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Review

Pathways of disulfide bond formation in *Escherichia coli*

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Abstract

Disulfide bond formation is required for the correct folding of many secreted proteins. Cells possess protein-folding catalysts to ensure that the correct pairs of cysteine residues are joined during the folding process. These enzymatic systems are located in the endoplasmic reticulum of eukaryotes or in the periplasm of Gram-negative bacteria. This review focuses on the pathways of disulfide bond formation and isomerization in bacteria, taking *Escherichia coli* as a model.

Keywords: Disulfide; DsbA; DsbC; DsbD; Periplasm; *Escherichia coli*; Protein folding; Electrons

Contents

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1. Introduction

Proteins face an obstacle race on their way to successful folding in the cell. The first hurdle is the crowding of the cytoplasm. When they emerge from the ribosome, nascent polypeptides expose hydrophobic surfaces and are prone to aggregation. To prevent this fatal process, cells possess a complex machinery of molecular chaperones that helps nascent proteins to fold. Another obstacle awaiting many secreted proteins is the formation of a disulfide bond between two cysteine residues.

Disulfide bonds are vital for the correct folding and tertiary structure stability of many secreted proteins. The formation of a disulfide bond between two cysteine residues stabilizes a protein structure, mainly by decreasing the conformational entropy of the denatured state. The stabilizing effect can be up to approximately 4 kcal/mol per disulfide formed (Clarke & Fersht, 1993; Pantoliano et al., 1987; Shaw & Bott, 1996). Failure to form proper disulfide bonds is likely to cause protein misfolding, leading to aggregation and degradation by proteases.

Disulfide bonds can form spontaneously in the presence of molecular oxygen. However, air oxidation is a rather slow process: in vitro, it can take several hours or even days of incubation to allow the formation of all the native disulfide bonds required for the correct folding of a protein. In contrast, disulfide bond formation in the cell is a much more rapid process and occurs within minutes or even seconds after synthesis. For instance, the refolding of RNAse A, a protein with four disulfide bonds, takes several hours in vitro but less than 2 min in vivo. The discrepancy between these in vivo and in vitro rates led to the discovery of the first catalyst of disulfide bond formation, protein disulfide isomerase (PDI). PDI is a eukaryotic protein present in the endoplasmic reticulum where it is part of a complex network of proteins involved in disulfide bond formation. The eukaryotic pathways of disulfide bond formation will not be discussed here (for a review, see Sevier & Kaiser, 2002). This review will focus on the pathways of disulfide bond formation and isomerization in *E. coli*. The pathways of disulfide bond formation in other bacteria have recently been reviewed by Kadokura, Katzen, and Beckwith (2003).

2. The chemistry

A thiol–redox reaction between a molecule A containing two reduced cysteine residues and a molecule B containing one disulfide bond can be seen as the transfer of two electrons from A to B or as the transfer of a disulfide bond from B to A. The rate of the reaction depends on the accessibility of the reactive groups, on the difference of the redox potential between the redox partners and on the probability of the sulfur atoms to come within the distance required for thiol/disulfide exchange (Englander & Kallenbach, 1983).

3. The Dsb protein family

In *E. coli*, disulfide bonds are rare within cytoplasmic proteins. This may be because cysteine residues often play vital roles in the catalytic site of proteins and the formation of disulfide bonds where they are not normally found introduces three-dimensional constraints that may lead to protein misfolding and aggregation. To protect the cell against oxidizing reagents that could damage cytoplasmic proteins, *E. coli* possesses several overlapping pathways to keep these proteins reduced, including the thioredoxin–thioredoxin reductase system and the glutaredoxin–glutaredoxin reductase system (for a review, see Carmel-Harel & Storz, 2000). The glutathione buffer in the cytoplasm overall is relatively reducing. The glutathione concentration (about 5 mM) and the GSH/GSSG ratio, which ranges from 50/1 to 200/1, help to prevent disulfide bond formation in proteins (Hwang, Sinskey, & Lodish, 1992; Kosower & Kosower, 1978).

In contrast, proteins secreted into the periplasm are oxidized. The periplasm contains a family of proteins that catalyze disulfide bond formation in newly translocated proteins. These proteins belong to the "Dsb" family (Dsb stands for disulfide bond).