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COMMENTARIES

Crystal Structure of the Extracellular Domain of the PTH/PTHrP Receptor

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Commentary on: Pioszak AA, Xu HE. Molecular recognition of parathyroid hormone by its G protein-coupled receptor. *Proc Natl Acad Sci U S A*. 2008 Apr 1;105(13):5034-9.

A major advance towards the goal of understanding the molecular mechanisms by which PTH binds to its receptor, the PTH1R, has been made through the determination of the crystal structure of the receptor's extracellular amino-terminal domain (ECD) in complex with the PTH(15-34) fragment, representing the ligand's principal binding domain (1). The PTH1R is a class II G protein-coupled receptor that plays critical roles in bone and mineral ion metabolism. As a class II GPCR, the PTH1R has a relatively large ECD, which has been shown by extensive mutagenesis and cross-linking analyses to play a key role in the ligand binding process, mainly by providing a docking site for the ligand's principal binding domain (2-4). As revealing as such mutagenesis and cross-linking studies may be, they are generally very limited in their capacity to define the specific contacts that occur between the ligand and receptor, and the molecular topology of the bimolecular complex. The new report by Pioszak and Xu (5) describes the crystal structure of the PTH1R ECD in complex with the PTH(15-34) fragment and thus fills a substantial gap in our knowledge of how PTH interacts with its receptor (Figure 1).

How Was It Done?

The structure was solved to a resolution of 1.95 A˚, which is high enough to define molecular interactions and surface topologies with an atomic level of precision. This high level of resolution was afforded by the high quality of the crystals, which, in turn, reflects the purity and conformational uniformity of the protein material used. For the PTH1R ECD, obtaining such purity and conformational uniformity is a challenging task, in large part because of the six cysteine residues that must be paired in the correct disulfide linkage pattern for proper protein folding and functionality. The authors met this challenge by engineering a protein overexpression and purification system that is generally suited for large, disulfide-bonded proteins. The PTHR1 ECD was produced as a fusion protein tagged at the N-terminus, with bacterial maltose-binding protein (MBP), and at the C-terminus, with a hexahistidine sequence. The intervening segment comprised human PTH1R residues Asp29-Leu187, essentially the complete ECD. The two terminal tags enabled a two-step affinity purification scheme that yielded only the intact fusion protein. The MBP tag also helped maintain protein solubility, and facilitated the crystallization/structure determination processes. The *E. coli* strain used for protein expression bore mutations in the genes for thioredoxin reductase (*trxB*) and glutathione reductase (*gor*), which together make for a relatively oxidative cytoplasmic environment conducive to disulfide bond formation. A bacterial chaperone-like protein, disulfide isomerase protein, or DSBC, was used during both the expression and purification steps to help resolve mismatched disulfide bonds. The final product migrated as a single band on native gels, exhibited an expected affinity of about 1 micromolar for PTH(15-34) in binding assays, and readily formed diffraction-quality crystals in the presence of added hPTH(15-34) peptide.

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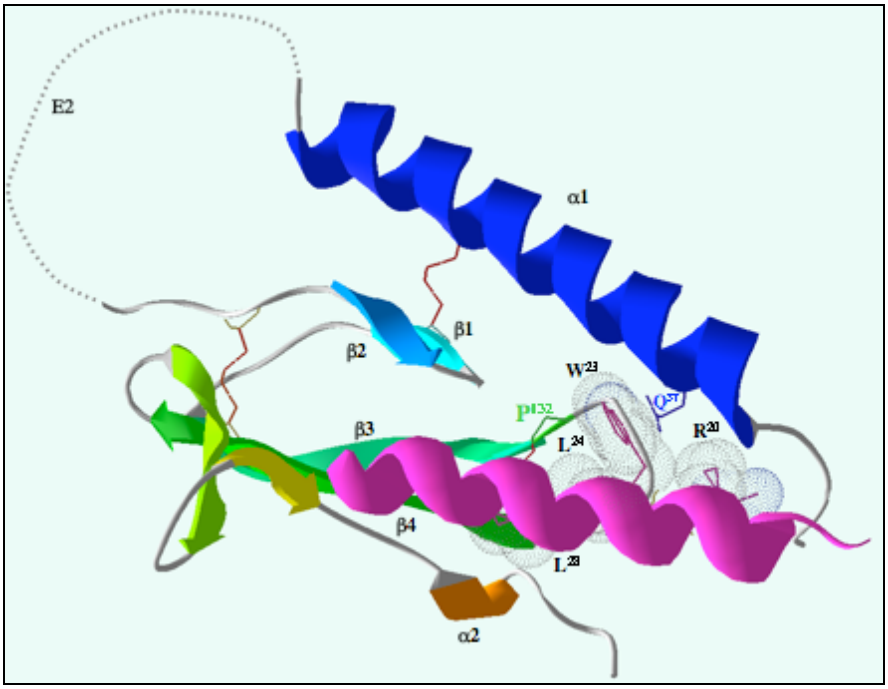

Figure 1. Crystal structure of PTH(15-34) docked to the PTH1R extracellular domain. The PTH(15-34) helix is shown in magenta, with the side-chains of core binding residues: Arg[20], Trp[23], Leu[24] and Leu[28] in van der Waals format projecting into the face of the structure. Receptor residue Gln[37] and Pro[132] are also labeled, and two disulfide linkages (red) are evident. Schematic prepared by T.J.G. from the crystal structure coordinates reported by Pioszak and Xu (5).

What Does the PTH1R ECD Look Like?

The overall ECD structure appears as a somewhat flattened shape, with dimensions 40A˚ x 25A˚ x 10A˚. A central groove, which accommodates the ligand, runs laterally across the front face of the structure. The overall tertiary fold follows an α-β-βα pattern, and is roughly organized into three layers of secondary structure components. The top layer consists of an N-terminal α-helix (α1) formed by residues Thr33-Gln57. The middle layer is formed on the left by a two-strand β sheet (β1-β2) and its interconnecting β-hairpin (Asp113-Leu116), and, on the right, by a turn connecting β-strands 3 and 4 of the lower layer. The lower layer consists of the β3-β4 sheet, a short α-helix (α2) (Ser168-Lys172) and a loop connecting β4 to α2 (Tyr169-Phe173). The three disulfide bonds interconnect the three framework components: Cys48-Cys117 connects upper layer α1 to the middle layer β-sheet; Cys108-Cys148 connects the middle layer β-sheet to the bottom layer β-sheet, and Cys131-Cys170 connects the mid-region turn to the bottom layer α2. A series of packing and H-bond interactions involving residues on the middle and bottom layers and centering around the mid-layer β-hairpin integrate with the disulfide bond scaffold and thereby provide the main stabilizing forces in the structure. The β sheet and turn arrangement of the mid and lower portion resembles the short consensus repeat (SCR) motif found in the immunoglobins.

Along with the six cysteine residues, the residues involved in the packing interactions (*e.g.*, Trp154, Trp118, Tyr167, Phe138, Ile135, Glu111, Asp113, and Arg46), are generally well-conserved across the class II GPCRs, supporting the idea that the same architectural plan is used for the ECDs of each of these receptors. One of these packing residues in the PTH1R is Pro132, which is the site of a homozygous leucine mutation in Blomstrand's chondrodysplasia (6). The structure thus suggests a plausible mechanistic basis for this perinatal lethal skeletal defect--destabilization of the PTH1R ECD. Not defined in the structure is the segment Arg58-Arg104, which overlaps closely with the non-essential segment

Ser61-Gly105 encoded by exon E2 of the PTH1R gene. Also not defined are the four N-linked glycosylations, and the C-terminal 12 amino acid segment: Asn176-Leu187, which may be more involved in interaction with the mid- or N-terminal region of PTH(1-34) (4).

How Does the Ligand Dock and Achieve Affinity and Specificity?

The PTH(15-34) domain is bound in the central groove as a continuous amphipathic α-helix. The PTH helix runs approximately parallel to α1, which forms the top ridge of the binding groove, and anti-parallel to α2, on the bottom ridge. This alignment places the N-terminus of the PTH helix flush with the mouth of the groove at the right side of the front face, such that the PTH(1-14) segment, not present in the structure, would extend in some direction from there. The ligand helix makes extensive contact with all surfaces of the groove. The overall appearance suggests to the authors a "hot-dog-in-a-bun" analogy for the bimolecular complex. A similar arrangement is apparent in the structures of two other class II GPCR ECDs that have recently been solved as complexes with their cognate peptide ligand: the corticotropin-releasing factor receptor-2 (7), and the gastro-insulinotropic peptide receptor (8). This general mode of ligand binding is thus likely to be relevant for most, if not all, of the class II GPCRs. Moreover, the finding of parallel structures alleviates doubt about potential artifact imposed by the MBP and His6 tags, or absence of certain residues.

In the structure, the PTH(15-34) amphipathic α-helix engages the ECD largely through its hydrophobic face, as formed by Val21, Trp23, Leu24, Leu28, and Val31, which are almost completely buried in the complex. The cognate contact surface in the receptor is formed largely by hydrophobic residues, and describes a contour surface that is complementary to that of the ligand's binding surface. This suggests that a shape-matching process involving a broad, yet precisely defined topological interface, comprises a key component of the mechanisms that determine ligand binding affinity and specificity for the PTH1R. Unlike the scaffolding and packing residues, the ECD residues that form the ligand contact surface are not conserved among the class II GPCRs.

The binding scheme revealed by the structure agrees remarkably well with the bulk of the data generated from previous functional and cross-linking studies, as these have consistently highlighted the importance of residues on the hydrophobic face of the predicted C-terminal amphipathic α-helix (9-12). The new data show the exact locations and molecular geometries of the key intermolecular contacts. For example, we now see that the indole ring of Trp23 packs, on one face, against Gln37, Ile38 and Leu41 projecting from the N-terminal helix α1, while the opposing face of the ring lies against a hydrophobic patch formed by Ile135, Phe138 and Ile115 on the floor of the binding groove. This hydrophobic patch also forms the contact surface for Leu24 and Leu28. The side chain of Arg20, the most conserved residue in the PTH series of ligands, and among the most critical (13), is completely buried, and involved in an extensive network of H-bond and polar interactions with a ring of oxygen atoms provided by M32, Asp29, and Gln37 at the N-terminus of α1. Thus it can now be better appreciated how these residues contribute so importantly to binding affinity and receptor specificity. As the results overall agree well with the previous functional studies performed on PTH ligands and the intact PTH1R, it seems safe to conclude that the binding mode and interactions seen in structure accurately reflect those used by intact PTH and the intact receptor in native cells.

We are thus much less in the dark about how the binding domain of PTH docks to the ECD of the PTH1R. This important advance sets the stage for further studies that will hopefully resolve the binding mechanism to the next higher levels. For example, the relative energetic contributions that the different contacts and proximities seen in the structure make to the binding process will need to be evaluated and defined. This can most likely be achieved by further receptor mutagenesis and pharmacological work, which, in any case, will be more efficient

now that a solved structure is in hand. Another goal will be to determine how the identified binding mechanism for PTH relates to that used by PTH-related protein (PTHrP), which differs both structurally from PTH, particularly in the (15-34) domain, as well as functionally, as revealed, for example, in kinetic receptor binding assays (14). Then there is the important matter of determining how the ECD is oriented with respect to the membrane-embedded portion of the receptor, and how this orientation facilitates movement of the PTH N-terminal pharmacophore domain into this juxtamembrane region to induce receptor activation. Finally, it will be interesting to see how the structure might enable the design of new PTHR ligands, particularly ones that have greater therapeutic efficacies than PTH(1-34). These are questions and problems that can now be better approached in the light provided by the newly solved, high resolution crystal structure.

Conflict of Interest: None reported.

Peer Review: This article has been reviewed by Michael Rosenblatt.

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