Molecular Basis of Zinc-Dependent Regulation of ERp44 for Protein Quality Control in the Early Secretory Pathway

ERp44 is a PDI family member involved in the transport of various secretory proteins and some ER-resident proteins from the endoplasmic reticulum (ER) to the Golgi. Here, we found that Zn^{2+} binds to ERp44 with high affinity, modulating its intracellular localization and ability to retrieve clients. Crystal structures of Zn^{2+}-bound ERp44 reveal that Zn^{2+} binds to a conserved histidine-cluster, inducing large conformational changes. Consequently, the substrate-binding region of ERp44 is fully exposed to solvent, enhancing interactions with clients. These observations suggest a new physiological role of Zn^{2+} as a key regulator for protein quality control in the early secretory pathway.

ERp44, a member of the protein disulfide isomerase (PDI) family, is involved in the transport of various secretory proteins and several ER-resident proteins from the endoplasmic reticulum (ER) to the Golgi. ERp44 traps its clients in the Golgi, retrieving them to the ER in a pH-dependent manner. High-resolution crystal structures of ERp44 at pH 7.2 and 6.5 revealed that protonation of key histidine and cysteine residues induces pH-dependent conformational changes in the protein, leading to exposure of the positively charged client-binding site including the essential cysteine Cys29 [1]. The structures also suggested that metal ions can bind to a histidine-clustered site (His-cluster) comprising three highly conserved histidines (His299, His328, His332).

In this study, we found that ERp44 specifically binds Zn^{2+} with submicromolar affinity to form a Zn^{2+}-dependent homodimer [2]. Depletion of intracellular zinc by a Zn^{2+} chelator altered subcellular localization of ERp44. Furthermore, silencing the Zn^{2+} transporters localized at the Golgi resulted in dysfunction of this chaperone, and increased secretion of its clients. To elucidate the detailed mechanism of how Zn^{2+} regulates ERp44, we determined the crystal structure of the Zn^{2+} bound form of ERp44 at 2.45Å resolution by the SAD method using the anomalous signals of Zn^{2+}. X-ray diffraction data were collected on the BL-1A beamline.

The overall structure of ERp44 consists of three thioredoxin (Trx)-like domains (**a**, **b**, and **b'**) and a C-terminal tail (C-tail). The crystal structure of the Zn^{2+}-bound ERp44 reveals that ERp44 forms a Zn^{2+}-bridged homodimer, where the **b'** domains from two protomers are bridged by a Zn^{2+} ion (**Fig. 1**). Three types of Zn^{2+}-binding sites (sites 1, 2 and 3) are found at the interface of the ERp44 homodimer. Site 1 is formed by the His-cluster in each protomer, at which a Zn^{2+} ion is coordinated by His299, His328, His332 and a water molecule in a tetrahedral geometry (**Fig. 1**, left lower inset). At the center of the dimer interface (site 2), two histidine pairs (His277 and His288) from the **b'** domains

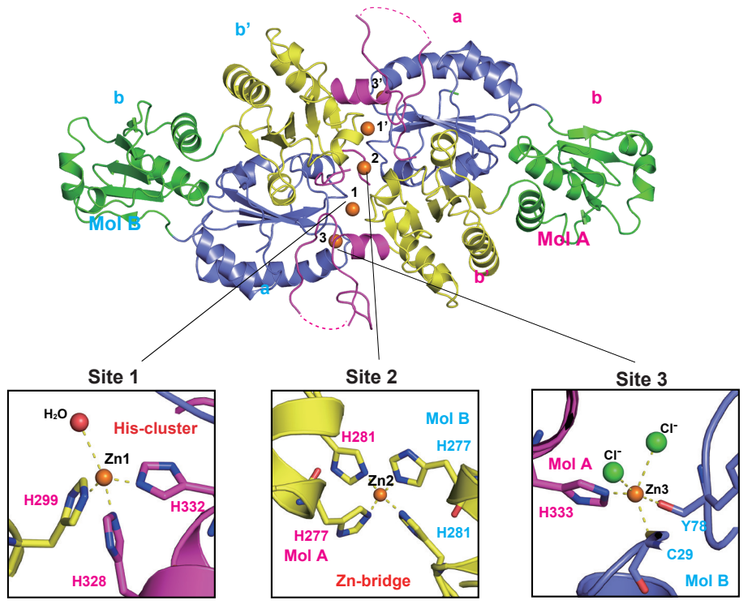

Figure 1: Crystal structure of the Zn-bridged ERp44 homodimer. The **a**, **b**, **b'** domains and C-tail of Mol A and Mol B are shown in blue, green, yellow, and magenta, respectively. The Zn^{2+} ions are shown as orange spheres. The lower insets display close-up views of the three Zn^{2+}-binding sites: site 1 (left), site 2 (middle) and site 3 (right).

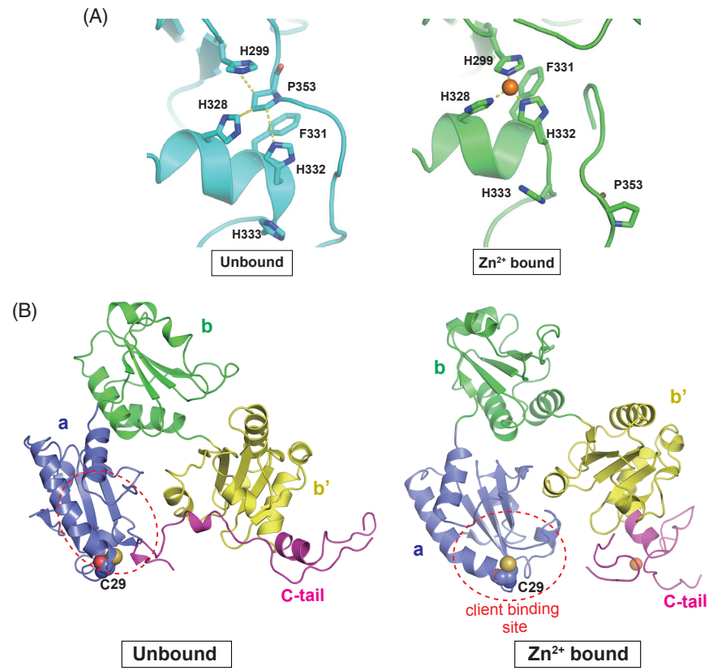

Figure 2 : Zn^{2+}-dependent conformational changes of ERp44 **(A)** Structural comparison of the His-cluster region in the metal-unbound (left) and Zn^{2+}-bound (right) ERp44. **(B)** Comparison of the overall domain arrangement in the metal-unbound (left) and Zn^{2+}-bound (right) states of ERp44 monomer. The essential cysteine (Cys29) is represented as spheres. Red-dashed circles indicate a client binding site.

coordinate a Zn^{2+}, playing an essential role in homodimerization of ERp44 (**Fig. 1**, middle lower inset). The Zn-bridged ERp44 homodimer possesses a third Zn^{2+}-binding site (site 3), which is unexpected because our ITC analyses suggested the presence of only two Zn^{2+}-binding sites in ERp44. At site 3, Zn^{2+} is coordinated by the thiol group of Cys29, the main-chain carbonyl oxygen of Tyr74 of one protomer, the Nδ of His333 of another protomer, and two chloride ions (**Fig. 1**, right lower inset). The coordination mode at site 3 is rarely found in other metalloproteins of known structures. Based on the ITC and other biochemical results, Zn^{2+} binding to site 3 does not seem necessary for dimerization.

The present structure further reveals that Zn^{2+} binding to site 1 induces large displacement of the C-tail, resulting in significant movements of the Trx-like domains (**Fig. 2**). In the metal-unbound state, Pro353 intervenes between His299, His328 and His332 in the His-cluster [**Fig. 2(A)**, left]. In the Zn^{2+} bound state, however, Pro353 is largely moved out and these three histidines get closer to one another, adopting a configuration suitable for Zn^{2+} binding (site 1) [**Fig. 2(A)**, right]. The large movement of Pro353 also accompanies a striking C-tail movement toward the **b'** domain, impairing the interactions between the C-tail and the **a** domain. As a result, the client binding site in the Zn^{2+}-bound form becomes fully exposed to the solvent [**Fig. 2(B)**, right], in contrast to the closed conformation in the metal-unbound state [**Fig. 2(B)**, left]. These observations suggest that the Zn^{2+}-bound form of ERp44 recognizes its client proteins with higher affinity than the metal-free form. Indeed, our ITC analysis revealed that interactions of ERp44 with its clients were further enhanced in the presence of Zn^{2+}.

Based on the present results, we propose a new model of Zn^{2+}-dependent protein quality control in the early secretory pathway [2]. Relative to pH changes, Zn^{2+} binding to ERp44 induces even larger conformational changes, leading to the enhanced interactions between ERp44 and its clients. These findings shed light on a new physiological role of cellular Zn^{2+} as a key regulator for protein quality control.

REFERENCES

[1] S. Watanabe, M. Harayama, S. Kanemura, R. Sitia and K. Inaba, *Proc. Natl. Acad. Sci. U.S.A.* **114**, E3224 (2017).
[2] S. Watanabe, Y. Amagai, S. Sannino, T. Tempio, T. Anelli, M. Harayama, S. Masui, I. Sorrentino, M. Yamada, R. Sitia and K. Inaba, *Nat. Commun.* **10**, 603 (2019).

BEAMLINE

BL-1A

S. Watanabe[1] and K. Inaba[1] ([1]Tohoku Univ. -IMRAM)