Proteome-wide Light/Dark Modulation of Protein Thiol Oxidation in Cyanobacteria Revealed by Quantitative Site-Specific Redox Proteomics

Jia Guo[1], Amelia Y. Nguyen[4], Ziyu Dai[3], Dian Su[1,5], Matthew J. Gaffrey[1], Ronald J. Moore[1], Jon M. Jacobs[1], Richard D. Smith[1,2], David W. Koppenaal[2], Himadri B. Pakrasi[4], and Wei-Jun Qian[1]*
[1]Biological Sciences Division, [2]Environmental Molecular Sciences Laboratory, and [3]Energy and Efficiency Division, Pacific Northwest National Laboratory, Richland, WA USA.
[4]Department of Biology, Washington University, St. Louis, MO, USA. [5]Current address: Genentech Inc, South San Francisco, CA 94080, USA

Pacific Northwest NATIONAL LABORATORY

Introduction

- Reversible protein thiol oxidation is an essential regulatory mechanism of signal transduction, metabolism, and gene expression.
- In photosynthetic organisms, reversible thiol oxidation modulates the activation or inactivation of many enzymes of photosynthetic electron transport linked to photosystems I and II in light/dark cycles.
- It is still largely unknown how broadly the redox process is involved beyond photosynthesis and what are the specific cysteine (Cys) sites serving as redox switches in photosynthetic organisms.
- We have investigated the extent of protein thiol oxidation under light, dark and DCMU (a photosystem II inhibitor) in cyanobacteria by a quantitative site-specific redox proteomic approach.
- Broad redox dynamic changes on individual cysteine residues in response to physiological perturbations were quantified.
- The functional significance of redox-sensitive Cys-sites in proteins (e.g., glucose 6-phosphate dehydrogenase, NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase, and peroxiredoxin Sll1621) was further confirmed by site-mutagenesis and biochemical studies.

Methods

Enrichment and processing workflow

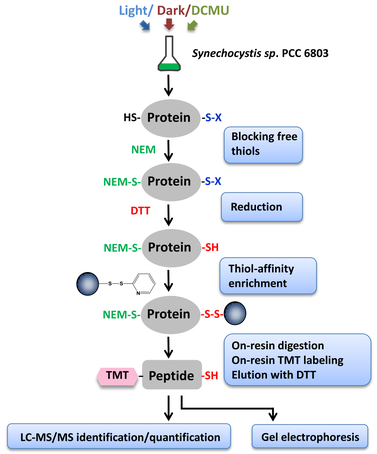

Figure 1. Workflow for the enrichment and site-specific quantification of thiol oxidation.

- *Synechocystis* were cultured under continuous light, dark, or DCMU for 2 h. Cells were lysed and free thiols were blocked with N-Ethylmaleimide (NEM). The reversible oxidized Cys were reduced by dithiothreitol (DTT). Enrichment of Cys-proteins was carried out by using Thiopropyl Sepharose 6B resin, followed by on-bead tryptic digestion and TMT labeling.
- Samples were analyzed by a Waters nanoAquity UPLC system. The system was operated with a gradient starting from 100% of mobile phase A (0.1% (v/v) formic acid in water) to 60% (v/v) of mobile phase B (0.1% (v/v) formic acid in acetonitrile) over 3 h. MS analysis was performed on a Thermo Scientific LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA).
- LC-MS results were then analyzed using the program MSGF+. Biological function analysis of the identified proteins was based on gene ontology and KEGG pathway analyses.

Results

Broad redox changes on Cys-sites modulated by light/dark

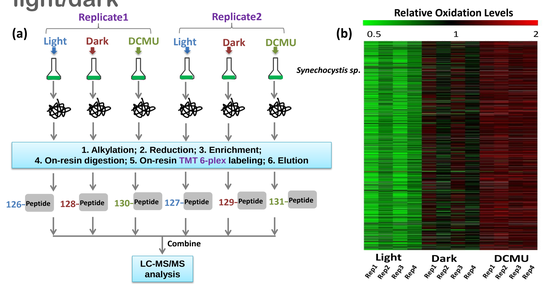

Figure 2. (a) Experimental workflow to study cyanobacteria oxidation. (b) Heatmap of the relative levels of Cys oxidation under light, dark or DCMU conditions.

Stoichiometry of Cys oxidation

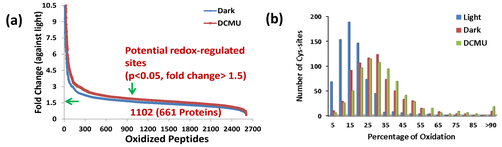

Figure 3. Stoichiometry of Cys oxidation. (a) The redox sensitivity of individual Cys-sites. (b) Distribution of Cys-sites in terms of the percentage of Cys oxidation.

Functional Category

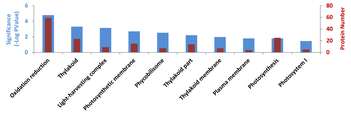

Figure 4. Top significant biological functions and pathways based on gene ontology and KEGG pathway analysis.

Levels of thiol oxidation for selected proteins

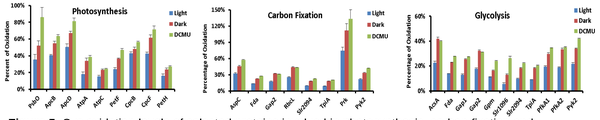

Figure 5. Cys oxidation levels of selected proteins involved in photosynthesis, carbon fixation and glycolysis.

Redox-sensitive proteins in important biological processes

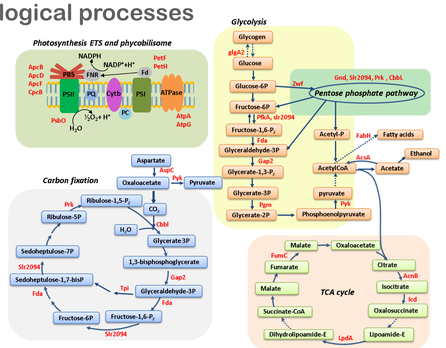

Figure 6. Redox-sensitive proteins involved in photosynthesis, carbon fixation, glycolysis and TCA cycle. The redox-sensitive protein are labeled in red.

Site-specific redox sensitivity predicts functional cysteine sites

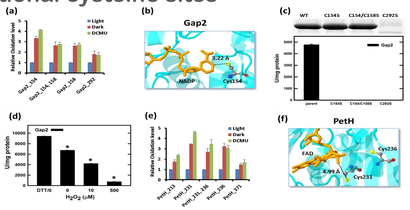

Figure 7. Functional cysteine sites. (a) Relative oxidation levels individual Cys-sites of glyceraldehyde 3-phosphate dehydrogenase (Gap2). (b) Structure of Gap2. (c) Gap2 activities. (d) Redox regulation of Gap2 activities. (e) Relative oxidation levels of individual Cys-sites of PetH. (f) Structure of ferredoxin NADP⁺ reductase (PetH).

Novel redox regulatory proteins

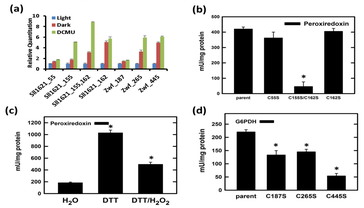

Figure 8. Functional sites of peroxiredoxin (Sll1621) and glucose 6-phosphate dehydrogenase (G6PDH, Zwf). (a) Relative oxidation levels. (b) Enzymatic activity of purified Sll1621 mutants. (c) Redox regulation of Sll1621. (d) Enzymatic activity of G6PDH activity.

Conclusions

- Proteome-wide coverage of redox changes on ~2,100 Cys-residues was achieved, which significantly expanded the current repertoire of thiol-based redox modifications under physiological conditions.
- The redox sensitivity data for individual Cys-residues provides important predictive information for identifying functional sites of given proteins as supported by functional validation.
- The stoichiometry information for individual Cys-sites provided additional evidence of functional relevance.

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CONTACT: Jia Guo, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: jia.guo@pnnl.gov

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