

“Biochemical Basis of Oxidative Protein Folding in Endoplasmic Reticulum”

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Chemistry 345
November 3, 2003

The major pathway for protein disulfide-bond formation in the endoplasmic reticulum (ER) is known to require protein disulfide isomerase (PDI) to deliver oxidizing equivalents from the ER-membrane resident protein (Ero1p) to the secretory proteins. In carboxypeptidase Y (CPY), a protein in the *Saccharomyces cerevisiae* yeast strain, five disulfide bonds are essential for proper tertiary protein folding and export from the ER. Three central components of the cellular redox machinery are tested in this study to determine the source of Ero1p oxidizing potential. Using reverse genetics, the influence of ubiquinone, heme, and FAD on the kinetics of CPY folding is conveniently monitored with SPS-polyacrylamide gel electrophoresis (PAGE). Deletion of CoQ5 or HEM1, which prevents biosynthesis of ubiquinone or heme respectively, is found to have no effect on disulfide bond formation. However, the suppression of RIB5, the gene responsible for biosynthesis of riboflavin and its metabolic derivative flavin adenine dinucleotide (FAD), inhibits CPY folding and results in reduced PDI and Ero1p accumulation. Additionally noncovalently bonded FAD is detected in purified Ero1p when analyzed with reverse phase high performance liquid chromatography coupled with a scanning fluorescence detector.

Bibliography

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