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Redox-Dependent Domain Rearrangement of Protein Disulfide Isomerase Coupled with Exposure of Its Substrate-Binding Hydrophobic Surface

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Protein disulfide isomerase (PDI) is a major protein in the endoplasmic reticulum, operating as an essential folding catalyst and molecular chaperone for disulfide-containing proteins by catalyzing the formation, rearrangement, and breakage of their disulfide bridges. This enzyme has a modular structure with four thioredoxin-like domains, a, b, b′, and a′, along with a C-terminal extension. The homologous a and a′ domains contain one cysteine pair in their active site directly involved in thiol–disulfide exchange reactions, while the b′ domain putatively provides a primary binding site for unstructured regions of the substrate polypeptides. Here, we report a redox-dependent intramolecular rearrangement of the b′ and a′ domains of PDI from *Humicola insolens*, a thermophilic fungus, elucidated by combined use of nuclear magnetic resonance (NMR) and small-angle X-ray scattering (SAXS) methods. Our NMR data showed that the substrates bound to a hydrophobic surface spanning these two domains, which became more exposed to the solvent upon oxidation of the active site of the a′ domain. The hydrogen–deuterium exchange and relaxation data indicated that the redox state of the a′ domain influences the dynamic properties of the b′ domain. Moreover, the SAXS profiles revealed that oxidation of the a′ active site causes segregation of the two domains. On the basis of these data, we propose a mechanistic model of PDI action; the a′ domain transfers its own disulfide bond into the unfolded protein accommodated on the hydrophobic surface of the substrate-binding region, which consequently changes into a "closed" form releasing the oxidized substrate.

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Abbreviations used: PDI, protein disulfide isomerase; SAXS, small-angle X-ray scattering; ANS, 1-anilino-8-naphthalenesulfonate; SPR, surface plasmon resonance; RNase A, ribonuclease A; DTT, dithiothreitol; HSQC, heteronuclear single-quantum coherence; NOESY, fnuclear Overhauser enhancement spectroscopy.

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Introduction

Maturation of proteins in cells is assisted by molecular chaperones, which inhibit aggregation of unstructured proteins, and by enzymes that accelerate protein folding.[1,2] Protein disulfide isomerase (PDI), a major protein in the lumen of the endoplasmic reticulum and a member of the thioredoxin superfamily, operates as an essential folding catalyst and a molecular chaperone.[3,4] This enzyme introduces disulfide bonds into protein substrates and catalyzes the rearrange-

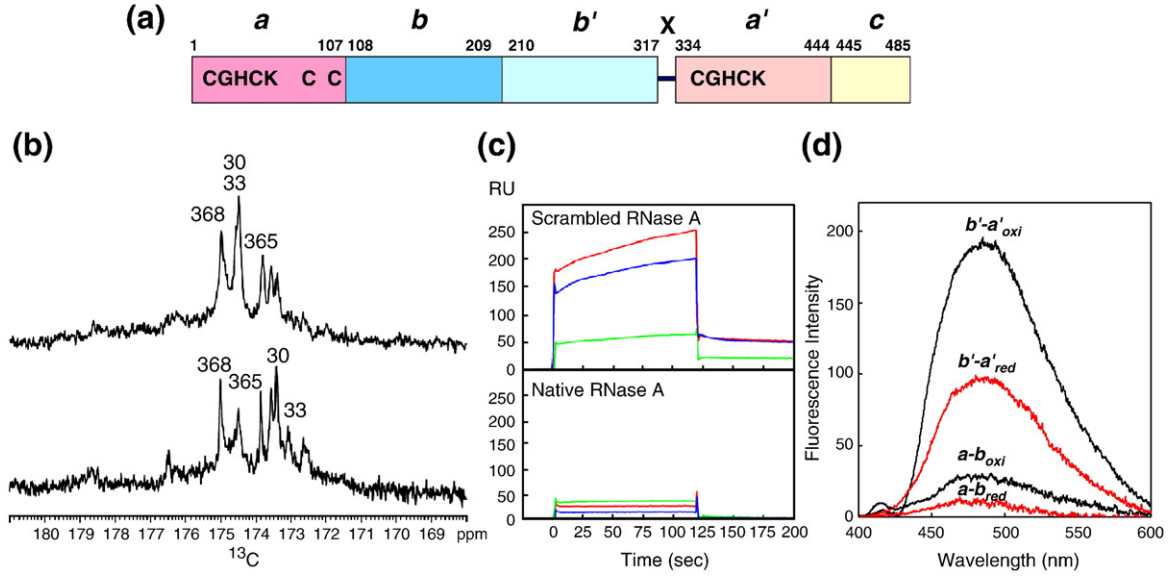

Fig. 1. Structural and functional asymmetry of the PDI domains. (a) Domain organization of *H. insolens* PDI based on the crystal structure of yeast PDI. (b) ^{13}C NMR spectra of the PDI labeled with ^{13}C selectively at the carbonyl carbon atoms of cyst(e)ine residues. The spectra were measured in the absence (top) and presence (bottom) of 0.8 molar equivalent of reduced glutathione. The residue numbers are displayed on top of the peaks. (c) SPR analyses of the interactions of the full-length PDI (red), a–b (green), and b′–a′ (blue) with disulfide-scrambled (top) and native RNase A (bottom). (d) Fluorescence spectra of ANS in the presence of b′–a′$_{oxi}$, b′–a′$_{red}$, a–b$_{oxi}$, and a–b$_{red}$.