we have measured PGK folding kinetics from 295K to 320K both in vitro and in vivo. Kinetics of PGK as multiplet-state folder can be fitted to stretched exponential. Folding rate and folding mechanism of PGK are correlated and both are strongly dependent on temperature, which can be explained by solvent viscosity and hydrophobic interactions.

283-Pos Board B69
Folding Mechanism of a Precursor Protein of a Peptide Hormone Mediated by an Intra-Molecular Chaperone
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Prouroguanylin is a precursor of uroguanylin. The mature form of uroguanylin contains intra-molecular disulfide bonds (Cys74-Cys82 and Cys77-Cys85). The propeptide region functions as an intra-molecular chaperone in the formation of the native conformation and the disulfide pairings of uroguanylin. To elucidate the mechanism of the propeptide-mediated folding, the pathway associated with the disulfide-coupled folding of prouroguanylin was examined in detail. Prouroguanylin, when prepared using an E. coli expression system, was obtained as an inclusion body. Therefore, it was purified as a reduced/denatured protein by reversed-phase HPLC after solubilization in urea. The folding reaction was carried out 0.1 M Tris/HCl (pH 8.0) at various concentrations of glutathione in the presence and absence of protein disulfide isomerase which catalyzes the disulfide exchange reaction. Kinetic analyses of the oxidative folding revealed that two types of intermediates containing mixed-disulfide disulfide bonds (namely, isomers 1 and 2 in which the disulfide bonds were between Cys74-Cys82 and Cys74-Cys77 and Cys82-Cys85 in the mature region, respectively) are predominantly included in the folding. However, only one type of intermediate containing mis-disulfide bonds, isomer 2, was able to proceed to the native conformation of prouroguanylin, regardless of the presence of protein disulfide isomerase. The results of these experiments will be discussed in this presentation.

284-Pos Board B70
Role of Leu66 in the Folding of Uroguanylin Assisted by Intra-Molecular Chaperone
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Uroguanylin is a matured via the processing of a precursor protein, prouroguanylin. The pro-peptide region of the precursor protein of uroguanylin regulates the formation of the native structure of uroguanylin, by serving as an intra-molecular chaperone. To estimate the role of the individual amino acid residues of the pro-peptide region in chaperon function, we previously prepared Gly or Ala mutants and the folding of the mutant proteins were examined. The results revealed that, except for Cys residues, only the Leu66 residue critically affected the folding of the mature region, uroguanylin. To further investigate the role of the Leu66 residue in the folding of uroguanylin, it was mutated to several different amino acid residues, such as Gly, Ala, Val, and Ile. The cDNA’s encoding the mutant proteins were amplified by polymerase chain reaction and inserted into pET17b vector. The mutant proteins were expressed using the T-promoter expression system in E. coli BL21(DE3) cells. The mutant proteins were obtained as inclusion bodies and solubilized in 0.1 M Tris/HCl (pH 8.0) containing 8 M urea and dithiothreitol. The reduced forms of the mutant proteins were purified by reversed-phase high performance liquid chromatography (HPLC) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analyses. The oxidative folding of the mutant proteins was carried out in the absence of reduced and oxidized forms of glutathione and the progress monitored by HPLC. The results of these experiments will be discussed in this paper.

285-Pos Board B71
Evaluating a Key Player in Acute Heart Failure: Interaction Surfaces and Structural Details of Interleukin-33
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The newest member of the Interleukin-1 family of proteins is IL-33. IL-33 was recently discovered in 2005 and since then has been identified as a key participant in immune and inflammatory responses through association with the IL-1 receptor family member ST2. However, the structural homology between IL33 and other members of the interleukin family are low- presenting unique sequence identity, unique receptor interactions, and potentially unique signaling mechanisms associated with its activity. The current, limited understanding of