Identification of Domain Structures in the Propeptide of Corin Essential for the Processing of Proatrial Natriuretic Peptide*

Received for publication, May 6, 2004, and in revised form, June 9, 2004
Published, JBC Papers in Press, June 10, 2004, DOI 10.1074/jbc.M405041200

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Corin is a type II transmembrane serine protease and functions as the proatrial natriuretic peptide (pro-ANP) convertase in the heart. In the extracellular region of corin, there are two frizzled-like cysteine-rich domains, eight low density lipoprotein receptor (LDLR) repeats, a macrophage scavenger receptor-like domain, and a trypsin-like protease domain at the C terminus. To examine the functional importance of the domain structures in the propeptide of corin for pro-ANP processing, we constructed a soluble corin, EKshortCorin, that consists of only the protease domain and contains an enteropeptidase (EK) recognition sequence at the conserved activation cleavage site. After being activated by EK, EKshortCorin exhibited catalytic activity toward chymotryptic substrates but failed to cleave pro-ANP, indicating that certain domain structures in the propeptide are required for pro-ANP processing. We then constructed a series of corin deletion mutants and studied their functions in pro-ANP processing. Compared with that of the full-length corin, a corin mutant lacking frizzled 1 domain exhibited ~40% activity, whereas corin mutants lacking single LDLR repeat 1, 2, 3, or 4 had ~49, ~12, ~53, and ~77% activity, respectively. We also made corin mutants with a single mutation at a conserved Asp residue that coordinates Ca2+-binding in LDLR repeats 1, 2, 3, or 4 (D300Y, D336Y, D373Y, and D410Y) and showed that these mutants had ~25, ~11, ~16, and ~82% pro-ANP processing activity, respectively. Our results indicate that frizzled 1 domain and LDLR repeats 1–4 are important structural elements for corin to recognize its physiological substrate, pro-ANP.

Human corin is a trypsin-like serine protease first cloned from the heart (1, 2). Corin mRNA and protein are abundantly expressed in cardiacomyocytes of the atrium and ventricle. In transfected human embryonic kidney (HEK)1 293 cells, recombinant human corin converted pro-atrial natriuretic peptide (pro-ANP) to biologically active ANP (3), a cardiac hormone essential in controlling blood pressure and maintaining electrolyte and body fluid homeostasis (4–6). In cultured cardio- myocytes, overexpression of an active site mutant corin or transfection of small interfering RNAs directed against the corin gene completely blocked the processing of pro-ANP (7). These data indicate that corin is the pro-ANP convertase that had remained elusive for many years.

Human corin is a polypeptide of 1042 amino acids that contains a short cytoplasmic tail at the N terminus followed by an integral transmembrane domain (1, 2). In the extracellular region, there are two frizzled-like cysteine-rich domains, eight LDLR class A repeats, a macrophage scavenger receptor-like domain, and a trypsin-like protease domain at the C terminus (see Fig. 1A). Topologically, corin belongs to a newly defined type II transmembrane serine protease family, which includes enterokinase (EK), hepsin, matriptases, TMPRSS2-5, human airway trypsin-like protease, and polyserase-I (8–12). The combination of the domain structures in corin is unusual among this family of serine proteases. Corin, for example, contains two frizzled-like cysteine-rich domains that are common in Wnt-interacting proteins but not in trypsin-like proteases. Most recently, we expressed and purified a soluble corin that consisted of only the extracellular fragment (13). The soluble corin converted human pro-ANP to biologically active ANP in a highly sequence-specific manner, indicating that the transmembrane domain of corin is not necessary for pro-ANP processing. It remained unknown, however, whether the other domains such as frizzled-like cysteine-rich domains and LDLR repeats contribute to the pro-ANP processing activity of corin.

In this study, we assessed the functional importance of the domain structures in the propeptide of corin for pro-ANP processing. We showed that a soluble corin that consisted of only the serine protease domain retained the catalytic activity toward small peptide substrates but was inactive in processing pro-ANP. We further identified a region in the propeptide of corin comprising frizzled 1 domain and LDLR repeats 1–4 that is critical for pro-ANP processing.

EXPERIMENTAL PROCEDURES

Materials—Cell culture medium, G418, anti-V5 and anti-Xpress antibodies, transfection reagent LipofectAMINE 2000, expression vectors pSecTag/FRT/V5-His-TOPO, and pcDNA4/HisMaxC were from Invitrogen. Fetal bovine serum was from SeraCare Life Sciences, Inc. (Oceanside, CA). HEK 293 cells were from the American Type Culture Collection (Manassas, VA). Oligonucleotide primers were synthesized by BIOSOURCE International Inc. (Camarillo, CA). Restriction enzymes and DNA polymerases were from New England Biolabs Inc. (Beverly, MA). Recombinant bovine light chain EK and EK capture beads (EK-uptore) were from Novagen Inc. (Madison, WI). Chromogenic substrates were from DiaPharma (West Chester, OH). Phenylmethylsulfonyl fluoride was from Bachem Bioscience Inc. (King of Prussia, PA). Protease inhibitors pepstatin, leupeptin, and EDTA-free protease inhibitor mixture tablets were from Roche Diagnostics. All other chemical reagents were from Sigma.

Expression Vectors—The expression vector, pSECEKsolCorin, encoding a soluble corin (EKsolCorin) that consists of an IgG signal peptide at the N terminus followed by a 919-amino acid sequence from the extracellular region of corin (residues 124–1042) and a viral V5 tag at the C terminus, was described previously (13). In EKsolCorin, the conserved activation cleavage sequence (RMNKR) was replaced by an EK recognition sequence (DDDDK), which allows for the activation of corin by

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The abbreviations used are: HEK, human embryonic kidney; ANP, atrial natriuretic peptide; EK, enterokinase; LDLR, low density lipoprotein receptor.
EK (see Fig. 1A). The plasmid pSECEKsolCorin was used as a template to construct the expression vector pSECEKshortCorin, encoding a protein that contains all of the propeptide of corin followed by a signal peptide from the N terminus of the soluble corin precursor. The plasmid pSECEKshortCorin was used as a template to amplify a second cDNA fragment flanked by an XhoI recognition sequence at the conserved activation cleavage site. Oligonucleotide primers 5'-GTT TAG GAA GGT CTT GTG GAT GTA AAT CTG-3' and 5'-AAA CAA GAC TGG GGC CCG CGG-3' and Pfu polymerase were used in PCR to amplify a human corin cDNA fragment (2452–3222 bp). After 30 cycles, 1 unit of Taq polymerase was added to the reaction and incubated at 72 °C for 10 min. This step allows adding an extra base of adenine at the 3'-end of PCR products, facilitating the cloning of DNA fragments into the pSTag/FRT/V5-His-TOPO vector.

To construct expression vectors encoding corin deletion mutants, a two-step PCR strategy was used. The full-length human corin cDNA was used as a template. The first step was to amplify a cDNA fragment flanked by an EcoRI site at the 5'- and an XhoI site at the 3'-end. The PCR products were digested with restriction enzymes EcoRI and XhoI and cloned into the vector pcDNA4/HisMaxC (Invitrogen). The PCR products were digested with restriction enzymes XhoI and ApaI and cloned into the pcDNA4/HisMaxC vector that contained the first cDNA fragment. The segments derived from PCR in each expression plasmid were verified by DNA sequencing. Table I summarizes the corin cDNA sequences that were used, 5'-GAC TGT GTG GAT for mutant D336Y, 5'-GAC TGT GTG GAT for mutant D373Y, and 5'-GAC TGT GTG GAT for mutant D410Y. Like the deletion mutants described above, these corin mutants also contain an Xpress tag at their N termini. All corin mutants in this series contain an Xpress tag at their N termini. To construct plasmids expressing corin mutants D300Y, D336Y, D373Y, and D410Y, site-directed mutagenesis was performed using the QuikChange II site-directed mutagenesis kit (Stratagene) and a full-length corin cDNA as template. The following oligonucleotide primers were used, 5'-C TGT GAC GAC TAC TGG TGT GAC GAT TGC AAC-3' for mutant D300Y, 5'-GAC TGT GAC TAC TGG TGT TGT GAC GAA AAT TGG TGT TGC-3' for mutant D336Y, 5'-GAC TGT GAC TGG TAC AGG TAC GAT TGG TGT TAC TGC AGC-3' for mutant D373Y, and 5'-GAC TGG AAC GAT GGG CAT TGT GAT GAC AAC TGC TGC TGC-3' for mutant D410Y. Like the deletion mutants described above, these corin mutants also contain an Xpress tag at their N termini.

**Expression, Purification, and Activation of EKshortCorin**—HEK 293 cells were co-transfected with the expression vector pSECEKshortCorin and a plasmid expressing the neomycin resistance gene using LipofectAMINE 2000. Stable clones expressing EKshortCorin were selected in α-minimum essential medium containing 500 μg/ml G418. Positive clones were identified by Western blotting and adapted for growth in serum-free Opti-MEM I medium. To purify EKshortCorin, the conditioned medium was collected, passed through a 0.2-μm filter, and dialyzed against Buffer A (50 mM Tris-HCl, pH 7.5, 300 mM NaCl) using a Dialyse Direct L module (Qiagen, Valencia, CA). The medium was loaded onto a 25-ml nickel-nitrilotriacetic acid Superflow column (Qiagen) that was subsequently washed with Buffer A containing 10 mM imidazole and eluted with a 10–250 mM imidazole linear gradient in Buffer A. Fractions containing the soluble corin were combined. The procedure had a purity of >95%, as determined by SDS/PAGE followed by silver staining and Western blotting. To activate EKshortCorin, 50 μg of the purified protein was incubated with 15 units of recombinant EK in 5 ml of activation buffer (100 mM Tris-HCl, pH 7.5, 10 mM CaCl₂) at 25 °C for 2 h. EK was then removed using EKapture beads by centrifugation (10000 g, 10 min). As a control, an assay buffer without corin protein underwent the same EK activation and removal procedures.

**Enzyme Kinetics**—Kinetic constants were determined using two selected chromogenic substrates S-2366 (pyroGlu-Pro-Arg-p-nitroanilide-HCl) and S-2403 (pyroGlu-Phe-Lys-p-nitroanilide-HCl), which were used previously for a full-length soluble corin (EKsolCorin) (13). For each assay, 50 μl of substrates (final concentrations from 0.2 to 2 mM in 100 mM Tris-HCl, pH 7.5, 10 mM CaCl₂) were mixed with 50 μl of activated EKshortCorin (final concentration of 40 nM) in a 96-well plate. The plate was incubated at 37 °C and read at 405 nm wavelength over 15 min at 20 s intervals in a plate reader (Spectra MAX 200, Molecular Devices Corp., Sunnyvale, CA). In these experiments, controls included purified EKshortCorin that was not activated by EK and an assay buffer that underwent the same EK treatment and removal procedures. Readings from these controls, which were minimal, were subtracted as the background. The Kₘ and Vₘₐₓ values were determined by a Lineweaver-Burk double-reciprocal plot. Each assay was carried out in triplicate and repeated at least three times.

**Effects of Protease Inhibitors**—Effects of protease inhibitors on EKshortCorin and EKsolCorin were tested in an assay using the chromogenic substrate S-2403. In each experiment, 45 μl of activated EKshortCorin or EKsolCorin (final concentration of 50 nM) was added to 1 μl of an inhibitor (final concentrations ranging from 50 to 200 μM) and incubated at 37 °C for 30 min. The substrate S-2403 (final concentration of 500 μM) was added to the mixture, and the absorbance was measured at 405 nm after 2 h. Each assay was performed in triplicate and repeated at least twice.

**Detection of Corin Protein**—For detection of the cell surface—HEK 293 cells in 100-mm dishes were transiently transfected with plasmids expressing either human pro-ANP or corin using LipofectAMINE 2000 according to the manufacturer’s instructions. Conditioned medium containing recombinant human pro-ANP was collected after 16 h, added to cells expressing either the full-length or mutant corin, and incubated at 37 °C for 4 h. Recombinant pro-ANP in cell lysates and recombinant corin derivatives in the conditioned medium were analyzed by Western blotting using an anti-Xpress- or anti-V5 antibody. To examine the pro-ANP processing activity of the soluble corins, EKshortCorin or EKsolCorin was first activated by EK and then added to the conditioned medium containing pro-ANP. Pro-ANP processing was analyzed by Western blotting. To quantify the conversion of pro-ANP to ANP, protein bands were analyzed using the ChemiImager™ 4400 instrument (Alpha Innotech Corp., San Leandro, CA). The optical density of the bands representing pro-ANP and ANP was measured, and the percentage of pro-ANP conversion was calculated. For each corin mutant, at least three independent experiments were performed.
Corin Propeptide Domains

**A**

[Diagram of full-length and soluble corins]

**B**

Table II

### Effects of Protease Inhibitors on the Hydrolytic Activity of EKshortCorin and EKsolCorin

| Inhibitor       | Concentration | Inhibition EKshortCorin | Inhibition EKsolCorin |
|-----------------|---------------|-------------------------|-----------------------|
| EDTA            | 20 mM         | 0                       | 0                     |
| Pepstatin       | 50 μM         | 0                       | 0                     |
| Benzamidine     | 5 mM          | 92.9 ± 2.3              | 90.8 ± 0.6            |
| Phenylmethylsulfonyl fluoride | 50 μM      | 42.9 ± 1.1              | 41.0 ± 1.6            |
| Leupeptin       | 50 μM         | 92.2 ± 2.5              | 91.9 ± 2.1            |
| Soybean trypsin inhibitor | 0.5 μM    | 93.0 ± 2.1              | 95.5 ± 0.6            |

The data are presented as the means ± S.D. from at least three independent experiments.

*Fig. 1. Processing of pro-ANP by EKsolCorin and EKshortCorin.* A, schematic diagrams of the full-length and soluble corins. The transmembrane domain (TM), frizzled-like cysteine-rich domains (Fz), LDLR class A repeats (LDLR), scavenger receptor cysteine-rich domain (SR), and protease catalytic domain (Catalytic) with active site residues histidine (H), aspartate (D), and serine (S) are indicated. The activation site is indicated by an arrow. The disulfide bond (-S-S-) connecting two polypeptide chains after the activation is shown. In soluble EKsolCorin and EKshortCorin, the cytoplasmic tail and transmembrane domain are replaced by a signal peptide from human Ig.

and EKshortCorin (right panel) migrated on SDS-PAGE as single bands of ~150 and ~38 kDa, respectively. The observed size of EKshortCorin was consistent with its calculated mass of ~33 kDa. EKshortCorin also contains one putative N-linked glycosylation site. When treated with increasing concentrations of EF, corinzymogens were converted to two-chain proteins in a dose-dependent manner. Because the V5 tag is located at the C termini of the proteins, the antibody detected only the C-terminal protease domain, which appeared as a band of ~35 kDa under reducing conditions (Fig. 1B). When activated EKshortCorin and EKsolCorin were added to the conditioned medium containing recombinant pro-ANP, only EKsolCorin, but not EKshortCorin, converted pro-ANP to ANP (Fig. 1B).

**Catalytic Activity of EKshortCorin**—One possibility for the failure of EKshortCorin to cleave pro-ANP could be that the protein was not properly folded in the absence of the propeptide, making it catalytically inactive. We then compared enzyme kinetics of EKshortCorin and EKsolCorin, which is biologically active (13), using the chromogenic substrates S-2366 and S-2403. The $K_m$ values for EKshortCorin were similar to those of EKsolCorin (3.63 ± 0.48 versus 3.29 ± 0.85 μM for S-2366 and 1.52 ± 0.20 versus 1.40 ± 0.38 μM for S-2403). Similar $k_{cat}$ values were also found for EKshortCorin and EKsolCorin (0.50 ± 0.06 versus 0.43 ± 0.14 s⁻¹ for S-2403 and 0.46 ± 0.06 versus 0.51 ± 0.12 s⁻¹ for S-2366). The results are consistent with the previously published data for EKsolCorin (13) and show that EKshortCorin is catalytically active toward small peptide substrates.

**Effects of Protease Inhibitors**—We next examined the effects of protease inhibitors on the catalytic activity of EKshortCorin and EKsolCorin in a chromogenic substrate-based assay. As summarized in Table II, the catalytic activity of EKshortCorin and EKsolCorin was inhibited similarly by trypsin-like serine...
protease inhibitors including benzamidine, phenylmethylsulfonyl fluoride, leupeptin, and soybean trypsin inhibitor but not by metallo- and cysteine-protease inhibitors such as EDTA and pepstatin. The data show that the active center of EKshort-Corin is properly formed consistent with the results from the kinetic studies described above. Together, these data indicate that the propeptide of corin is not necessary for its interactions with small peptide substrates and inhibitors but is required for its interaction with the physiological substrate pro-ANP.

Membrane-bound Corin with Internal Domain Deletions—To understand how different extracellular domains of corin contribute to its activity in pro-ANP processing, we constructed a series of mutants by deleting increasing numbers of domains at the N terminus of the extracellular region (Fig. 2A). All of these mutants were designed to contain the transmembrane domain, allowing us to examine pro-ANP processing in cell-based assays. The plasmids encoding these mutants were transfected in HEK 293 cells. Expression of recombinant corin proteins was allowed us to examine pro-ANP processing in cell-based assays. Cells transfected with the plasmid expressing the full-length corin but not biotinylated (FL0) or transfected with plasmids expressing soluble EKsolCorin and EKshortCorin or a control vector and biotinylated were included as controls (lower panels).

Expression of Corin Mutants on the Cell Surface—To exclude the possibility that the corin deletion mutants lost their activity because of the lack of cell surface expression, we examined the cell surface expression using a biotin-labeling method. HEK 293 cells were transiently transfected with plasmids encoding the full-length corin or corin mutants ΔFz1, ΔFz1R1-5, and ΔR1-5. Cell surface proteins were then labeled with biotin. Cells transfected with plasmids encoding soluble EKsolCorin and EKshortCorin or a parental vector were included as controls. Cells transfected with a plasmid expressing the full-length corin but that were not biotinylated were used as an additional control. By SDS-PAGE and Western blotting, we showed that the full-length corin and corin mutants ΔFz1, ΔFz1R1-5, ΔR1-5, EKsolCorin, and EKshortCorin proteins were present in cell lysate (Fig. 2C, upper panels). When the same blots were reprobed with horseradish peroxidase-conjugated streptavidin, which binds to biotin-labeled cell surface proteins, the full-length corin and mutant corin ΔFz1, ΔFz1R1-5, and ΔR1-5 proteins, but not control soluble EKsol-
Corin and EKshortCorin, were detected (Fig. 2C, lower panels). No specific bands were detected in cells transfected with the plasmid encoding the full-length corin but not biotinylated or cells transfected with a control vector and biotinylated (Fig. 2B, lower panels). The results show that mutant corin ΔFz1, ΔFz1R1–5, and ΔR1–5 proteins were indeed present on the cell surface.

Functional Importance of Individual LDLR Repeats—To determine the functional importance of each individual repeat within the fragment containing LDLR repeats 1–5, we first generated a series of plasmids expressing corin mutants lacking frizzled 1 domain and increasing numbers of LDLR repeats (ΔFz1R1, ΔFz1R12, ΔFz1R1–3, ΔFz1R1–4) (Fig. 3A). These data show that in the absence of frizzled 1 domain deletion of LDLR repeat 1 alone or together with LDLR repeats 2–4 abolished the pro-ANP processing activity of corin.

We then constructed a second series of corin mutants that retained frizzled 1 domain but lacking increasing numbers of LDLR repeats 1–4 (ΔR1, ΔR12, ΔR1–3, and ΔR1–4) (Fig. 4A) and expressed them in HEK 293 cells (Fig. 4B, upper panel). As shown in pro-ANP processing assays, mutant corin ΔR1 had ~49% activity, whereas mutant corin ΔR12 had ~26% activity when compared with that of the full-length corin (Fig. 4B, lower panel). Mutants ΔR1–3 and ΔR1–4 had no detectable activity in pro-ANP processing (Fig. 4B, lower panel). These data show that in the absence of frizzled 1 domain amino acid sequence-
spanning LDLR repeats 1–3 are important for corin-mediated pro-ANP processing.

To examine the contribution of individual LDLR repeats, we constructed a third series of corin mutants lacking each LDLR repeat (ΔR2, ΔR3, and ΔR4) (Fig. 5A) and expressed them in HEK 293 cells transfected with plasmids encoding the full-length corin (FL) and corin mutants ΔR2, ΔR3, and ΔR4, or a control vector (vector) were detected by Western blotting using an anti-Xpress antibody (upper panel). Pro-ANP and its derivatives in the conditioned medium were analyzed by immunoprecipitation and Western blotting using an anti-V5 antibody (lower panel).

These results show that LDLR repeats 1–4 each contributes to the function of pro-ANP processing with LDLR repeat 2 being the most important.

**Effect of Point Mutations in LDLR Repeats 1–4**—To further confirm the importance of each repeat in the LDLR1–4 fragment, we constructed a new series of corin mutants by replacing a highly conserved Asp residue with a Tyr residue in LDLR repeats 1, 2, 3, or 4 (D300Y, D336Y, D373Y, and D410Y) (Fig. 6, A and B). In the LDLR, the corresponding Asp residue has been shown to coordinate Ca$^{2+}$ binding (14, 15) (Fig. 6A), which
is required to maintain the structural integrity of the protein. A point mutation at the Asp residue is expected to alter the conformation of individual LDLR repeats without causing structural perturbation to its neighboring repeats (16). The mutant corin proteins were expressed in HEK 293 cells (Fig. 6C, upper panel), and their pro-ANP processing activities were analyzed by Western blotting (Fig. 6C, lower panel) and quantified by densitometry. Corin mutants D300Y, D336Y, D373Y, and D410Y had 25, 11, 16, and 82% of pro-ANP processing activities, respectively, compared with that of the full-length corin. These results are consistent with data from corin mutants with single LDLR repeat deletions (Figs. 4 and 5) demonstrating the individual contribution of LDLR repeats 1–4 in pro-ANP processing.

**DISCUSSION**

In this study, we examined the functional importance of the domain structures in the propeptide of corin for pro-ANP processing. We expressed a soluble corin, EKshortCorin, that consists of only the serine protease domain and contains an EK recognition sequence at the conserved activation cleavage site. We showed that EKshortCorin was activated by EK and retained the catalytic activity when examined in chromogenic substrate-based assays. Unlike the full-length soluble corin, EKsolCorin, which is biologically active (13), EKshortCorin failed to cleave pro-ANP indicating that certain domain structures in the propeptide are required for this activity of corin. We constructed a series of corin mutants by deleting increasing numbers of domain structures starting at the N terminus of the extracellular region. We showed that a deletion of frizzled 1 domain reduced the pro-ANP processing activity, whereas a deletion of LDLR repeats 1–5 abolished the activity. By analyzing additional corin mutants that either lacked individual LDLR repeats or contained point mutations at a conserved Ca\(^{2+}\) binding site in the LDLR repeats, we showed that a region spanning from frizzled 1 domain to LDLR repeat 4 is critical for the activity of corin in processing pro-ANP. Fig. 7 summarizes the corin mutants described in this study and their activities in pro-ANP processing.

Corin is a mosaic protease that contains a transmembrane domain near the N terminus and several distinct domain structures in its C-terminal extracellular region. In a recent study, we showed that the transmembrane domain is not required for the activity of corin in pro-ANP processing (13), but the functional importance of other extracellular noncatalytic domains of corin was not determined. Previous studies have shown that noncatalytic domains in the extracellular region of other type II transmembrane serine proteases are important for their biological functions. For example, Lu et al. (17) have reported that a soluble bovine EK consisting of only the protease domain is catalytically active toward small peptide substrates but fails to activate trypsinogen indicating that the propeptide of EK plays a role in recognizing trypsinogen. In this study, we showed that frizzled 1 domain and LDLR repeats 1–4 in the propeptide of corin are required for the processing of pro-ANP. To our knowledge, EK and corin are the only two members from the type II transmembrane serine protease family for which physiological substrates have been identified. These data suggest that the propeptide in other type II transmembrane serine proteases...
may also have an important function in interacting with their physiological substrates. It will be important to test this concept in future studies.

The frizzled-like cysteine-rich domain, which is ~120 amino acids in length and contains 10 conserved cysteine residues, was first discovered in members of the Frizzled family of seven-transmembrane receptors for Wnt signaling proteins (18, 19). Subsequently, the frizzled-like cysteine-rich domain also has been found in soluble Frizzled-related proteins that act as antagonists of Wnt signaling (20–22). Studies have shown that the frizzled-like cysteine-rich domain interacts directly with Wnt proteins (23, 24). In addition to Wnt receptors and inhibitors, other proteins such as human carboxypeptidase Z (25), mouse collagen (XVIII) α1 chain (26), and several receptor tyrosine kinases including muscle-specific kinase and Smoothened (27, 28) also contain frizzled-like cysteine-rich domains. A recent study (29) has indicated that the frizzled-like cysteine-rich domain in chick carboxypeptidase Z binds to Wnt proteins and plays a role in the formation of skeleton. At this time, the functional significance of the frizzled-like domain in collagen and plays a role in the formation of skeleton. At this time, the frizzled-like cysteine-rich domain in chick carboxypeptidase Z binds to Wnt proteins (XVIII) α1 chain, muscle-specific kinase, and Smoothened is not known. In this study, we showed that the deletion of frizzled 1 domain reduced the activity of corin in pro-ANP processing assays, mutants D300Y, D336Y, D373Y, and D410Y. The mutation is expected to affect only the function of the individual LDLR repeat in which it resides but not the overall protein structure.

Unlike the deletion mutants, the mutants with a single amino acid substitution shall also maintain the distance between the cell membrane and individual LDLR repeats, which may be important for the binding of macromolecular substrates. In pro-ANP processing assays, mutants D300Y, D336Y, D373Y, and D410Y had ~25, ~11, ~16, and ~82% activities, respectively. The overall data are consistent with the results from corin mutants R1Δ, R2Δ, R3Δ, and R4Δ, showing that LDLR repeats 1–4 all contribute to the binding of pro-ANP and that among these LDLR repeats repeat 2 appears to be most critical, whereas repeat 4 is least important. Based on these results, we propose a model in which pro-ANP binds to corin by interacting with frizzled 1 domain and LDLR repeats 1–4 (Fig. 8). The binding of pro-ANP to this region of corin may allow the protease domain to cleave the substrate more efficiently. It is equally possible that the binding induces conformational changes in pro-ANP, making its activation cleavage site accessible to the protease domain of corin. This model may explain why pro-ANP is resistant to the cleavage by EKshortCorin, which contains only the catalytic domain. At this time, the specific amino acid residues in frizzled 1 domain, and LDLR repeats 1–4 of corin that make contact with pro-ANP are not known. Further mutagenesis experiments shall help to verify and refine this model.

Acknowledgments—We thank René Pagila, Silke Finster, and Dr. Brent Larsen for their advice in protein purification, Dr. Marc Whitlow for helpful discussions on protein structures, Drs. John Morser and Bill Dole for their support, and Prof. Peter Donner for his encouragement.

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J. Biol. Chem. 2004, 279:34464-34471.
doi: 10.1074/jbc.M405041200 originally published online June 10, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M405041200

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