論文概要

○　論文題目　　Role of Persulfides/Polysulfides in Reversibility of *S*-Oxidation of Sensor Proteins during Oxidative Stress
（酸化ストレス下におけるセンサータンパク質の酸化修飾の可逆性に対するパーサルフィド/ポリサルフィドの役割）

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目的：

Reversibility of post-translational modifications on proteins plays a critical role in regulation of protein functions, thereby, maintaining intracellular homeostasis. Protein *S*-oxidation to form P-SOH, P-SO_2H or P-SO_3H inhibits its activity, resulting in activation of redox signaling. Regulation of P-SO_2H and P-SO_3H, which are known to be irreversible, is still unclear. Protein-bound persulfides/polysulfides (P-SSH/P-SS_nH) have been recently proposed to act as cellular protectants against irreversible oxidation by excessive reactive oxygen species (ROS), since the S–S bond can be reduced by reductants (Ida et al., *PNAS* 2014; Abiko et al., *Chem Res Toxicol* 2015; Akaike et al., *Nat Commun* 2017). However, the contribution of P-SSH/P-SS_nH to cellular homeostasis has not been understood well. Hence, the purposes of this study are to clarify 1) the significance of oxidative modification through protein tyrosine phosphatase 1B (PTP1B), which has reactive thiols, by using 9,10-phenanthraquinone (9,10-PQ) as a model of environmental ROS producer and 2) the importance of persulfides/polysulfides in maintaining the reversibility of the sensor proteins during oxidative stress.

方法：

Cells: Human epithelial carcinoma cells (A431 cells). Detection of oxidation: P-SOH and P-SSOH, which were labeled by dimedone, P-SO_nH or P-SSO_nH were detected by Western blotting with anti-oxidized PTP active site or anti–2-thiodomedone antibodies and analyzing by UPLC-MS^E. Measurement of PTPs activity: Using *p*-nitrophenyl phosphate as a substrate.

結果：

9,10-PQ caused inhibition and *S*-oxidation of cellular proteins such as PTPs in A431 cells, in which PTP1B was determined as a main target. Incubation of recombinant hPTP1B with 9,10-PQ-produced H_2O_2 resulted in *S*-oxidation and inhibition of the enzyme activity. UPLC-MS^E analysis revealed that 9,10-PQ oxidized hPTP1B at Cys215 to yield -SOH, -SO_2H, and -SO_3H. Incubation of hPTP1B with persulfides caused formation of hPTP1B-SSH, concomitant with inhibition of enzyme activity. Subsequent addition of H_2O_2 to the reaction mixtures resulted in formation of hPTP1B-SSOH derivatives at Cys215, Cys32, and Cys121. Although, reductants such as dithiothreitol (DTT) or thioredoxin system could slightly reduce the H_2O_2-mediated inhibition of hPTP1B activity, the suppressed activity following persulfides treatment was more robustly

recovered by these reductants. Identification of thiodimedone released from hPTP1B-SS−dimedone during treatment with DTT validated the reversible formation of hPTP1B-SSOH. We also found a significant proportion of Cys residues existing as P-SSOH in various cellular proteins such as PTP1B, PTEN, Keap1, and HSP90 under not only physiological but also oxidative conditions.

考察：

These results suggest that 9,10-PQ could serve as a model of environmental ROS producer to cause *S*-oxidation of critical Cys residues on sensor proteins through its redox cycling, thereby altering their functions and resulting in adaptive responses or cellular toxicology based on the exposed levels. We also propose that persulfides/polysulfides orchestrate reversibility of *S*-oxidation of protein thiols through reversible formation of P-SSO_nH, thereby, protecting cellular proteins against irreversible oxidation during oxidative stress. In addition, detection of P-SSOH in cells under physiological condition implicates the possible involvement of these species in modulation of redox signaling. These suggest that persulfides/polysulfides might be a target for the development of novel therapies as well as the discovery of new drugs to treat diseases related to oxidative stress.

結論：

Taken together, the present study indicates that 9,10-PQ through redox cycling could cause *S*-oxidation of sensor proteins, thereby changing their functions. In addition, *S*-sulfuration mediated by persulfides/polysulfides appears to be essential for reversibility of sensor proteins to tolerate oxidative stress.