Functional Analysis of Proteins Based on Their Interactions

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In this thesis, the author focused on the functional analysis of proteins based on protein-medicine or protein-protein interactions. In the process of screening of proteins binding to aminoglicoside antibiotics, which cause hepatopathy or nephrotoxicity, these antibiotics were found to bind to protein disulfide iosmerase(PDI) and to inhibit its chaperone activity. Human P5 (hP5), a PDI homologue, showed the same behavior as PDI regarding the interaction with antibiotics. Human P5 is one of PDI superfamily members, and has two CGHC sequences and the ER retention signal KDEL, which are also found in PDI. The author showed that hP5 has both isomerase and chaperone activities, but both activities are weaker than those of hPDI. Moreover, hP5 was observed to have peptide binding ability, and its chaperone activity was confirmed with rhodanese and citrate synthase as substrates, but not with D-glyceraldehyde-3-phosphate dehydrogenase, showing that hP5 has substrate specificity with respect to chaperone activity. PDI is a multifunctional protein, and indispensable for protein folding in the cell. To examine the invivo functions of PDI in more detail, the author screened for PDI-binding proteins, and found that cyclophilin B (Cyp B) interacted with PDI, and that the chaperone activity of PDI was increased by Cyp B. However, Cyp B completely inhibited its chaperone activity in the presence of cycloosporin A (Cs A), which is an inhibitor of Cyp B. These results indicate that the protein interaction sometimes increases the chaperone activity of PDI, and sometimes decrease it.

Based on the observations obtained in this thesis, the author suggests that PDI and Cyp B cooperate the folding and stability of newly synthesized polypeptides. On the other hand, P5 exerts the chanperone activity for the substrate, which is the same as or different from PDI. Together with the report that secreted proteins decrease after the retrograde transport of aminoglycoside antibiotics, it is suggested that the chaperone activity of PDI is inhibited by aminoglycoside antibiotics, and as a result, folding and stability of newly synthesized polypeptide might be affected by the inhibition. Whether these influences cause the side effect in liver and kidney still remains not clear, however the author suggests that this is one possibility, although further study will be required to establish this clearly.

This thesis will be available for the further elucidation of side effect, which is caused by aminoglycoside antibiotics or quality control system in the ER.