V. Summary

Corin is a cardiac mosaic serine protease that contains an integral transmembrane domain near the N-terminus. In the extracellular region of corin, there are two Fz domains, eight LDLR repeats, one SR domain, and a trypsin-like serine protease domain at the C-terminus. In previous cell-based studies, corin converted pro-ANP to biologically active ANP, suggesting that corin might be the long-sought pro-ANP convertase. This notion is supported by a recent study of corin-deficient mice in which the conversion of pro-ANP to ANP was abolished. In the first half of this study, we assessed the requirement of the transmembrane domain and activation cleavage in human corin for pro-ANP processing. We showed that a recombinant soluble corin that consists of only the extracellular region was capable of processing recombinant human pro-ANP in cell-based assays. In contrast, a mutation at the conserved activation cleavage site, R801A, abolished the function of corin, demonstrating that activation cleavage is essential for corin activity. These results allowed us to design, express, and purify a mutant soluble corin, EKsolCorin, that contains an EK recognition sequence at the activation cleavage site. Purified EKsolCorin was activated by EK in a dose-dependent manner. Activated EKsolCorin had hydrolytic activity toward peptide substrates with a preference for Arg and Lys residues in the P1, position. This activity of EKsolCorin was inhibited by serine protease inhibitors but not inhibitors of aspartic- or metallo-proteases. In pro-ANP processing assays, purified active EKsolCorin converted recombinant human pro-ANP to biologically active ANP in a highly sequence-specific manner. The pro-ANP processing activity of EKsolCorin was not inhibited by human plasma in the absence or presence of heparin. Together, these data indicate that the transmembrane domain is not necessary for the biological activity of corin but may enable a mechanism to localize corin at specific sites, while the proteolytic cleavage at the activation site is an essential step in controlling the activity of corin.

In the second half of this study, we examined the functional importance of the domain structures in the propeptide of corin for pro-ANP processing. We constructed, expressed, and purified a soluble corin, EKshortCorin, that consists of only the protease domain and contains an EK recognition sequence at the conserved activation cleavage site. After activation by EK, EKshortCorin failed to cleave pro-ANP in a functional assay. In chromogenic substrate-based assays, however, EKshortCorin exhibited catalytic activity and substrate specificity similar to those of EKsolCorin. In addition, protease inhibitors had similar effects on EKshortCorin and EKsolCorin, indicating proper folding of the protease domain in EKshortCorin. Together, these results demonstrated that certain domain structures in the propeptide of corin are required for pro-ANP processing, whereas the recognition of small molecule substrates does not depend on the propeptide. We then constructed different series of corin mutants lacking one or more domains within the propeptide and studied their functions in pro-ANP processing. For example, corin mutants lacking either the Fz1 or Fz2 domain exhibited ~40 % and ~32 % activity compared to that of the full-length corin, whereas corin mutants lacking single LDLR repeat 1, 2, 3, 4, or 5 had ~47, ~13, ~50, ~71 and ~80 % activity, respectively. We also made corin mutants with a single amino acid substitution at a conserved Asp residue that coordinates Ca^{2+}-binding in LDLR repeats 1, 2, 3, and 4 (D300Y, D336Y, D373Y, and D410Y) and showed these mutants had ~25, ~11, ~16, and ~75 % pro-ANP processing activity, respectively. Our results indicate that the Fz domains and LDLR repeats 1-5 within the propeptide of corin are critical structural elements for corin to recognize its physiological substrate, pro-ANP.