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Mini Review

Oxidative Protein Folding: An Update

ADAM M. BENHAM

INTRODUCTION

TWO YEARS might seem like an eternity when one considers how rapidly a protein can fold. But 2 years can pass quickly, and since the last *Antioxidants & Redox Signaling* forum on the topic was commissioned, the oxidative protein folding community has spent that time very fruitfully. In this brief overview, I will consider the progress that has been made in the field since the articles and reviews were accepted for the August 2003 issue.

DISULFIDE BOND FORMATION IN THE BACTERIAL PERIPLASM

Disulfide bond formation in the periplasm is catalyzed by the oxidant DsbA, which is maintained in the oxidized state by DsbB. Disulfide bond rearrangement is catalyzed by the isomerase DsbC, which is maintained in a reduced state by DsbD (31). Subsequent work by the Beckwith group has used genetics coupled with alkylation and proteolytic studies to redress the topology of DsbD. Two essential cysteine residues (C163 and C285), thought to be located within a membrane spanning region, have now been shown to form a disulfide bond and may be available for disulfide exchange with cytosolic thioredoxins (18). How redox information is conducted from the DsbD cytosolic cysteine residues to its periplasmic cysteine residues is not entirely clear. However, the solution of a 2.5-Å DsbD crystal structure has shown that an N-terminal periplasmic cysteine (C109 in the DsbD Ig-like domain) can form a transient intramolecular disulfide with a C-terminal periplasmic cysteine (C146 in the thioredoxin-like domain) (34). As with many redox enzymes, large spatial reorientations seem to occur when the binding partner changes: in this case, when the DsbD Ig domain binds alternatively to DsbC or to the DsbD thioredoxin domain.

Evidence presented by Blank *et al.* (5) suggested that in *V. cholerae*, amino acids H94 and P149 influence the catalytic activity of this organism's DsbA protein. Remarkably, mutating P151 in the *E. coli* DsbA enzyme has allowed covalent intermediates in the oxidation process to be isolated. At least 13 DsbA substrates, five of which were not previously known, have been trapped and identified by mass spectrometry using this approach (17). Another trapping approach using osmotic shock has enabled *in vivo* substrates of both DsbA and DsbC to be identified (14). Engineering the cytosolic (reductive) thioredoxin pathway to support oxidative disulfide bond formation by forced evolution has been achieved (28), and the control of DsbA/DsbB under anaerobic conditions is also yielding to experimental analysis (36).

REDOX SWITCHES

Linke and Jakob drew our attention to the reversibility of disulfide bond formation, and how this is regulated in compartments where the redox potential was traditionally thought to disfavor the formation of disulfide bonds (26). Leichert and Jakob have now introduced a methodology that permits large-scale analysis of protein thiol modifications *in vivo* (24). By coupling trapping techniques and two-dimensional polyacrylamide gel electrophoresis to genetic studies, these authors have shown that a significant number of proteins obtain modified thiols after oxidative stress. This approach should facilitate the comprehensive identification of targets of cytoplasmic redox regulation under various growth and stress conditions.

The bacterial transcription factor OxyR is regulated by peroxide-induced redox switching (26). Detailed kinetic and mass spectrometric studies of this protein have demonstrated that specific S-S bond formation is required for this process, and that the rapid kinetics of the oxidation–reduction cycle relies upon conformational strain (23). In addition to reactive oxy-

Department of Biological Sciences, Durham, England.

gen species, it appears that the response to reactive nitrogen species will also involve sensing by redox-active cysteine residues in transcription factors (30). Other redox sensors are emerging. The bacterial ArcB protein, for example, is a kinase inhibited by a quinone-driven switch (27).

GENOMICS AND THE PROTEIN DISULFIDE ISOMERASE (PDI) FAMILY

Fomenko and Gladyshev outlined the power of genomics as a tool for investigating disulfide bond formation in the endoplasmic reticulum (ER), periplasm, and other compartments (10). To emphasize the richness of this approach, the Gladyshev group has used comparative genomics to identify and characterize the elusive phosphoseryl-tRNA[Ser]Sec kinase involved in selenoprotein biosynthesis (6), and to map the eukaryotic (22) and prokaryotic selenoproteomes (21). Genomics has proved useful for identifying potential new folding catalysts and chaperones, too. PDI is the prototypical ER disulfide bond catalyst (19), and since 2002, a number of novel eukaryotic PDI family members have been identified, including ERp18 (2), the endothelial EndoPDI (35), and the testis-specific PDILT (40). The publication of more tightly defined domain boundaries for the PDI family should help in the functional analysis of the new additions to this protein family (1). Exciting recent work also suggests that PDI family members are involved in morphological processes. The zebra fish P5 PDI homologue is required for appropriate bilateral asymmetrical gene expression (15), whereas in nematodes, the *pdi-3* gene (an ERp57 homologue) is involved in maintaining body morphology through its role in extracellular matrix assembly (9). Biochemical studies in concert with animal models should continue to break fresh ground in this area.

The PDI homologue ERp57 interacts with tapasin, a molecule involved in loading major histocompatibility complex (MHC) class I complexes with antigenic peptides for antigen presentation (3). The biochemistry of MHC class I complex formation in the ER requires oxidative protein folding, and is the focus of much attention because of its relevance to the immune response. The Ig domain of tapasin and the cysteine residues within it are important for its intermolecular interactions (38). Although tapasin acts as a bridge between some MHC class I molecules and the antigen transporter, TAP, its precise role is still being defined. There is some debate about whether tapasin functions as a peptide editor or facilitator (25, 43), and tapasin does appear to improve antigen presentation of low-affinity peptides by increasing their half-lives (16). The exact function of ERp57 within the MHC class I loading complex still requires more scrutiny (41).

ER OXIDOREDUCTASES

Ero1p is the prototypical ER oxidoreductase, and Ero1p is essential for disulfide bond formation in *S. cerevisiae* (37). We reported the sequencing and primary characterization of two Ero1p homologues in plants, AERO1 and AERO2, whose gene expression is likely to be differentially regulated (8). Support for differential regulation of ER oxidoreductases has come from work on rats and in cell lines, showing that mammalian Ero1α expression is regulated by oxygen tension (11). This is in contrast to mammalian Ero1β, which is regulated by the unfolded protein response (33). *S. pombe* also has two Ero proteins, SpEro1ap and SpEro1bp, with SpEro1bp being the functional equivalent of Ero1p (20). Why is the synthesis of two Ero proteins required in *S. pombe*? The answer is not yet known.

Undoubtedly, the most significant advance in this area has been the publication of the 2.2-Å and 2.8-Å crystal structures of the Ero1p core protein (12). This work gives a clear impression of how intramolecular electron transfer is facilitated in a protein that is constrained by two long-range disulfide bridges (between C90-C349 and C295-C150). The solution of two crystal structures, in an "in" and an "out" conformation, reveals how the crucial active-site cysteine residues (C100-C105 and C352-C355) can contact each other and transfer electrons, presumably upon binding and release of PDI. The unique single-domain and mainly α-helical structure also reveals the architecture of a novel FAD binding motif. Although the structure is elegant, we still do not know precisely how PDI binds, how the nonconserved Ero1p C-terminal tail fits in, or how electrons are handed on to the final electron acceptor, oxygen. Answers to these questions will surely emerge in the next few years as the molecular understanding of oxidation in the ER deepens.

ANTIOXIDANTS, REDOX STATE, AND PROTEIN FOLDING

There has been considerable interest in how the ER communicates with the cytosol during times of stress. Oxidative stress induced by peroxide can disrupt ER protein folding (39), and the redox state of the cystosol, in particular glutathione load, can influence the rate of disulfide bond formation in the ER by controlling disulfide isomerization (rather than oxidation directly) (7, 29). Eukaryotic cells mount an unfolded protein response (UPR) in situations where more protein folding capacity is required in the ER. It has been shown recently in yeast that sustained UPR results in the accumulation of reactive oxygen species and eventually apoptotic cell death, and that ER-associated degradation can alleviate this problem (13). Microarray analysis of HeLa cells placed under hypoxic stress nicely reveals how the ER stress response and cellular hypoxia may be integrated by activation of the transcription factor ATF4 in tandem with the phosphorylation of the initiation factor eIF2α by the ER membrane-associated kinase PERK (4). Although there is along way to go before we fully understand how physiological cytosolic stress and ER stress are coordinated, considerable progress in this area is expected in the next few years. Big leaps are already being made with respect to the role of ER stress in insulin-deficient diabetes (32, 42). No doubt that there will be further significant advances in the next two years.

ABBREVIATIONS

ER, endoplasmic reticulum; MHC, major histocompatibility complex; PDI, protein disulfide isomerase; UPR, unfolded protein response.

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Address reprint requests to:
Adam M. Benham, Ph.D.
Department of Biological Sciences
University of Durham
South Road
Durham DH1 3LE, U.K.

E-mail: adam.benham@durham.ac.uk

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