Crystal Structure of Hormone-Bound Atrial Natriuretic Peptide Receptor Extracellular Domain: Rotation Mechanism for Transmembrane Signal Transduction*

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Running Title: Crystal structure of the ANP receptor-hormone complex

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SUMMARY

A cardiac hormone, atrial natriuretic peptide (ANP), plays a major role in blood pressure and volume regulation. ANP activities are mediated by a single-span transmembrane receptor carrying intrinsic guanylate cyclase (GCase) activity. ANP binding to its extracellular domain stimulates GCase activity by an as yet unknown mechanism. Here we report the crystal structure of dimerized extracellular hormone-binding domain in complex with ANP. The structural comparison with the unliganded receptor reveals that hormone binding causes the two receptor monomers to undergo an inter-molecular twist with little intra-molecular conformational change. This motion produces a Ferris wheel-like translocation of two juxtamembrane domains in the dimer with essentially no change in the inter-domain distance. This movement alters the relative orientation of the two domains by a shift equivalent to counter-clockwise rotation of each by 24°. These results suggest that transmembrane signaling by the ANP receptor is initiated via a hormone-induced rotation mechanism.
INTRODUCTION

Atrial natriuretic peptide (ANP) is a hormone produced in the cardiac atrium and secreted into the circulation in response to atrial distension. ANP stimulates salt excretion (1) and dilates arterial vessels (2,3). Through these activities, ANP plays a major role in the regulation of blood pressure and salt-fluid volume homeostasis. Transgenic animals devoid of the ANP gene develop salt-sensitive hypertension (4), and those lacking the ANP receptor gene develop salt-insensitive essential hypertension accompanied by severe cardiac hypertrophy, fibrosis, and dilatation (5), implicating the ANP and ANP receptor systems in cardiovascular pathophysiology. An analogous hormone, B-type natriuretic peptide (BNP), is also produced and secreted mainly by the heart and has hormonal activities similar to ANP (6). The activities of ANP and BNP are mediated by the ANP receptor, or the A-type natriuretic peptide receptor (NPR-A), carrying intrinsic guanylate cyclase (GCase) catalytic activity. Binding of the hormone to the receptor stimulates GCase catalytic activity, thereby elevating intracellular cGMP levels. cGMP, in turn, mediates the hormonal actions through cGMP-regulated ion-channels, protein kinases, and phosphodiesterases. The ANP receptor occurs as a dimer of a single-span transmembrane polypeptide, each containing an extracellular hormone-binding domain and an intracellular domain consisting of a protein kinase-like, ATP-dependent regulatory domain and a GCase catalytic domain (7). The molecular mechanism by which ANP binding to the extracellular domain stimulates the catalytic activity of the intracellular GCase domain is not understood.

A closely related receptor, the B-type natriuretic peptide receptor, mediates actions of C-type natriuretic peptide (CNP) which occurs mostly in the brain. CNP and the B-type receptor are thought to play a role in the central nervous system-mediated control of the blood pressure and
salt-fluid balance (6,8,9). The B-type receptor has approximately 60% sequence identity with the A-type receptor and has a similar overall molecular topology. It is likely then that the B-type receptor has a signaling mechanism similar to that of the A-type receptor. Yet another related receptor, the natriuretic peptide clearance-receptor, lacks the GCase domain and is not GCase-coupled (10). The clearance-receptor binds ANP, BNP, and CNP as well as some of their biologically inactive fragments with equally high affinity and removes the excesses of these peptides from the circulation (11,12). The clearance-receptor has not been linked to any of the known hormonal actions of natriuretic peptides.

The GCase-coupled, A-type and B-type natriuretic peptide receptors belong to the family of membrane-bound receptor-GCases that include guanylin and enterotoxin receptors (13), retinal GCases (14), and olfactory cell GCases (15). These receptor-GCases, in turn, belong to the super-family of single-span transmembrane receptors, for which the mechanism of transmembrane signaling has not been well defined.

Toward elucidating the signaling mechanism of the ANP receptor, we have expressed and purified the extracellular hormone-binding domain of the receptor (ANPR) in a soluble form (16) and have characterized its biochemical properties, including the disulfide bond structure (17), glycosyl structure (18), and requirement for chloride ion for its binding with ANP (19). We have also crystallized the ANPR without the hormone (apoANPR) and determined its X-ray structure (20). The apoANPR was originally described to occur in a tail-to-tail dimer form associated through its membrane-proximal domain (20). However, it was later recognized that the crystal packing of apoANPR also contained an alternative dimer pair, a head-to-head dimer, associated through the membrane-distal domain. Both the tail-to-tail and head-to-head dimer interfaces
involve a large buried surface area (1,680 Å² and 1,100 Å², respectively) and multiple residues
contacts. Thus, from the crystallographic data alone, it has not been possible to distinguish which
dimer form represents the physiological ANP receptor dimer structure. We have recently
reported site-directed mutagenesis studies of the residues involved in the two possible dimer
interfaces in the full-length ANP receptor expressed on COS cells. We have found that certain
mutations at the head-to-head dimer interface cause the receptor to become either uncoupled or
constitutively GCcase-active, while mutations at the tail-to-tail dimer interface cause no such
effect (21). These results strongly suggest that the extracellular domain of the native ANP
receptor on the cell surface assumes the head-to-head dimer structure and that the tail-to-tail
dimer previously described represents an artificial crystallographic dimer pair occurring in the
crystal packing of apoANPR. The head-to-head dimer structure for the apoANPR is similar to
that proposed for the extracellular domain of the natriuretic peptide clearance-receptor (22).

In the present study, we have determined the crystal structure of the ANPR complexed with
the hormone ANP. The comparison of the complex structure, also occurring in the head-to-head
configuration, with the unbound structure reveals a structural change caused by ANP binding and
suggests a structural basis for transmembrane signaling by the ANP receptor.

EXPERIMENTAL PROCEDURES

**Crystallization of ANPR-ANP complex and Data Collection**—Expression and purification
of the ANPR (16) and crystallization of the ANPR-ANP complex were carried out as described
elsewhere (23). Briefly, the ANPR was expressed in CHO cells and purified by ANP affinity
chromatography. The ANPR was obtained N-glycosylated (18). The ANPR was treated with
sialidase to reduce heterogeneity in the glycosyl structure and was again purified by ANP affinity
chromatography. The complex of the ANPR (10 mg/ml) and an ANP peptide consisting of residues 7 through 27 (sequence: Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg) was crystallized by hanging-drop vapour diffusion at room temperature with 1.6 M to 2.0 M ammonium sulfate in 0.1 M MES buffer, pH 6.5, containing 10 mM NaCl. These conditions differ from those used for crystallizing the apoANPR. Crystals were dialyzed against high concentration of ammonium sulfate solution and were frozen in liquid propane. The crystals had the space group of $P6_1$ with unit cell parameters $a = 100.1$ Å, $b = 100.1$ Å, and $c = 259.8$ Å. Two ANPR molecules are present in an asymmetric unit with a $V_M$ (Mathews coefficient) of 3.9 Å$^3$/dalton. Data were collected at 100 K at Advanced Photon Source beamline 19-ID, and National Synchrotron Light Source beamlines X4A and X25. Data were processed and scaled using the HKL package (24).

**Structure Determination and Refinement**—The structure was solved by molecular replacement with the program CNS (25) using one ANPR molecule in the apoANPR dimer structure (PDB accession number 1DP4) as the search model (Table I). Molecular replacement calculations were also performed using the individual membrane-proximal and membrane-distal domains as the model, which gave essentially the same structure. Refinement was done with the program REFMAC (26) without imposing non-crystallographic symmetry restraints. Rebuilding and correction of the model was guided by $\alpha A$ weighted $2F_o-F_c$ map with the program TURBO-FRODO (BioGraphics). The structure of bound ANP was traced in a single unique conformation occurring in two-fold symmetry orientations (Supplemental Fig. S1). The ANP structure was refined as alternate conformations with equal occupancy. Residues 427 to 435 of monomer A and residues 253 to 256 and 426 to 435 of monomer B had insufficient electron density to be
assigned. The stereochemistry of the final structure was analysed with PROCHECK (27). Assignment of the secondary structures was done using program DSSP (28).

RESULTS AND DISCUSSION

**Overall Structure of the ANPR-ANP Complex**—The extracellular hormone-binding domain of the ANP receptor (ANPR) consisting of residues 1-435 was expressed and purified as described (16). ANPR was crystallized with an ANP peptide with the sequence Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg (Cys 7 and Cys 23 are disulfide-bonded) representing ANP residues 7 through 27 (23). This peptide is fully hormonally active (29,30). The structure of ANP-bound receptor complex (Fig. 1a) was determined to 2.95 Å resolution (Table I). The asymmetric unit of the crystal contains two ANPR molecules bound with one molecule of ANP, forming a 2:1 complex. Because the ANP molecule has no internal symmetry, binding of ANP to the receptor is asymmetric. Bound ANP occurs in two alternate conformations (orientations) of equal occupancy (0.5) related by a two-fold axis of symmetry (Supplemental Fig. S1). The two-fold symmetry in the electron density for ANP suggests that these two alternate bound conformations are equally distributed in the crystal packing. The ANPR monomer has membrane-distal and membrane-proximal domains, each consisting of a central α-sheet surrounded by several α-helices. The membrane-distal domain contains a bound chloride ion (20) necessary for ANP binding (19). The two ANPR monomers, also related by non-crystallographic two-fold symmetry, form a head-to-head dimer through their membrane-distal domains (Fig. 1a).
**ANP-induced Structure Changes**—To examine the structural basis for ANP receptor signaling, the structure of the ANP-bound ANPR dimer complex (Fig. 1a) was compared with that of the apoANPR dimer (Fig. 1b). The ANP-induced structural change involves a shift in the relative positions of the two ANPR monomers as shown in Fig. 2 (also shown in Supplemental Animation S2). There is no appreciable intra-molecular conformational change in each individual ANPR monomer (root mean square deviation of Cα, 0.64 Å) (Fig. 3a). Upon ANP binding, the two ANPR molecules undergo a twist motion centered on a fulcrum point O (Fig. 2). In the front view, ANP binding leads to a seesaw-like motion, centered on point O, that causes the dimer interface to partially open and the membrane-proximal domains to close onto the bound ANP (Fig. 2a). Seen from the side, this motion is accompanied by a counter-clockwise rotation of each monomer, again centered on point O (Fig. 2b). In the juxtamembrane region, this twist motion of the two monomers results in translation of the two membrane-proximal domains by approximately 10 Å in opposite directions (arrows) (Fig. 2c, bottom view). The distance between the two domains is essentially unchanged. Near the membrane, amino acid residues Pro 417, Cys 423 (and its disulfide-bond counterpart Cys 432), and Phe 425 constitute the juxtamembrane signaling motif (31), a structure that is conserved among receptor-GCases and plays a critical role in transmembrane signaling. The movement of these residues shows that the two juxtamembrane domains undergo a parallel translocation. This ANP-induced parallel translocation alters the relative orientation of the two juxtamembrane domains, likely initiating transmembrane signaling.

**Comparison with the Natriuretic Peptide Clearance-Receptor**—The above hormone-induced structural change in the ANP receptor differs markedly from that found in the natriuretic peptide clearance-receptor upon CNP binding (22). In the clearance-receptor, the structural
change occurs within each receptor monomer. CNP binding causes each monomer structure to "open" at the flexible intra-molecular hinge connecting the membrane-distal and membrane-proximal domains (Fig. 3b). This bending causes the membrane-proximal domain to swing onto the bound ligand, leaving the membrane-distal domain and its dimerized structure essentially unchanged (Supplemental Fig. S3). This motion approximates the two membrane-proximal domains but does not change their relative orientation. Unlike the ANP receptor, the clearance-receptor lacks the GCase domain (10) and is not known to mediate any of the known hormonal activities of natriuretic peptides. The clearance-receptor binds ANP, BNP, and CNP as well as some of their biologically inactive fragments with equally high affinity in order to remove the excess of these peptides from the circulation (11,12). The difference in the ligand-induced motion in the ANP receptor from that in the clearance-receptor may reflect the fact that the ANP receptor mediates hormonal signaling whereas the clearance-receptor effects metabolic clearance. Additionally, the conformational flexibility at the intra-molecular hinge in the clearance-receptor (22) may be responsible for its broad ligand specificity.

**Dimer Interface Structure**—At the dimer interface in the apoANPR structure (Fig. 4, shown in cyan), inter-molecular contacts are made through hydrophobic interaction between Trp 74 of one monomer (monomer A) (residue Trp 74A) and Trp 74B of the other (monomer B), two hydrogen bonds (blue dotted lines) between Asp 71A and His 99B, and between His 99A and Asp 71B, and hydrophobic interaction between Phe 96A and Phe 96B. Upon ANP binding, the dimer interface partially opens (Fig. 4, shown in orange), breaking the hydrophobic contact Trp 74A-Trp 74B and hydrogen bonds Asp 71A-His 99B and His 99A-Asp 71B. The hydrophobic contact Phe 96A-Phe 96B, on the other hand, remains and apparently contributes to stabilizing the ANP-bound activated receptor complex. We have found that mutation of Asp 71 to Arg
(D71R) or W74R in the full-length ANP receptor as expressed on COS cells produced a receptor mutant that is constitutively GCase active (21). These mutations incorporate opposing charges at the dimer interface. The electrostatic repulsion between the charges may force the interface of the apo-receptor dimer to become partially open even in the absence of ANP and generate a structure that partially mimics the activated receptor complex. Such an effect may be responsible for the partial constitutive activation of GCase activity. On the other hand, F96D mutation produced an uncoupled receptor mutant that bound ANP but did not cause cGMP stimulation. F96D mutation incorporates two negatively charged residues facing each other near the center of the monomer movement point O. The repulsive force between the two charges may distort the structure of the bound complex and abolish signaling and GCase activation. Thus, the results of mutagenesis studies performed with the full-length ANP receptor expressed on the COS cell surface are consistent with the crystal structures of the apo and ANP-bound ANPR dimer and with the ANP-induced structural change identified from these structures. This finding, in turn, suggests that the hormone-induced structural change identified in this study likely reflects that occurring in the native full-length ANP receptor in the membrane.

ANP-Binding Site and Binding Interactions—Binding of ANP is asymmetric, and the binding site in one ANPR molecule (site A) differs from that in the other (site B) (Fig. 5a). Site A interacts mainly with the N-terminal part of the ANP peptide, while site B interacts mainly with the C-terminal part (Supplemental Table S1-3). Buried surface areas at the two sites are nearly equal (1,374 Å² and 1,367 Å² for sites A and B, respectively), suggesting significant contributions of both sites to hormone binding. The surface structures of both sites A and B have a ring-shaped groove lined with polar residues (Fig. 5b, c). ANP fits closely into the groove at both sites, consistent with the high ligand specificity of the ANP receptor. Upon binding, ANP
residue Arg 14 (Arg 14(ANP)) forms hydrogen bonds with Asp 62B and Glu 119A. These hydrogen bonds may play a key role in holding together the partially open dimer interface (Fig. 4) and stabilizing the structure of ANP-bound ANPR dimer complex. The critical role of Arg 14(ANP) is consistent with the finding that this residue is essential for the hormonal activity of ANP (30). An additional hydrogen bond occurring between Arg 95A and Asp 62B also contributes to the stability of the complex. Residue Phe 8(ANP), essential for hormonal activity, extends to and makes hydrophobic contact with a hydrophobic cavity in site A formed by residues Tyr 154A, Phe 165A, Val 168A, and Tyr 172A (Supplemental Table S1-3). The C-terminal segment of ANP (residues Asn 24(ANP) through Arg 27(ANP)) runs parallel with a stretch of $\alpha$-sheet in the ANPR (residues Gln186B through Phe188B), and a hydrogen bond occurs between Asn 24(ANP) carbonyl oxygen and Glu 187B amide nitrogen, suggesting partial formation of a parallel $\alpha$-sheet (Fig. 5a). An additional hydrogen bond occurs between Asn 24(ANP) and His185B. These interactions are consistent with the finding that the C-terminal residues of ANP are necessary for receptor binding and hormonal activities (30). The agreement found here between the ANP residues involved in binding and the structure-activity relationship for ANP (30) confirms the assigned ANP peptide structure in the complex and, at the same time, suggests that the crystal structure of this complex closely reflects the structure of the native ANP receptor bound with ANP.

ANP residues contributing to binding to the receptor are all conserved in BNP, including Arg14, Phe8, Asp24 (Asn24 in ANP), and Arg24 (Supplemental Fig. S1 e). The conservation of these critical residues suggests that the mechanism of BNP binding to this receptor as well as the resulting structural change in the receptor may be similar to those described here for ANP. The ANP receptor binds ANP and BNP with high affinity but shows weak affinity to CNP (16). The
weak affinity to CNP, which lacks C-terminal residues (Supplemental Fig. S1 e), may be due to its inability to form this interaction. The orientation of the bound ANP is consistent with the results of affinity labeling reaction in which ANP peptide analogs containing an electrophilic iodoacetyl-group at position 18 and 29 reacted with Met 173 and Asp 194 of the receptor, respectively (K. Misono, in preparation). In the crystal structure of the complex, Gln 18(ANP) is positioned close to Met 173A, and Arg 27(ANP) positioned near Asp 194A (Fig. 5b), corroborating the structure assignment for the bound ANP.

**Rotation Mechanism for ANP Receptor Signaling**—Fig. 6a illustrates schematically the movement of the two ANPR molecules induced upon ANP binding. Each ANPR molecule is shown by a solid cylinder because ANP binding causes only a slight intra-molecular conformational change. Upon ANP binding, the two molecules undergo a twist motion centered on point O and close onto the ligand ANP. In cross-section at the juxtamembrane region (arrow), seen from the top (Fig. 6b), this twist motion causes the juxtamembrane domains of the two molecules (depicted by circles) to translocate, by an angle of 24° with respect to point O, from the apo position (circle C_{apo}) to the complexed position (C_{com}) without appreciable change in the inter-domain distance. This parallel translation alters the relative orientation of the two juxtamembrane domains in the receptor dimer. This change in the orientation is equivalent to rotating each of the two domains by 24° counter-clockwise (Fig. 6b, inset). Earlier, Koshland and colleagues postulated models for transmembrane signaling mechanism that depended on the hypothetical motion in the transmembrane region (32). Their models included association, dissociation, piston, rotation, scissor, and seesaw models. The ANP-induced motion of the juxtamembrane domains in the ANP receptor corresponds closely to the rotation mechanism postulated by these investigators (shown schematically in Fig. 6b, inset).
Signaling by many single-span transmembrane receptors is thought to occur by the association (or clustering) mechanism. Association of the receptor molecules to a dimer (or an oligomer) brings their intracellular domains close together. The proximity of the intracellular domain, in turn, triggers the actions of the effector-enzyme domains inside the cell. Certain single-span transmembrane receptors, such as the erythropoietin (EPO) receptor, occur as a preformed dimer in the absence of the hormone. In the crystal structure of the extracellular domain of EPO receptor, two C-termini in the juxtamembrane region are apart by 73 Å in the apo-dimer (33). EPO binding causes a large conformational change in the dimer that closes the C-termini distance to 39 Å (33,34). This approximation of the two C-termini is suggested to trigger the intracellular signaling cascade. It has also been shown by fluorescence-conjugation assay that EPO binding brings the intracellular domains into proximity (35), supporting the association (or approximation) mechanism proposed for EPO receptor signaling (33). In contrast, the juxtamembrane domains of the ANP receptor dimer are close to each other even in the absence of the hormone, and ANP binding causes no appreciable change in the inter-domain distance (Fig. 2c, Fig. 6b). Instead, ANP binding causes a large 24° rotation of each of the two juxtamembrane domains, altering their relative orientations (Fig. 6b). In the intact ANP receptor, this hormone-induced rotation of the juxtamembrane domains, transduced through the transmembrane helices, may reorient the relative positions (or configuration) of the intracellular domains to cause GCase activation (Fig. 7).

The ANPR expressed in a soluble form lacks the transmembrane and intracellular domains and, for this reason, its apo and ANP-bound structures may not accurately reflect those of the native full-length receptor in the cell membrane. Nevertheless, we have shown earlier that the effects of mutations at the dimer interface in the full-length receptor on COS cells are consistent
with the crystal structures of the apo and hormone-bound ANPR, and with the ANP-induced structural change identified from those structures (21). Additionally, binding interactions found in the complex structure are in agreement with the structure-activity relationship for ANP (30). These findings together suggest strongly that the ANP-induced structural change identified in this study reflects that occurring in the native receptor in the cell membrane and support the proposed rotation mechanism initiating ANP receptor signal transduction. The structures of the intact receptor in the membrane with and without bound ANP should provide direct evidence for the mechanism in the future.

Soluble GCase, which has approximately 51% sequence identity with the GCase domain of the ANP receptor, is active only as a dimer even though each monomer carries a GCase catalytic site (36). The GCase domain of ANP receptor, when expressed in a soluble form by truncation of the extracellular, transmembrane, and kinase-like domains, spontaneously forms a dimer and is catalytically active (37). It is possible that, in the intact ANP receptor dimer, the GCase catalytic activity is suppressed because the two GCase domains are unable to form an active dimer configuration. We speculate that, upon ANP binding and in the presence of ATP (an obligatory and positive allosteric effector for GCase activation by ANP (38,39)), ANP-induced rotation of the juxtamembrane domains may alter the relative positions (or the conformation) of the intracellular domains such that the catalytic sites of the two GCase domains are brought to an optimal proximity and orientation, thereby giving rise to GCase catalytic activity (Fig. 7).

The rotation mechanism initiating transmembrane signaling by the ANP receptor uncovered in the present study represents to our knowledge the first such mechanism identified for any known class of single-span transmembrane receptors. At the same time, this finding introduces a
new paradigm whereby a transmembrane conformational change involving a rotation, rather than simple association or approximation of the receptor molecules, mediates transmembrane signal transduction.

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REFERENCES


Footnotes:

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1 The abbreviations used are: ANP, atrial natriuretic peptide; ANPR, the extracellular hormone-binding domain of the ANP receptor; GCase, guanylate cyclase; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; EPO, erythropoietin.
Figure Legends

Fig. 1. **Crystal structures of ANPR with and without bound ANP hormone.**  

*a*, The structure of the ANPR dimer complexed with ANP(7-27). The ANPR structure is shown in ribbon model and the ANP peptide shown in green in space filling model. The ball and stick model shows the carbohydrate structures.  

*b*, The structure of the *apo*ANPR dimer. The *apo*ANPR dimer structure was originally proposed in a tail-to-tail dimer configuration (20). However it has been shown recently by site-directed mutagenesis and chemical modification studies that the ANP receptor dimer exists in the head-to-head configuration (21). The two-fold axis relating two ANPR monomers in both ANP-bound and unbound structures runs through the center of the dimer, parallel to the face of the page. Chloride ions are drawn as magenta balls. The figures were drawn with MOLSCRIPT (40) and rendered with RASTER3D (41).

Fig. 2. **ANP-induced quaternary structure change in the dimerized ANPR.**  

*a*, The structure of the ANP-bound complex (orange) superimposed onto the *apo* structure (cyan) is shown in front view. α-Helices are shown as cylinders and α-sheets as ribbons. ANP is shown as green sticks. Point O is the fulcrum of the movement of the two monomers and is on the axis of two-fold symmetry. The point O was identified such that the structure of an ANPR monomer in the *apo*ANPR dimer, rotated around point O as the center, superimposes onto that of a corresponding monomer in the ANP-bound structure.  

*b*, Side view.  

*c*, Bottom view seen from the membrane. Conserved residues Pro 417, Cys 423, and Phe 425 (shown in space-filling model) anchor the juxtamembrane signaling motif invariably found in GCase-coupled receptors (31). Parallel shifts of these residues exemplify the translation of the juxtamembrane domains that alters the relative orientation of the two domains.
Fig. 3. Intra-molecular conformational change induced by ligand-binding in the ANPR and in the natriuretic peptide clearance-receptor extracellular domain. *a*, Super-positioning of the structure of the ANP-bound ANPR (orange) onto that of unbound ANPR (cyan) in front view. ANP binding causes little intra-molecular conformational change (root mean square deviation of Cα, 0.64 Å). *b*, Super-positioning of the clearance-receptor extracellular domain structure in the CNP-bound form (red) onto the unbound form (green) (22). In the clearance-receptor, ligand binding causes each subunit to bend at the flexible intra-subunit hinge structure from “closed” to “open” position, allowing the membrane-proximal domain to swing toward the bound ligand.

Fig. 4. Close-up view of the dimer interface in the *apo* (cyan) and ANP-bound (orange) structures. The dimer interface in the *apo* and in the ANP bound receptor involves buried surface areas of 1,100 Å² and 770 Å², respectively. Dimer contacts in the *apo* structure include two inter-molecular hydrogen bonds between Asp 71 of one monomer (monomer A) (Asp 71A) and His 99B of the other (monomer B) and between His 99A and Asp 71B (blue dotted lines) and hydrophobic interactions Trp 74A-Trp 74B and Phe 96A-Phe 96B. Upon ANP binding, the dimer interface becomes partially open, breaking the inter-molecular contacts Asp 71A-His 99B, His 99A-Asp 71B, and Trp 74A-Trp 74B. The remaining contact Phe 96A-Phe 96B and newly formed hydrogen bonds, Arg 14(ANP)-Glu 119A, Arg 14(ANP)-Asp 62B, and Arg 95A-Asp 62B (orange dotted lines), help stabilize the complex. The figures were drawn with MOLSCRIPT.

Fig. 5. ANP binding-site structure. *a*, Close-up stereo view of receptor and ANP interface. ANP (green) and the interacting amino acid residues of ANPR (yellow) are shown in ball-and-
stick presentation. ANPR monomers A and B, bearing binding sites A and B respectively, are shown in ribbon model. Chloride ions are shown as magenta balls. Hydrogen bonds are represented by red dotted lines. Figures were drawn using MOLSCRIPT and RASTER3D. 

**b** and **c**, Open-page view of the ANP binding surfaces of the receptor generated with GRASP (42). Positive and negative charges are shown in blue and red, respectively. Bound ANP is drawn as green sticks. Receptor residues Met 173 and Asp 194, that are reacted by the stepwise affinity alkylation method (43), are denoted by broken-line circles. ANP peptide analogs containing an electrophilic iodoacetyl-group at positions 18 and 29 (peptide derivatives, \(N_{4a}\)-acetyl-\(N_{18e}\)-IAc-[Lys\(^{18}\)]ANP(4-28)) and \(N_{4a}\)-acetyl-\(N_{29e}\)-IAc-[Lys\(^{29}\)]ANP(4-29), respectively) reacted with Met 173 and Asp 194, respectively (K. Misono, in preparation).

**Fig. 6.** Change in the quaternary structure of dimerized ANPR caused by ANP binding, and proposed rotation mechanism for signaling. **a**, Schematic illustration of the movement of the ANPR molecules upon ANP binding. Each monomer ANPR is shown as a cylinder. ANPR molecules in apo-dimer (cyan cylinders) undergo a twisting motion (arrows) upon binding of ANP (green triangle) centered on the fulcrum point O to the complexed position (orange cylinder). **b**, The bottom of the cylinders, corresponding to the juxtamembrane regions (indicated by the open arrow in a), are approximated by circles. The juxtamembrane domains in the apoANPR dimer (circles \(C_{\text{apo}}\), in cyan) translate by an angle of 24° relative to point O upon ANP binding to the complex position (circles \(C_{\text{com}}\), in orange) with little change in the interdomain distance. The directions in which two \(C_{\text{apo}}\) circles face each other are depicted with the cyan arrows. The arrows move parallel with the movement of the circles (orange arrows) in a non-rotational displacement. This motion, in effect, produces the two juxtamembrane domains to
rotate counter-clockwise by 24° relative to the line connecting the two centers (inset), altering their relative orientation.

Fig. 7. Hypothetical rotation mechanism for transmembrane signaling by the ANP receptor. ANP binding causes a twist motion of the two extracellular domains. This motion produces a rotation of the two juxtamembrane domains relative to each other. This rotation, transduced across the transmembrane helices, reorients the two intracellular domains. In the presence of ATP bound to the protein kinase-like regulatory domain (PKLD), the reorientation of the intracellular domains brings two active sites of GCase domains to optimal proximity and orientation, thereby giving rise to the GCase catalytic activity.
Table I.  

*Crystallographic data and refinement statistics*

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<td>(R_{\text{free}})</td>
<td>26.9 (30.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average (B)-factors (Å(^2))</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP receptor</td>
<td>52.4</td>
</tr>
<tr>
<td>ANP</td>
<td>44.0</td>
</tr>
</tbody>
</table>
Oligosaccharides 70.1

Chlorides 53.1

r.m.s. deviation from ideality

Bond length (Å) 0.009

Bond angles (°) 1.5

Bonded B-factors (Å²)

(main chain, side chain) 1.6, 2.6

Ramachandran plot (%)

(Favored, allowed, generous, disallowed) 84.2, 15.4, 0.4, 0

*Rmerge = Σhkl |I - <I>| / Σhkl I, where I is the intensity of unique reflection hkl, and <I> is the average over symmetry-related observation of unique reflection hkl. †Rcryst = Σ |Fo -Fc| / Σ |Fo|, where Fo and Fc are the observed and calculated structure factors, respectively. ‡Rfree is R with 7.5% of reflections sequestered before refinement.
misono_fig1
misono_fig2
misono_fig6
misono_fig7
Supplementary Data

"Crystal Structure of Hormone-Bound Atrial Natriuretic Peptide Receptor Extracellular Domain: Rotation Mechanism for Transmembrane Signaling"

Haruo Ogawa, Yue Qiu, Craig M. Ogata & Kunio S. Misono
### Table S1. Hydrogen bonds between ANPR and ANP contributing to binding

<table>
<thead>
<tr>
<th>ANP atoms</th>
<th>Binding site A</th>
<th>ANP atoms</th>
<th>Binding site B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe8 O</td>
<td>E169 Oδ2</td>
<td>Arg14 Nδ1</td>
<td>D62 Oδ2</td>
</tr>
<tr>
<td>Arg14 Nδ2</td>
<td>E119 Oδ1</td>
<td>Arg14 Nδ2</td>
<td>D62 Oδ2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asn24 Oδ1</td>
<td>H185 Nδ2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ans24 O</td>
<td>E187 N</td>
</tr>
</tbody>
</table>

### Table S2. Hydrophobic interactions between ANPR and ANP

<table>
<thead>
<tr>
<th>ANP residue</th>
<th>ANPR residues in binding site A</th>
<th>ANPR residues in binding site B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe8</td>
<td>Y154, F165, V168, Q169, Y172, H185</td>
<td>none</td>
</tr>
</tbody>
</table>

### Table S3. List of all buried residues (<4.5 Å) in the ANP-bound ANPR dimer complex

<table>
<thead>
<tr>
<th>ANP residues</th>
<th>ANPR residues in binding site A (total buried surface area 1,374 Å²)*</th>
<th>ANPR residues in binding site B (total buried surface area 1,367 Å²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys7</td>
<td>Y154</td>
<td></td>
</tr>
<tr>
<td>Phe8</td>
<td>Y154, F165, V168, Q169, Y172, H185</td>
<td></td>
</tr>
<tr>
<td>Gly9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg11</td>
<td>L112, V116, M173</td>
<td></td>
</tr>
<tr>
<td>Ile12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp13</td>
<td>V87, Y88, A91, A111</td>
<td></td>
</tr>
<tr>
<td>Arg14</td>
<td>R95, Q119</td>
<td>D62, Y88</td>
</tr>
<tr>
<td>Ile15</td>
<td></td>
<td>Y88, A91, P92, R95, Y95, Y120</td>
</tr>
<tr>
<td>Gly16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala17</td>
<td></td>
<td>R95</td>
</tr>
<tr>
<td>Glu18</td>
<td></td>
<td>A91, A111, I114, Y120, F166</td>
</tr>
<tr>
<td>Ser19</td>
<td>L158</td>
<td></td>
</tr>
<tr>
<td>Gly20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu21</td>
<td></td>
<td>Y172</td>
</tr>
<tr>
<td>Gly22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn24</td>
<td></td>
<td>H185, Q186</td>
</tr>
<tr>
<td>Ser25</td>
<td></td>
<td>Q187</td>
</tr>
<tr>
<td>Phe26</td>
<td></td>
<td>K198, R201</td>
</tr>
<tr>
<td>Arg27</td>
<td></td>
<td>M173</td>
</tr>
</tbody>
</table>

*The buried surface area was calculated with a 1.4 Å probe radius.
**Fig. S1.** SIGMAA-weighted 2Fo-Fc map near the dimer interface (a) and for the bound ANP molecule (b, c, d). a, The density map is shown with the assigned structure in stereo presentation. b, The peptide structure was refined as alternate conformations with equal occupancy (0.5). The two structures are shown in green and orange in the electron density map. The two-fold symmetry of the peptide structures is reflected in the electron density map. c, d, The ANP structures in two different orientations are shown individually in the electron density map. The figures were prepared using program PyMOL (1). e, Amino acid sequences of ANP, BNP, and CNP from rat. Residues conserved are shaded green. In all peptides, the two Cys residues (in bold) are disulfide linked.
**Animation S2.** Animation showing the change in the quaternary structure of the ANPR dimer caused by ANP binding, viewed from the a front, b side, and c bottom.

[This animation is attaches as a QuickTime movie file.]
Fig. S3. Ligand-induced conformational change in the clearance-receptor extracellular domain. The dimer structure of the *apo* (green) and peptide-bound complex (red) in the a front, b side, and c bottom view modeled based on the structures reported by He et al. (2). The peptide ligand (CNP) is shown in blue. Binding causes the membrane proximal-domain to swing in by 13° toward the bound peptide, while the dimerized structure of the membrane-distal domains remains essentially unchanged. The models were drawn using the coordinates at the Protein Data Bank (PDB accession numbers 1JDP and 1JDN for the CNP-bound and unbound forms of clearance-receptor extracellular domain, respectively).
References


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Crystal structure of hormone-bound atrial natriuretic peptide receptor extracellular domain: Rotation mechanism for transmembrane signal transduction

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