Persulfide shield as a newly identified endogenous electrophile detoxification mechanism in biological system

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学　位　論　文　要　約

博士論文題目　Persulfide shield as a newly identified endogenous electrophile detoxification mechanism in biological system（親電子物質の解毒メカニズムを介するパースルフィドの新規生体保護作用）

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The efforts in identifying biological function of reactive sulfur species (RSS) are currently gaining momentum and attention from scientists worldwide. Previously, remarkable amount of polysulfides derivatives were found to be generated endogenously and ubiquitously in both prokaryotic and eukaryotic organisms. Nonetheless, the polysulfides are far from fully understood, especially due to their reactivity and intricate redox-active properties. In the beginning of this study, as representatives of RSS, glutathione polysulfide (GS-SS-SG) was used to test RSS stability and degradation kinetics. The GS-SS-SG showed severe degradation following the environment alkalinity suggesting that heterolytic scission triggered by hydroxyl anion (OH^-) underlying its mechanism. Consequently, polysulfides cleavage produces thiolates and sulfenic acids, which thereby accelerated by alkylating reagents (e.g., IAM) and dimedone. However, a hydroxyphenyl containing derivative, *β*-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM), has shown potent stabilizing effect on polysulfides. In fact, tyrosine, an amino acid that shares similar group with the HPE-IAM exhibited more potent polysulfides stabilizing properties and prevented the polysulfides degradation occurring in alkaline pH. Thus, the polysulfides degradation-protective effect is likely caused by inhibitory action of hydroxyphenyl residues against alkaline hydrolysis. Hence, using HPE-IAM based LC-MS/MS analysis, the endogenous RSS level is expected can be quantified with better precision. Recombinant cysteinyl tRNA-synthetase (CARS); mouse cytosolic CARS (CARS1) and human mitochondrial CARS (CARS2) showed PLP-dependent cysteine persulfide synthase (CPERS) activity. Evidently, knocking out the CARS2 significantly reduces the endogenous RSS as compared to the CARS1 in HEK293T cell line. Moreover, the heterozygous CARS2 mutant (*Cars2*$^{+/-}$) mice also exhibited marked reduction of intracellular RSS level indicating that CARS2 plays major role in CPERS activity. The homozygous CARS2 KO *in vivo* model however was unable to obtain potentially due to embryonic lethality. Another *in vitro* model, Schizosaccharomyeces pombe (*S. pombe*) with deficient sulfide quinone:oxidoreductase (SQR) function was developed. The impairment of SQR function leads to the accumulation of sulfide. Hence, knocking out either CARS2 or SQR simulates the reduction and accumulation of intracellular RSS level respectively. Indeed, the CARS2 KO HEK293T cell was found to be more susceptible towards methylmercury (MeHg) compared to the wild type which is believed due to the lack of chelation agent provided by RSS. Knocking out the SQR increases *S. pombe* susceptibility towards cadmium chloride ($CdCl_2$) which in line with previous reports but the model exhibited significant resistance towards

MeHg. As a conclusion, different electrophiles may exhibit different polysulfides degradation properties. HPE-IAM is currently the best option to be used as a probe for LC-MS/MS-based polysulfides detection. CARS2 greatly involves in governing CPERS activity. The intracellular level of RSS which is primarily modulated by CPERS can influence cellular susceptibility towards electrophiles such as MeHg. The presence of endogenous RSS potentially acts as a shield against electrophiles.