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NMR study on the structural changes caused by the oxidation to the active cysteine residue in proline cis/trans-isomerase, Pin1

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Human Pin1 (*h*Pin1) recognizes the phosphor-serine/threonine-proline sequences and isomerizes the proline peptide bond. The isomerization to the phosphor-proteins by *h*Pin1 changes their fate in the cells, which also regulate the cell function [1]. In spite of the functional importance of *h*Pin1, its catalytic mechanism still remains elusive. *h*Pin1 contains WW and PPIase domains. The WW domain of *h*Pin1 mainly recognizes the phosphorylated substrate, while the *h*Pin1 PPIase domain is responsible for catalysis. The cysteine at 113 sequence position, C113, in the PPIase domain is known to be the key in catalytic process [2, 3]. We have found that the C113 is easy to be oxidized in the cell. The C113D mutant, which mimics the C113 oxidized form, shows the reduced activity. In the isomerization process, the thiol in C113 functions as a nucleophile to the carbonyl in the target Pro peptide bond, which could be activated by the hydrogen bonding to the neighboring histidine in the active site. The hydrogen bond network in the active site, therefore, was focused in this work.

We determined the solution structures of the PPIase domains of the wild-type *h*Pin1 and C113D mutant by NMR. No apparent structural difference in the backbone structures were found between them (Fig. 1A). In the C113D mutant, however, the signal intensities of the amides protons for S114

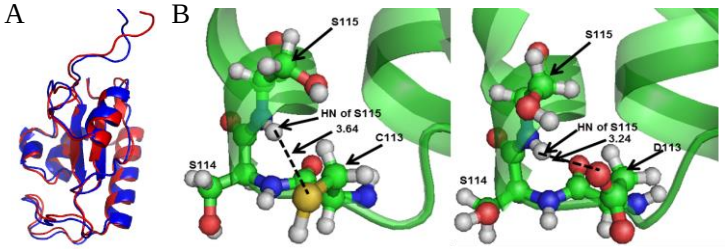

Fig.1. (A): Superposition of ribbon representations of PPIase domains of wild-type *h*Pin1 (red) and C113D mutant (blue). (B): Ribbon diagram of PPIase domains of wild-type *h*Pin1 (left) and C113D mutant (right).

and S115 were weaker than those in the wild-type, suggesting these residues are more flexible in the mutant relative to the corresponding residues in the wild-type. In addition to the change in the structural flexibility of S115, its amide proton has the hydrogen bonding to different atoms in the wild-type and the mutant; S115 amide proton in the wild-type was in the vicinity of C113 thiol group, whilst the corresponding was close to D113 carbonyl oxygen in the mutant (Fig. 1B). Our results may suggest that the catalytic function of *h*Pin1 PPIase domain may be related to the hydrogen bonding among the residues in the active site.

[1]Bayer E et al., *J. Biol. Chem*, 278, 26183-26193 (2003).

[2]Ranganathan R et al., *Cell*, 89, 875-886 (1997).

[3]Behrsin C.D et al., *J. Mol. Biol*, 365, 1143-1162 (2007).