wild-type LDL-R (7). LDL-R also binds to very low density lipoprotein (VLDL), β-VLDL, interme- diate density lipoprotein (IDL), and chylomicron remnants via the 33-kD apolipoprotein E (apoE) (8, 9); disrupting R5 decreases β-VLDL binding to 30 to 50% of that of the wild-type receptor, whereas disrupting R4 or R6 reduces binding only slightly (7). At neutral pH, negative charges on repeats R1 to R7 are thought to interact with positive charges on apoB and apoE. Indeed, LDL binding to LDL-R can be disrupted competitively with polycations or permanently by selective chemical modification of positively charged residues on apoE or apoB (1, 10, 11). Further, the LDL-R cysteine-rich repeats contain clusters of acidic residues implicated not only in ligand binding but also in calcium binding, as shown at pH = 5.0 crystallographically by Fass et al. (12) and at pH = 6.7 with the use of nuclear magnetic resonance (NMR) imaging (13). The calcium ions enable the repeats to bind to the ligand (14, 15).

Dissociation of ligands is crucial for receptor recycling and hence proper receptor function; mutations in LDL-R that impair ligand release produce FH (2). Deletion mutagenesis studies in LDL-R and the related VLDL-R have indicated that, although the ligand-binding domain is sufficient for binding lipoprotein particles, the receptor requires the EGF precursor homology domain for ligand release (16–18).

The structural basis for LDL-R’s ability to recognize a diverse group of lipoprotein particles, all varying in size, and release them at acidic pH is unknown. High-resolution crystal structures of modules R5 (12) and β propeller–C (5) are known, and solution NMR structures are known for single and tandem repeats, including R1, R2, R5, R6, A, and B (13, 19–26). These analyses have been carried out at a wide range of pH values (from 3.9 to 7.5), with most of the NMR studies done at acidic pH. Electron microscopic studies have imaged a variety of shapes (27). Here we present the crystal structure of the extracellular portion of LDL-R at pH = 5.3 and at 3.7 Å resolution; this structure should represent the conformation of LDL-R adopted in endosomes.

Structure determination. The extracellular domain of human LDL-R (residues 1 to 699) was crystallized at pH = 5.3, with the symmetry of space group P21 (28). Soaking crystals in sodium 12-tungstophosphate (Na3PW12O40) improved their diffractive quality and incorporated large anomalous scatterers. The asymmetric unit contains a single protein molecule and two tungsten clusters as well as half of a tungsten cluster on a crystallographic twofold axis. Data collection, structure determination, and model statistics are given in Table 1 and in (29). The combination of good experimental phases and well-determined structures for most modules enabled us to build a reliable model at 3.7 Å that indicates the relative orientation of the modules; however, a discussion of atomic-level interactions (exact side-chain conformations) at this resolution is not possible.

Monomer description. There is clear electron density in the crystal structure for the modules R2, R3, R4, R5, R6, R7, A, B, β propeller, and C (Fig. 1A); module R1 is present but not seen because of disorder in the crystal. The modules of the ligand-binding domain (R2 to R7) form an arc that folds back onto the EGF precursor homology domain, a linear array of modules A, B, the β propeller, and C (Fig. 1, B and C). The arc from R2 to R7 (Phe65 to Met777) spans 140 Å, and the array of A, B, β-propeller, and C (Met777 to Ser462) spans 105 Å. The height of the molecule is 90 Å (Fig. 1B, Gin170 to Thr669). The density for two large carbohydrates and the linkers between the modules are well resolved, with the exception of the linker between R4 and R5. The modules have the same fold as observed in previously determined (related) fragments. Each cysteine-rich repeat contains two loops connected by three disulﬁde bonds. The second loop carries conserved acidic residues and forms a calcium-binding site with the consensus sequence Xn (main chain carboxyl)-X-X-Asp/Asn+3 (side-chain)-Xn+5 (main chain carboxyl)-X-X/Asp/ Glu+7 (side-chain)-X-X-X-X-X-A5p+13 (carboxylate)/Asp/Glu+14 (carboxylate), with the functional groups given in parentheses, X indicating any residue, and n indicating the residue number. The A, B, and C modules also contain three disulﬁde bonds each. Consistent with the structural studies on fragments, the modules R2, R3, R4, R5, R6, R7, and A and B appear to contain calcium ions in their calcium-binding sites. These ions were identified as features in the electron density that far exceed the average protein density. The majority of the extracellular domain of LDL-R is thus well ordered in the crystal structure.

In the crystal, each monomer forms major contacts with five neighboring symmetry-related molecules. Although the relative orien-
tation of some of the LDL-R modules is determined by their intramolecular interactions, other modules are positioned by their intermolecular interactions, and these interactions should also occur in solution.

The modules in the ligand-binding domain (R2 to R7), apart from their linkers, do not interact with each other directly. The linkers are short, four or five residues, except for linker R4–R5, which is 12 residues. R2 contacts module B of a symmetry-related molecule, whereas R3 makes direct contact with the β-propeller of another symmetry mate. Tungsten clusters (10 Å in diameter) stabilize these interactions, one packing between R2 and R3 and another packing between R3, β-propeller, and a neighboring β-propeller (Fig. 2). R4 and R5 bind side by side to one end of the β-propeller “barrel” via extensive interactions, and the protein moieties do not contact each other, symmetry-related molecules, or tungsten clusters. R6 makes extensive crystal contacts to two neighboring molecules (β-propeller and C module of one molecule and R7 of another). Lastly, R7 is held in place by module A and a symmetry-related molecule (side of β-propeller barrel and R6 module). Thus, in the ligand-binding domain, whereas R2, R3, and R6 are fixed by crystal contacts and R7 makes intramolecular as well as crystal contacts, R4 and R5 are held in place solely by β-propeller.

The modules of the EGF precursor homology domain interact with each other in addition to contacting the ligand-binding domain. The junctions between R7, A, B, the β-propeller fold, and C direct their relative orientations. At the kink formed by R7 and A, where the ligand-binding domain folds back on the EGF precurso-n-homology domain, hydrophobic areas of side chains pack together to form a core [disulfide Cys272–Cys292 (of R7), Val274 (R7), Lys312 (A), and Ile313 (A)]. At the next junction, the carboxyl-terminal end of A interacts tightly with the amino-terminal part of B. The linker residues are sandwiched on one side by a loop from A and on the other side by a loop from B. In the middle of the linker, Asp339 (of A) ligands to the calcium ion in B, and Phe342 (of A) and Glu333 (B) pack on either side. Module A does not make contact with symmetry-related molecules and is held in place by R7 and B. The B module is packed against the β-propeller but also contacts the R2 of a neighboring molecule. Although the individual A and B modules are similar to those observed by NMR spectroscopy on A–B fragments (25), sharing 2.6 and 2.5 Å root mean square deviation (rmsd) for 39 and 41 Cα atoms, respectively, their relationship in the extracellular domain is different. A and B still form an extended rod, but B has swiveled about 40° with respect to A in a rigid body motion (roughly around the rod axis). Three loops from the carboxyl-terminal half of module B pack against the β-propeller (Gln338 to Gln345, Glu359 to Phe362, and Ala370 to Gly375) to form an extensive interface. The polypeptide chain reverses direction between B and β-propeller with a sharp kink at Gly375, packing hydrophobic residues together [Phe362 (of B) and Leu357 and Phe393 (β-propeller)]. A mutation of Gly375 to Ser375 is found in FH patients (3), suggesting that nonglycine residues are incompatible with the main chain conformation. A number of interacting charged residues are buried at the interface between B and β-propeller: Asp339 (of B) or Asp341 (B with Arg553, Asp348, Glu594, and Arg612 (β-propeller). The mutations of Asp341 to Ala341, Gln345 to Arg345, Arg553 to Cys553 (R553C), Glu594 to Lys594, and Arg612 to Cys612 (R612C) are found in FH patients (3); all five mutations would change the balance and/or location of charges, likely disrupting the B–β-propeller interface, whereas R553C and R612C might also affect folding and proper disulfide bond formation. The presence of additional modules (R1 to R7 and A) in our structure and/or the acidic pH seems to promote the interface between B and β-propeller, because in the crystal structure of the β-propeller–C-fragment at neutral pH the B module is present but disordered (5). The C module packs against the β-propeller as was seen at neutral pH in the crystal structure of the fragment (5). The modules in the EGF precursor homology domain observed in our crystal structure influence each other by imposing relative orientations through their junctions; they influence the ligand-binding domain as well.

The electron density for linker R4–R5 is ambiguous in our crystal structure due to flexibility or static disorder. The R4–R5 linkers from two different monomers pack on either side of a two-fold crystallographic axis, so that module R4 could conceivably connect to module R5* instead of module R5 and coexist in the crystal (Fig. 2). Linker length, electron density, and the monomeric state observed in gel filtration studies at pH 5 make a domain-swapped scenario unlikely. In any case, chemical interactions between R4, R5, and the β-propeller fold would not change, and our observations would remain the same.

Our crystal structure reflects three important aspects of the LDL-R molecule: (i) The loops in
the cysteine-rich repeats, with their lack of secondary structure elements, are pliable. They mold in different ways to form crystal contacts with apparent ease, as no helical or β-sheet interactions need to be broken. In the NMR studies, this flexibility has manifested itself in ensembles of widely divergent conformations.

(ii) The length and flexibility of the linkers between the cysteine-rich repeats enable them to adjust with respect to each other under the influence of other modules or crystal contacts. R5 and R6 fold toward each other and R3 and R4 extend away, whereas R4 and R5 align side by side. (iii) At endosomal pH, some modules are constrained with respect to each other (R4, R5, R7, A, B, β propeller, and C), whereas others apparently are not.

**Interface of R4 and R5 with β propeller.** The modules R4 (residues Cys127 to Cys163) and R5 (Cys170 to Cys219) bind β propeller (Ile277 to Gly642) via extensive interactions (Fig. 3). The calcium-binding loops of both R4 and R5 dock side by side, one on the hub and one to the side of the six-bladed β-propeller barrel, placing the calcium ions circa (ca.) 21 Å apart. In contrast, the calcium-binding sites in the other repeats point in different directions, exposed to the solvent (R6 and R7) or partially involved in crystal contacts (R2 and R3). Though the precise location of the amino acid side chains cannot be determined given the resolution of the diffraction data, the following nevertheless appears clear: In R4, the calcium-binding loop provides the chelating residues Trp144, Asp147, Asp149, and Asp151—in addition to His140, Pro141, and Asp151—to the interface. Hydrophobic contacts are made between R4 (Ala130, Ile140, Pro141, and Trp144) and His156, and Lys159, and His162. Salt bridges are formed as well (Asp147 to Lys208 and Asp149 to His206 and His208). In R5, the calcium-binding ligands Trp193, Asp196, Glu581, and Asp200—in addition to His190, Ser191, and Lys202 from the same loop—also bind β propeller. Hydrophobic interactions are made (Trp193 between Glu581 and Lys582), and salt bridges are formed (His190 to Glu581, Asp196 and Asp200 to Lys582, and likely Lys202 to Glu263). A cluster of histidines (His190, His562, and His566) is found at the three-way junction between R4, R5, and β propeller. All together, R4 and R5 provide β propeller with 14 major residues to form an interface mediated by hydrophobic and charged interactions.

The relative dispositions of R4, R5, and β propeller appear highly specific, and in particular the R4–β propeller interface is highly conserved. Eleven out of 14 residues from R4 and data, $R_{merge}$ is the multiplicity weighted $R_{merge}$ with respect to $\lambda_{mean}$ (i.e., $R_{merge}$ and their symmetry-related mates). The traditionally reported $I/\sigma$ (where $\sigma$ is the rms scatter of observations) are as follows (outer-shell statistics are in parentheses); peak data, $I/\sigma = 3.2 (2.5)$; inflection data, $I/\sigma = 4.1 (1.9)$; HER, $I/\sigma = 4.8 (1.6)$; and LER, $I/\sigma = 2.6 (2.0)$. The data show a more or less linear fall-off and are weak. Wilson $B$-factor values calculated by TRUNCATE/C CCP4 (43) from lowest to highest resolution shell exemplify this: peak, 133 Å$^2$; inflection, 122 Å$^2$; and LER, 110 Å$^2$. The MAD phasing statistics (Table 1) show that the resolution is 13 Å and the completeness is 64%. Wilson $B$ factors and $I/\sigma$ values were calculated by TRUNCATE/C CCP4 (43) from lowest to highest resolution shell:

Table 1. Statistics from the crystallographic structure determination (space group P3₁2₁). HER, high energy remote; LER, low energy remote; and Infl, inflection. The number of crystals used per data set is given in parentheses after the unit cell dimensions. Outer-shell statistics are given in parentheses for data resolution, data completeness, $R_{merge}$ and $<I/\sigma$—$\sigma(I)$ is the corrected standard deviation [see SCALA/CCP4 (43)]. Entries marked by a dash are not relevant. Completeness in outer shells is low because of anisotropic diffraction and subsequent anisotropic integration. $R_{merge}$ is the multiplicity weighted $R_{merge}$ keeping $I^+\text{ and } I^-$ separate [see SCALA/CCP4 (43)]. Bijvoet and symmetry-related pairs were kept together for scaling but separate for merging in peak, inflection, and HER, but not LER, MAD phasing (Table 2).

![Fig. 2. Two monomers stabilized by tungsten clusters; the ligand-binding domains have been labeled R2 to R7 and R2* to R7*, respectively. The linkers between R4 and R5 and R4* and R5* meet at a crystallographic twofold axis. At a distance below, a cluster (center) sits on the twofold as well.](image-url)

**R S E A R C H A R T I C L E**

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<table>
<thead>
<tr>
<th>Data set</th>
<th>$\lambda$(Å)</th>
<th>Unit cell (Å)</th>
<th>Resolution (Å)</th>
<th>Total/unique reflections</th>
<th>Completeness (%)</th>
<th>$R_{merge}(\lambda,\lambda)$ (%)</th>
<th>$R_{merge}(\lambda,\lambda)$ (%)</th>
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<tr>
<td>Peak</td>
<td>1.2136</td>
<td>184.80 184.80 84.96 (3)</td>
<td>39.3–3.8 (3.9–3.80)</td>
<td>101571/14057</td>
<td>84.6 (20.7)</td>
<td>11.4 (39.6)</td>
<td>19.3 (42.5)</td>
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<td>Infl</td>
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<td>184.80 184.80 84.88 (1)</td>
<td>49.4–4.0 (4.1–4.00)</td>
<td>43739/13002</td>
<td>91.4 (51.2)</td>
<td>12.0 (50.1)</td>
<td>17.5 (49.0)</td>
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<td>45.2–3.8 (3.9–3.80)</td>
<td>47496/17898</td>
<td>89.6 (32.0)</td>
<td>9.5 (59.6)</td>
<td>11.4 (55.6)</td>
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<td>LER</td>
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<td>33.7–3.7 (3.83–3.70)</td>
<td>64801/17303</td>
<td>95.6 (64.5)</td>
<td>–</td>
<td>9.0 (44.9)*</td>
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**MAD phasing statistics**

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<th>$R_{merge}$ (Infl)</th>
<th>$R_{drop}$ (Infl)</th>
<th>$R_{merge}$ (HER)</th>
<th>$R_{drop}$ (HER)</th>
<th>$R_{merge}$ (LER)</th>
<th>$R_{drop}$ (LER)</th>
<th>$\Delta$iso</th>
<th>$\Delta$ano</th>
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<tr>
<td>Peak</td>
<td>50–3.8</td>
<td>0.166</td>
<td>0.12</td>
<td>0.12</td>
<td>0.75/0.82</td>
<td>0.53</td>
<td>10.3/0.75</td>
<td>5.24/0.75</td>
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<tr>
<td>Infl</td>
<td>50–4.0</td>
<td>0.14</td>
<td>0.14</td>
<td>0.17</td>
<td>0.80/0.78</td>
<td>0.69</td>
<td>2.39/1.42</td>
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<tr>
<td>HER</td>
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<td>0.106</td>
<td>0.12</td>
<td>0.75/0.82</td>
<td>0.53</td>
<td>0.60/0.78</td>
<td>2.78/0.78</td>
<td>1.81/0.78</td>
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**Table 2. MAD phasing statistics.** The nonmultiplicity weighted (traditional) $R_{merge}$ for LER is 7.7% (38.8% in the outer shell).

**Figure 2.** Two monomers stabilized by tungsten clusters; the ligand-binding domains have been labeled R2 to R7 and R2* to R7*, respectively. The linkers between R4 and R5 and R4* and R5* meet at a crystallographic twofold axis. At a distance below, a cluster (center) sits on the twofold as well.
R5 contacting β propeller are identical or highly conserved in nine LDL-R sequences and six VLDL-R sequences (the equivalent modules R5 and R6) (Fig. 4A, top). Of the 10 counterpart residues on β propeller, 6 are identical or highly conserved, and at two other sites adjacent residues in the sequence could provide similar chemical interactions (not shown). Structural features such as the three salt bridges between R4 and β propeller and the cluster of histidines are conserved in the 15 sequences, as well as three out of four salt bridges between R5 and the β-propeller fold in many species, suggesting functional relevance. In fact, the region obscured by R4 and R5 on β propeller belongs to the largest area of contiguous sequence conservation between LDL-R and VLDL-R on the molecular surface (Fig. 4B).

To confirm that the ligand-binding domain binds to the β-propeller fold at endosomal pH in solution, we cleaved the protein between R4 and R5 with H9251-chymotrypsin at pH 6 and pH 8 and performed size exclusion studies. At pH 6, the 33-kD fragment R1-R4 remains associated with the rest of the molecule (Fig. 5; lanes 1, 2, and 3). At pH 8, the fragment R1-R4 dissociates (Fig. 5, lanes 6 and 7). When the cleavage reaction is performed at pH = 5.5 and the pH subsequently raised to 8, the fragment R1-R4 dissociates, whereas cleaving LDL-R in two at pH = 8 followed by a shift to acidic pH results in R1-R4 being retained with the rest of the molecule (39).

Implications of the LDL-R crystal structure—a mechanism for ligand release at acidic pH. The compact shape of LDL-R seen at pH = 5.3 confirms the protein properties observed during our quest for crystals. Gel filtration studies at pH = 5 indicated a hydrodynamic radius corresponding to a monomer with an apparent mass of 95 kD. In contrast, at pH = 8 and in the presence of either CaCl2 or EDTA, gel filtration and dynamic light-scattering studies revealed a hydrodynamic radius too small for a dimer unless completely globular and more consistent with an elongated monomer (apparent mass of 160 kD). Studies with other techniques have been hampered by the low solubility of LDL-R at acidic pH. The association of R1-R4 with the rest of the molecule was suspected early, because proteolysis of the linker R4-R5 took place on occasion during crystal
growth (confirmed by amino-terminal sequencing), yet both fragments remained in the crystal. Our structure suggests that, at endosomal pH, β propeller might act as an alternative substrate for the ligand-binding domain. Biochemical and structural observations support this proposal. Module R5 is essential for LDL and β-VLDL binding, and R4 is important for LDL-R binding as well (7). In the crystal structure at acidic pH, R4 and R5 bind independently of each other to β propeller. Deleting the A and B modules creates a receptor that binds ligand but does not recycle, indicating that ligand release is not possible (31). Removing A and B would hinder R4 and R5 from reaching β propeller, and other modules are apparently not functionally equivalent (Fig. 4A, bottom). Mutant LDL-R and VLDL-R lacking the complete EGF precursor homology domain (with its β propeller) still bind ligand at the cell surface (though with reduced affinity for LDL-R) and are internalized but fail to recycle (16–18). Although the heparan sulfate analog suramin (binding LDL- and β-VLDL) successfully dissociates lipoproteins at the cell surface in vitro from LDL-R and the mutant LDL-R lacking the EGF precursor homology domain, exposure to acidic pH only releases lipoproteins from the ligand-binding domain when the EGF precursor homology domain is present (1, 16). Many of the residues that bind the calcium ion in R4 and R5 also form the interface with β propeller, and these residues (or ones close by) likely bind to apoB and apoE as well. For example, two of these, Trp144 and Trp194, donate only a main-chain carbonyl to calcium coordination; nevertheless their side chains are strictly conserved in 15 sequences. R6 appears different in this respect; placing a Trp at the equivalent site appears detrimental (Fig. 4A, bottom), because the mutation from Arg323 to Trp323 in R6 is found in FH patients (3). The interface of R4, R5, and β propeller displays structural features that are well suited to support reversible, pH-regulated binding. Tryp- tophan residues can generate high binding affinity, as shown for the human growth hormone receptor (32). In the pH range from 5 to 7.5, the formation and strength of the interface salt bridges could change as functions of the pH and the pKa (where pK is the acid dissociation constant) of the aspartates, glutamates, and histidines involved. At acidic pH, histidine residues support a (partial) positive charge, explaining how a histidine cluster could stabilize buried negatively charged residues nearby. Mutations of two of the three histidines at the interface have been found in FH patients, His180 to Tyr180 and His562 to Tyr562 (33), again suggesting functional importance. Histidines have been implicated in other acid-regulated mechanisms (36, 37). For LDL-R, NMR studies of modules at acidic and more neutral pH have not identified any side chains with pH-specific conformations.

Thus, it is possible that, in the endosome, the EGF precursor homology domain of LDL-R promotes ligand release by presenting the ligand-binding domain with a better substrate, the β propeller, in an intramolecular displacement reaction. LDL-R exhibits a wide range of binding affinities to different lipoprotein particles, and the crystal structure even at acidic pH suggests a molecule accommodating ligand variation. The apoE and apoB epitopes vary with lipoprotein particle size (35, 36). The receptor-binding region of apoE forms a single bent helix that spans 70 Å in the presence of the lipid-mimicking trifluoroethanol; R5 together with a second cys

References and Notes
3. Databases for FH mutations are available online at www.uchc.edu/ fh and www.umde.necker.fr.
28. Twenty-two mutants of the extracellular domain of LDL-R (amino acids 1 to 699) with different combina-

Formation of a One-Dimensional Array of Oxygen in a Microporous Metal-Organic Solid

Ryo Kitaura, Susumu Kitagawa, Yoshihiko Kubota, Tatsuo C. Kobayashi, Koichi Kindo, Yoshiki Mita, Akira Matsuo, Michihiro Kobayashi, Ho-Chol Chang, Tadashi C. Ozawa, Megumi Suzuki, Makoto Sakata, Masaki Takata

We report the direct observation of dioxygen molecules physisorbed in the nanochannels of a microporous copper coordination polymer by the MEM (maximum entropy method)/Rietveld method, using in situ high-resolution synchrotron x-ray powder diffraction measurements. The obtained MEM electron density revealed that van der Waals dimers of physisorbed O2 locate in the middle of nanochannels and form a one-dimensional ladder structure aligned to the host channel structure. The observed O–O stretching Raman band and magnetic susceptibilities are characteristic of the confined O2 molecules in one-dimensional nanochannels of CPL-1 (coordination polymer 1 with pillared layer structure).

The confinement of molecules into low-dimensional nanospace may alter their properties and reactivity, especially in the case of terminal sequencing of the protein from crystals shows that the first residue is Asp, suggesting that while R1 is present it is disordered. As a starting point for model building, we used the fragments from PDB 1A1J, 1D2J, 1H0U, and 1T0Q. Including the model in the phase calculation and sharpening the data by applying a resolution-dependent B factor (exp [−(B/2)]) for the resolution and B = −120 Å2, derived from the Wilson plot of the data) resolved many of the side chains. The model includes amino acids 44 to 693, except Arg57, Val295, Asp75, Tyr99, Cys102, Val294, Val341, Gln314, and Ala324, for which there is poor density because of heavy atom Fourier ripples from the clusters. About 10% of the side chains are currently modeled as alamines. For molecular surface calculations, all side chains were incorporated. Restrained positional refinement with a maximum likelihood target function including experimental phase information was applied with CNS to regularize the model. Crystallographic refinement is problematic because of the poor ratio of observations to parameters (with 17,303 unique reflections and more than 5000 atoms) and the lack of noncrystallographic symmetry. The Rmerge is 38.8% with the use of all reflections F > 0 between 33.1 and 3.7 Å; the Rmerge is 39.2%, calculated with a test set of 7% of the total reflections. The model has root mean square (rms) bond-length deviations of 0.03 Å and 4.9° for bond angles. The model has 583 residues in the most favored and allowed regions and 58 in the generous region, whereas six residues are currently in the unfavorable regions of the Ramachandran plot (PROCHECK [43]). Figures were made with CLUSTAL W [48], MOLSCRIPT [49], RASTER 3D [50], and CRASP [51].

G. Rudek et al., unpublished data.


S. G. Young, Circulation 82, 1574 (1990).


Reports

终端的序列性蛋白质从晶体中显示，第一个残基是Asp，表明在R1的情况下它是紊乱的。作为开始模型构建的一步，我们使用了PDB 1A1J, 1D2J, 1H0U, 和1T0Q的片段。包括模型在 phases计算和尖峰锐化数据由应用一个 resolution-dependent B factor (exp (−B/2)), 其中B是分辨率的倒数和B = −120 Å2，来自Wilson plot的数据)解决了许多的侧链。这个模型包括氨基酸44到693，除了Arg57, Val295, Asp75, Tyr99, Cys102, Val294, Val341, Gln314, and Ala324，因为它们有恶劣的密度由于重原子Fourier 周期从簇。大约10%的侧链是目前被模型化为alamines。对于分子表面积计算，所有侧链被包括。限制位置的锐化与一个最大可能性目标函数包括实验的相位信息被应用于CNS以标准化模型。晶体学的精留是困难的，因为观察与参数的比低(有17,303独特的反射和超过5000个原子)和缺乏非晶体学的对称性。Rmerge是38.8%，使用所有反射F > 0之间33.1和3.7 Å；Rmerge是39.2%，计算在一个7%的总反射的测试集。模型有根均方(rms)的键长度偏差为0.03 Å和4.9°键角度。模型有583个残基在最favorable和allowed区域，并58个在generous区域，而六个残基是目前在 unfavorable区域的Ramachandran图[PROCHECK [43]]。图像是用CLUSTAL W [48], MOLSCRIPT [49], RASTER 3D [50], 和 CRASP [51]制成的。

G. Rudek et al.,未发表数据。


S. G. Young, Circulation 82, 1574 (1990).

