Functional Analysis of Protein Disulfide Isomerase Family Proteins

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I analyzed the functions of protein disulfide isomerase(PDI) family proteins *in vivo* and *in vitro* using a newly developed scFv - selected from a phage antibody library- that recognizes the CGHC motif present in these proteins.

Although the CGHC motif and sequences in its vicinity are conserved among PDI family proteins, polyclonal antibodies raised against individual PDI family proteins did not recognize the CGHC motif due to the elimination of autoimmune reactions. Then the use of an artificial phage antibody library made it possible to obtain the new antibody that is specific for the CGHC motif.

The isomerase activities of PDI family proteins differ from protein to protein, even when their active sites comprise the same sequence. I determined the affinity of selected scFvs to individual active sites in various mutant PDI family proteins. The results obtained indicated that isomerase activity correlates with the accessibility of the scFv to the active site on the protein. This provides the first evidence that environmental factors may dictate the strength of isomerase activity among proteins. The isomerase activity of Mpd1p, which is one of the 4 yeast PDI family proteins, is extremely lower than that of PDI, although Mpd1p has the exact same CGHC motif and surrounding amino acids sequence as PDI. In addition, yeast PDI family proteins all had a chaperone activity except Mpd1p, and only PDI had the isomerase activity. These observations indicate that the yeast growth is completely dependent on the isomerase activity of PDI. Thus, the specific motif, CGHC, is one of the important factors to determine the strength of isomerase activity.

By using the obtained scFvs, I demonstrated for the first time that a lysine residue following the CGHC motif significantly increases the isomerase activity in most PDI family proteins. Thus, the isomerase activity of PDI family proteins is dependent not only on the amino acid sequence of the active site, but also on the location of the active site within the protein.