氏　　名	LUONG CONG NHO
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学位論文題目	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）
主　　査	筑波大学教授　医学博士　加藤　光保
副　　査	筑波大学准教授　博士(薬学　大林　典彦
副　　査	筑波大学講師　博士(農学)　蕨　　栄治
副　　査	筑波大学講師　博士(医学)　松井　裕史

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Luong Cong Nho describes the role of persulfides/polysulfides in the reversibility of S-oxidation of sensor proteins during oxidation stress. The summary is as follows:

（目的　Purpose）

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 recentlyproposed to act ascellular protectants against irreversible oxidation by excessive reactive oxygen species (ROS), since the S–S bond can be reduced by reductants. However, the contribution of P-SSH/P-SS_nH to cellular homeostasis has not been understood well. Hence, the author aimed to clarify 1)the significance of oxidative modification of 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.

（対象と方法　Materials and Methods）

The author used human epithelial carcinoma cells (A431 cells) as a model cell line, protein tyrosine phosphatase 1B (PTP1B) as a target protein with reactive thiol and 9,10-phenanthraquinone (9,10-PQ) as an environmental

ROSproducer.Oxidized P-SH groups, P-SOH and P-SSOH, were detected by labeling with dimedone and P-SO_nH or P-SSO_nH were detected by Western blotting with anti-oxidized PTP active site.Anti–2-thiodomedoneantibodiesand UPLC-MS^Eanalysis were also used for the detection of oxidized conditions of PTP. PTP activity was measured using *p*-nitrophenyl phosphate as a substrate.

（結果　Results）

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 with9,10-PQ-produced H_2O_2resulted 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 ofhPTP1B-SSOH. The author 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　Discussion）

Based on the results obtained in this study, the author suggests 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. The author also proposesthat 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 is thought to implicate the possible involvement of these species in modulation of redox signaling. From these results, the authorsuggests 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.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

This 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 ofsensor proteins to tolerate oxidative stress. The study has high novelty and social relevance. Data are clear and convincing, and discussion is reasonable.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on January 15, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in

Medical Sciences.