学位論文要旨

Structure biological study on the peptidyl-prolyl *cis-trans* isomerization mechanism

（　プロリンペプチド結合異性化機構 の構造生物学的研究　）

広島大学大学院理学研究科数理分子生命理学専攻　徐　宁

1. Background

Previous studies have demonstrated that peptidyl-prolyl *cis-trans* isomerization plays an important role in physiological processes. The isomerization process is accelerated by peptidyl-prolyl isomerase (PPIase) in living cells. Pin1 catalyzes specifically pSer/pThr-Pro motif as one famous peptidyl-prolyl isomerase. The *cis–trans* isomerization mechanism of Pin1 has been studied by various approaches, including X-ray crystallography, site directed mutation to identify the functionally relevant residues, and kinetic isotope effect on the isomerization. However, it still remains elusive. Preceding studies proposed that C113 in Pin1 has a catalytic role in the conformational transition process through its nucleophilic attack or hydrogen bonding to substrate carbonyl moiety. The fact that C113D mutant Pin1 does not abolish the activity challenges the importance of C113 as a catalyst. To facilitate our understanding on the Pin1 isomerization process, we compared the structures and dynamics of the wild-type and C113D mutant Pin1 PPIase domains comprising the residues 51–163.

2. Results and discussion

Cis-trans isomerization rates for the wild-type and C113D mutant PPIase domains were measured using 2D EXSY (Figure 1). The isomerase activity of C113D was approximately 70-fold lower than that of wild-type. ITC experiments revealed that C113D mutant had very limited binding ability to the phosphor-peptide, apparently no binding ability was observed. The reduced isomerization rate of C113D mutant was ascribed to its faint substrate affinity.

Three dimensional structures of wild-type and C113D mutant were solved by NMR in the presence of sulfate and phosphate ions. The overlaid structures for the wild-type and C113D PPIases demonstrated the apparent structural changes were in the helix near the mutation site, and the active site loop (Figure 2). The extended helix in C113D could make the amide groups in S114 and S115 stay close to the side chain negative charge of D113 in longer residence time than that for the corresponding residues in the wild-type, which may explain the reduced signal intensities for S114 and S115 in C113D. The side chains of the residues in the basic triad (K63, R68 and R69) were thought to have hydrogen bonds to phosphor moiety in the substrate. The side chain disorientation of the basic triad in C113D mutant may explain the severely reduced binding to the phosphorylated substrate

Values from hNOEs showed the active loops in wild-type and C113D were rigid in the presence and absence of sulfate and phosphate ions; hNOEs comparison indicated that C113D mutation did not apparently affect the structural dynamics in ns-ps time range. The changes in the reduced spectral density function $J_{\mathrm{eff}}(0)$ values were particularly apparent for the residues in the phosphate binding pocket, especially residues 60–75, suggesting the binding pocket became structurally unstable in C113D. Most of the residues in C113D showed enhanced H/D exchange rates relative to the wild-type, implying C113D mutant PPIase structure was less stable than the wild-type, which was also evidenced by the melting temperatures for the wild-type and C113D were 49.4°C and 46.2°C, respectively. In addition, the less number of NOEs from imidazole rings

of H59 and H157 in the catalytic site of C113D mutant relative to the wild-type implicated the increased structural flexure of the histidines in the catalytic site.

We measured the tautomeric state of important dual histidines (H59 and H157) located in the active site for wild-type and C113D mutant PPIases in different buffer conditions. We found that H59 imidazole formed a tighter hydrogen bond to H157 in the wild-type, whilst it had a stronger hydrogen bond to D113 in C113D mutant.

Maleimide fluorescence labeling and mass spectroscopy experiments showed that C113 SH moiety had higher sensitivity to oxidation and C113D could be supposed to mimic the oxidized form of Pin1 PPIase domain that happens inside cells under oxidative stress.

3. Conclusions

This study has demonstrated a novel role of C113 in stabilizing the catalytic site fold. C113D mutation disturbs the hydrogen bonding network formed by the catalytic tetrad by unbalancing the tug-of-war for H59 between the residues at 113 and 157. Dual histidine motif comprising of the conserved H59 and H157 is important for keeping the catalytic site in an active fold. The imbalance of the tag of war for H59 in C113D mutant weakens the hydrogen bond among the dual histidine motif, thus destabilizes the catalytic site fold. The disturbed hydrogen bonding network in the catalytic tetrad leads to the allosteric disorder of the active loop including basic triad that binds to a phosphate moiety in the substrate, which explains the reduced substrate binding ability of C113D mutant. Pin1 oxidation happening in cells should reduce its isomerization activity through the alleviated binding to substrate, as anticipated from the work on C113D mutant being supposed to be a surrogate for the oxidized form of Pin1 PPIase domain.

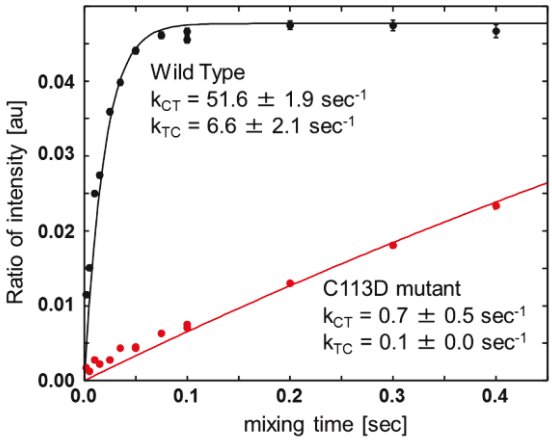

Figure 1: Experimental rate constants for the wild-type (black) and C113D mutant (red) were measured by EXSY with Cdc25C phosphorylated peptide

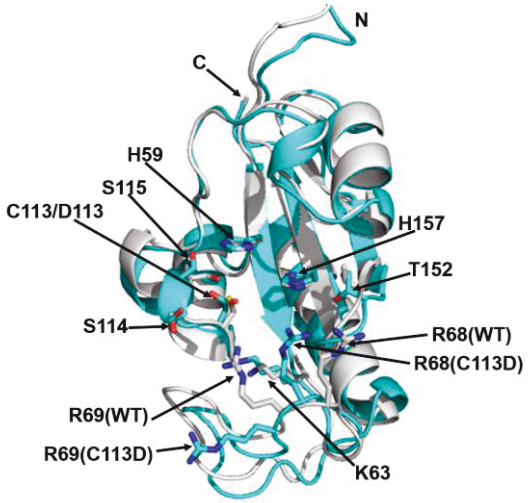

Figure 2: Structure overlap between the wild-type (gray) and C113D mutant (cyan) PPIase domains

Published paper

Xu, N., Tochio, N., Wang, J., Tamari, Y., Uewaki, J., Utsunomiya-Tate, N., Igarashi, K., Shiraki, T., Kobayashi, N., and Tate, S. (2014) C113D mutation in human Pin1 causes allosteric structural changes in the phosphate binding pocket of the PPIase domain through the tug of war in the dual histidine motif, *Biochemistry* 53, 5568-5578.