Polysulfide stabilization by tyrosine and hydroxyphenyl-containing derivatives that is important for a reactive sulfur metabolomics analysis

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Abbreviations: RSS, reactive sulfur species; CysSSH, cysteine hydropersulfide; OH\textsuperscript{−}, hydroxyl anion; CARS, cysteiny1-tRNA synthetase; LC, liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; HPE-IAM, β-(4-hydroxyphenyl)ethyl iodoacetamide; MBB, monobromobimane; NEM, N-ethylmaleimide; IAM, iodoacetamide; H\textsubscript{2}S, hydrogen sulfide; NaHS, sodium hydrogen sulfide; GSH, reduced glutathione; FA, formic acid; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; MRM, multiple reaction monitoring; R-S-R, bis-monosulfide alkyl adducts; GS-S-G, oxidized glutathione trisulfide; GS-SS-G, oxidized glutathione tetrasulfide; ANOVA, analysis of variance; HPE, β-hydroxyphenyl-ethyl; ROS, reactive oxygen species

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Abstract
The physiological importance of reactive sulfur species (RSS) such as cysteine hydropersulfide (CysSSH) has been increasingly recognized in recent years. We have established a reactive sulfur metabolomics analysis by using RSS metabolic profiling, which revealed appreciable amounts of RSS generated endogenously and ubiquitously in both prokaryotic and eukaryotic organisms. The chemical nature of these polysulfides is not fully understood, however, because of their reactive or complicated redox-active properties. In our study here, we determined that tyrosine and a hydroxyphenyl-containing derivative, β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM), had potent stabilizing effects on diverse polysulfide residues formed in CysSSH-related low-molecular-weight species, e.g., glutathione polysulfides (oxidized glutathione trisulfide and oxidized glutathione tetrasulfide). The protective effect against degradation was likely caused by the inhibitory activity of hydroxyphenyl residues of tyrosine and HPE-IAM against alkaline hydrolysis of polysulfides. This hydrolysis occurred via heterolytic scission triggered by the hydroxyl anion acting on polysulfides that are cleaved into thiolates and sulfenic acids, with the hydrolysis being enhanced by alkylating reagents (e.g. IAM) and dimedone. Moreover, tyrosine prevented electrophilic degradation occurring in alkaline pH. The polysulfide stabilization induced by tyrosine or the hydroxyphenyl moiety of HPE-IAM will greatly improve our understanding of the chemical properties of polysulfides and may benefit the sulfur metabolomics analysis if it can be applied successfully to any kind of biological samples, including clinical specimens.

1. Introduction
Reactive sulfur species (RSS) are now well known to be endogenously produced in abundance in many species [1–4], and they occur as diverse polysulfide forms with unique redox-active or reactive chemical properties [5–14]. We recently discovered a family of cysteine persulfide synthases, i.e. cysteinyl-tRNA synthetases (CARSs), which primarily possess moonlighting functions not only in

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translational processes during protein biosynthesis but also as specific enzymes that generate persulfides and polysulfides from the substrate cysteine [2,14,15]. Related to this discovery, of great importance is our recent finding that the mitochondrial isoform of CARS (CARS2) is the predominant enzyme responsible for most RSS formed in vivo and contributes to mitochondrial bioenergetics, i.e. sulfur respiration [2,15].

To investigate these sulfide metabolic pathways, we first developed a reactive sulfur metabolomics analysis [1,2], by utilizing liquid chromatography-mass spectrometry (LC-MS), combined with trapping or derivatizing RSS with means of β-(4-hydroxyphenyl)ethyl iodacetamide (HPE-IAM). The specificity of our approach relies on the selectivity of amidation of sulfhydryls of various hydropolysulfides, which will ensure specific detection and quantification of RSS through the sulfur metabolomics analysis that we recently produced in our laboratory [2].

As Fig. 1 illustrates, however, harsh electrophiles such as mono- bromobimane (MBB) and N-ethylmaleimide (NEM) may participate in a nucleophilic attack on a sulfur residue of polysulfides, which will lead to extensive polysulfide decomposition [2,16]. In fact, this process is initiated by alkaline-induced hydrolysis of polysulfides, which results in the generation of thiolate and sulfonyl moieties. The thiolate moiety is rapidly alkylated by strong electrophiles such as MBB and NEM. The sulfonyl moiety undergoes reactions with other thiol compounds or the sulfenic acid probe dimedone. Thus, electrophiles and dimedone may cause a shift in the equilibrium in alkaline hydrolysis, which would enhance polysulfide decomposition. The overall reaction is highlighted in the upper panel in (A). (B) Molecular structures of chemicals used in this study.

For example, one of the most serious issues in this context is that hydrogen sulfide (H₂S) adducts of these harsh reagents, i.e. NEM and MBB, were most often observed in the reactions of NEM/MBB and polysulfides bound to low-molecular-weight cysteine and protein cysteins [2]. These adducts are most likely artifactually generated during the processing of various samples with MBB/NEM. Because we now know that CARS can induce polysulfidation of all Cys-containing proteins during translational or co-translational processes [1,2,14,15], such artificial formation of bis-monosulfide alkyl adducts (R-S-R) is believed to be the most serious pitfall in metabolomics analysis using electrophilic compounds, regardless of the reactivity of these electrophiles [2,14]. This difficulty exists even if using HPE-IAM can minimize artificial degradation and R-S-R formation. To overcome this technical problem, we investigated a new mechanistic approach that can be applied to our method using HPE-IAM trapping combined with LC-tandem mass spectrometry (LC-MS/MS) that we are developing.

In addition to demonstrating the relatively selective RSS trapping by HPE-IAM, we discovered that HPE-IAM itself can stabilize polysulfides. In fact, we found here that tyrosine had a potent polysulfide-sustaining effect when added to a reaction mixture containing polysulfides, with the effect most likely resulting from the similar chemical structures of tyrosine and HPE-IAM. This polysulfide stabilization effect of tyrosine and its related hydroxyphenyl-containing compounds such as HPE-IAM has important implications for a better understanding of the molecular mechanism of the maintenance of extensive protein polysulfidation in many proteins. In addition, we may identify, for the first time, the unique chemical nature of polysulfides that are typically characterized by alkaline hydrolysis and electrophilic decomposition as physiologically regulated by tyrosine. We may thus successfully develop the most accurate and ideal reactive sulfur metabolome.

2. Materials and methods

2.1. Materials

IAM, MBB, sodium hydrogen sulfide (NaHS), reduced glutathione (GSH), L-tyrosine, glycerol, and sucrose were obtained from Nacalai Tesque (Kyoto, Japan). NEM, iodine, methanol, ethanol, 4-acetamidophenol, and dimedone were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). HPE-IAM was purchased from Molecular Biosciences (Boulder, CO, USA). Na₂³⁴S was obtained from Dojindo Laboratories (Kumamoto, Japan).

2.2. Synthesis of oxidized glutathione trisulfide/tetrasulfide (GS-S-SG/GS-SS-SG)

GSH (20 mM) was reacted with 20 mM NaHS in the presence of iodine (20 mM) in 20 mM Tris-HCl buffer (pH 7.4) at room temperature for 15 min. For purification of GS-S-SG and GS-SS-SG, the reaction mixture was subjected to high-performance LC (Prominence; Shimadzu Corporation, Kyoto, Japan) with a reversed-phase column (YMC-Triart C18 column, 50 × 2.0 mm inner diameter; YMC, Kyoto, Japan), as reported previously [1]. Isotope-labeled GS-³⁴S-SG and GS-³⁴S³⁴S-SG were synthesized by reacting GSH with Na³²S instead of Na³⁴S.

2.3. In vitro polysulfide stability assay

GS-S-SG or GS-SS-SG dissolved in 0.01% formic acid (FA) was added to an equal volume of 200 mM phosphate buffer (adjusted to the pH indicated in the figure legends) and was incubated at 37 °C in dark with the reagents indicated in the figure legends. Fig. 1B summarizes the reagents used in this study. The undegraded GS-S-SG or GS-SS-SG was quantified by using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) as described below.
2.4. Quantification of GS-S-SG and GS-SS-SG

The reaction mixtures for LC-MS/MS analysis were diluted 5-fold with 1% FA and were then mixed with an equal amount of 200 nM isotope-labeled GS-34S-SG and GS-34S34S-SG as internal standards (100 nM, final concentration), followed by quantification of GS-S-SG and GS-SS-SG via LC-ESI-MS/MS by using the triple quadrupole mass spectrometer LCMS-8050 (Shimadzu) coupled to the Nexera UHPLC system (Shimadzu), as described previously[2,3]. In brief, samples were subjected to the Nexera UHPLC system with a YMC-Triart C18 column (50×2.0 mm inner diameter) and were then eluted by using a linear methanol gradient of the mobile phase (0–90%, 15 min) in the presence of 0.1% FA at a flow rate of 0.2 ml/min at 40°C. GS-S-SG and GS-SS-SG were detected by using multiple reaction monitoring (MRM). MRM parameters were described previously[1,2].

2.5. Analysis of decomposition products of GS-SS-SG

GS-SS-SG (10 μM) was treated with alkylating reagents (e.g. MBB, HPE-IAM, or NEM) (1 mM each) in 100 mM phosphate buffer (pH 7.0) with or without 1 mM tyrosine at 37°C for 1 h in dark. The reaction samples were diluted 5-fold with 1% FA and then mixed with an equal volume of 100 nM isotope-labeled HPE-IAM, NEM, or MBB-adducts as an internal standard (50 nM, final concentration). Each alkylating reagent adduct was quantified via LC-ESI-MS/MS by the LCMS-8050 Nexera UHPLC system (Shimadzu) with a YMC-Triart C18 column (50×2.0 mm inner diameter) as described above. H2S (BiS-S-adduct), hydrogen disulfide (BiS-S-adduct), thiosulfate (HS2O3-adduct), GSH (GS-adduct), GSH persulfide (GSS-adduct), GSH trisulfide (GSSS-adduct), oxidized GSH (GSSG), and GS-S-SG were detected by using MRM [1,2,16].

2.6. Cell culture analysis for sulfur metabolites

HEK293T cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. To increase endogenous GS-SS-SG, HEK293T cells were treated with 0.2 mM GS-SS-SG in DMEM without FBS for 3 h at 37°C. Cells were washed twice with ice-cold PBS, followed by extraction with ice-cold methanol containing 1 mM HPE-IAM or 1 mM MBB. After centrifugation (14,000× g, 5 min, 4°C), the supernatants were mixed with an equal volume of 200 mM phosphate buffer (pH 7.5 or 8.0) containing HPE-IAM or MBB (2 mM each) in the presence or absence of 2 mM tyrosine and then incubated for 30, 60, and 90 min at 37°C in dark. The reaction samples were diluted 10-fold with 1% FA containing known amounts of isotope-labeled internal standards and subjected to LC-MS/MS analysis as just described.

2.7. Statistical analysis

Two-way analysis of variance (ANOVA) with Tukey’s test or Student’s t-test was used for multiple comparisons in GraphPad Prism 5.0 (GraphPad Software, CA, USA). P < 0.05 was accepted as the level of statistical significance.

2.8. Data availability

Data are available upon request from the Corresponding Author, Takaaki Akaike (takaike@med.tohoku.ac.jp).

3. Results

To determine the stabilities of polysulfides, we first analyzed the degradation kinetics of oxidized glutathione tetrasulfide (GS-SS-SG), as
a model for polysulfide species, in various conditions and in the presence or absence of different alkylating reagents. Utilization of our MS-based technique enables direct and absolute quantification of intact GS-SS-SG in samples without artifacts [1,2]. When incubated for 90 min at 37°C in pH 7.0, 7.5, or 8.0, the amount of GS-SS-SG (10 μM, initial concentration) was decreased to 50%, 60%, or 30% of the inputs, respectively (Fig. 2A, B, and C). We observed enhanced stability of GS-SS-SG in the presence of HPE-IAM at pH 7.0 and 7.5, whereas IAM did not significantly change the degradation kinetics of GS-SS-SG at both pH 7.0 and pH 7.5. Incubation with NEM or MBB, however, resulted in GS-SS-SG instability. We also analyzed products of GS-SS-SG after incubation with HPE-IAM, NEM, or MBB in pH 7.0 (Supplementary Fig. S1). It is shown herein that the GS-SS-SG was relatively stable in the incubation with HPE-IAM, NEM, or MBB in pH 7.0 (Supplementary Fig. S1). It is shown herein that the GS-SS-SG was relatively stable in the presence of HPE-IAM but became unstable and decayed readily by the treatment with other much reactive electrophiles like NEM and MBB. This profile is strongly supported by the finding that no major compounds other than GS-SS-SG were found in the reaction mixture of GS-SS-SG with HPE-IAM, whereas MBB caused cleavage of polysulfide into GSS- and GSSS-MBB adducts, and similar but much extensive fragmentation was evident with NEM, which generated the smallest decomposed products of GS-SS-SG, i.e., H2S and GSH adducts (NEM-S-GSH). Additionally, we conducted a cell culture study with HEK293T cells, which is an in vivo model for sulfur metabolism, as we established earlier [1,2]. The change in the profiles for the amounts of various polysulfide derivatives derived from GS-SS-SG is shown in Fig. S2A and B. This metabolic profiling further supported that alkaline-induced GS-SS-SG decay was promoted more greatly by MBB than was by HPE-IAM, as evidenced by marked increase in decomposed products of GS-SS-SG, such as each adduct of H2S (bis-S), GSH, and GSS- adducts, and a Cys-S-S-Cys adduct that is indirectly derived from the polysulfide. We also found that incubation at pH 8.0 caused rapid degradation of GS-SS-SG, possibly because of hydrolysis in a high pH environment. All alkylating reagents examined, including HPE-IAM, showed additional enhancing effects on GS-SS-SG degradation to different extents under weak alkaline conditions. These results confirm that some alkylating reagents can perturb the quantification of polysulfide species and suggests serious technical difficulties that may lead to underestimation of their absolute concentrations and also erroneous determination of artificial sulfide products, most typically H2S, especially when a high pH buffer, even pH 8.0, is used. Relative concentration of an alkylating reagent to GS-SS-SG appeared to be an important factor for degradation kinetics, because a high excess molar ratio of an alkylating reagent to GS-SS-SG conferred GS-SS-SG-degrading potency to IAM or enhanced degradation of GS-SS-SG caused by NEM or MBB (Fig. 2D, E, and F), when compared with the kinetics found with a lower molar ratio (Fig. 2A, B, and C). Even HPE-IAM, which prevented GS-SS-SG degradation at the lower molar ratio as shown in Fig. 2A and B, induced GS-SS-SG instability when administrated in excess (Fig. 2D and E). Also, we checked the effects of oxygen, light, and temperature on stability of GS-SS-SG in terms of alkaline hydrolysis and electrophilic degradation of GS-SS-SG (Fig. S2A, B, and C). These results indicated that its stability was not affected appreciably either by the oxygen content in solution or by light (at least, normal room illumination, around 500 lx) for 60 min. In contrast, the polysulfide became quite unstable as increasing the temperature up to 50°C.

Inasmuch as HPE-IAM is a derivative of IAM that has a β-hydroxymethyl (HPE) group (Fig. 1B), the different effects of HPE-IAM (protective) and IAM (degrading) on the stability of GS-SS-SG as noted above can be attributed to this specific HPE group. Therefore, we hypothesized that tyrosine, an amino acid that has a similar group, could stabilize polysulfides. As expected, we observed delayed degradation of GS-SS-SG (Fig. 2) and oxidized GSH trisulfide (GS-S-SG) (Fig. 3A, B and Table S1) in the presence of 1 mM tyrosine at pH 7.0, 7.5, and even 8.0, which indicates that tyrosine functions as a stabilizer of polysulfide species. The more important result was that exogenous introduction of tyrosine ameliorated degradation of GS-S-SG (Fig. 3A, B and Table S1) and GS-SS-SG (Fig. 3C, D, and Table S1) caused by alkylating reagents and even auto-degradation in pH 7.5 and 8.0, data that strongly support the possibility of utilizing tyrosine as an efficient protective agent for quantification of polysulfides. As shown in Fig. 3E, we examined the dose-dependent profile of tyrosine in terms of its effect on GS-SS-SG stability, which revealed herein a very nice dose-dependent stabilization with tyrosine. Notably, a higher concentration of tyrosine at 10 mM could inhibit almost completely the alkaline hydrolysis of GS-SS-SG, confirming again such a strong polysulfide-stabilizing activity of this hydroxyphenyl-containing compound. The in vivo analysis shown in Fig. S2A and B also indicated that GS-SS-SG formed in the cells was significantly stabilized by tyrosine added exogenously. Specifically, similar to the in vitro cell-free analysis, tyrosine could remarkably suppress the alkaline- and electrophile (both HPE-IAM and MBB)-accelerated GS-SS-SG degradation (Fig. S2A and B). In addition, various compounds containing hydroxyl moieties were also tested for their stabilizing effect on GS-SS-SG (Fig. 3E and F). A clear and potent degradation-inhibitory effect was evident with various phenolic or alcoholic substances similar to tyrosine. It is therefore reasonable to conclude that the polysulfide-protective effect is caused by their alcohol (OH) residues without being dissociated into their ionic forms, rather than caused by directly competing with the hydroxy anion formed in a pH-dependent manner.

Finally, we evaluated GS-SS-SG stability in the presence of dime-done, which reacts with sulfenic acid (-SOH) [17]. As shown in Fig. 4 and Table S2, dimedone destabilized GS-SS-SG in a concentration-dependent manner, especially at pH 8.0. This result indicates that dime-done promotes alkaline hydrolysis of GS-SS-SG, most likely by pushing the reaction equilibrium forward (Fig. 1A). Indeed, the presence of both IAM and dimedone enhanced the degradation of GS-SS-SG (Fig. 4A, B and Table S2). Moreover, we observed that tyrosine prevented dime-done-dependent degradation of GS-SS-SG at both pH 7.5 and pH 8.0, a result that confirms its polysulfide-stabilizing property (Fig. 4C, D and Table S2). The present results thus suggested that tyrosine can prevent GS-SS-SG degradation caused by alkylating reagents and dimerone, which probably indicates that tyrosine can inhibit hydrolysis of polysulfides by hydroxyl anion (OH-).

4. Discussion

The exact chemical nature of polysulfides has remained unclear. Nevertheless, our recent study found that particular sulfide moieties of polysulfides are susceptible to electrophilic degradation mediated by various alkylating agents, during alkaline hydrolysis [2,16], as Fig. 1 illustrates. As shown in the present work, simply because the alkaline pH is apparently promoting the degradation of polysulfides in the absence of any electrophiles added, which is empirically known as alkaline hydrolysis often occurring with all types of inorganic and organic polysulfides, we assumed that there is some equilibrium between e.g., [GS-SS-SG (+ OH⁻)] and [GSS⁻ + GSS-OH], in which if dimerone consumes GSS-OH, the equilibrium will shift to the right, resulting in enhanced decomposition of GSSSSG (see a scheme in Fig. 1A). The same is true for the reaction of GSS with an electrophile (E), as schematized in Fig. 1A. The most logical and consistent interpretation is, therefore, that both hydrolysis and electrophilic alkylation of polysulfide residues may need to occur at the same time to mediate effective polysulfide degradation. For instance, a particular sulfur residue, e.g., ‘-SS-’ in GS-SS-SG can be cleaved off by electrophiles to generate GSS-E adducts by the electrophilic alkylation, only when its vicinal sulfur is hydroxylated (OH- addition) to leave out GSS-OH, which may eventually result in the electrophilic polysulfide decomposition involving hydrolysis (Fig. 1A).

We report here, for the first time, that such alkaline hydrolysis is partly but significantly prevented by hydroxyphenyl-containing compounds including tyrosine and HPE-IAM. As a notable finding, even an alkylating agent containing the electrophilic IAM such as HPE-IAM, as long as its HPE hydroxyl moiety is functionally active so as to inhibit
alkaline hydrolysis, could have a potent polysulfide-stabilizing effect. Although the chemical reaction mechanism of alkaline-induced decomposition of polysulfides is not fully understood, several earlier reports showed that simple disulfides undergo hydrolysis only under strong alkaline conditions\[18–20\]. Therefore, the degradation-protective effect is likely caused by an antagonistic action of hydroxyphenyl residues of tyrosine and HPE-IAM during alkaline hydrolysis of polysulfides via heterolytic scission triggered by the \(\text{OH}^-\) acting on polysulfides that are cleaved into thiolates and sulfenic acids. Thus, compared with authentic diaryl disulfides, polysulfides appear to be more reactive in terms of alkaline hydrolysis of sulfide residues that may be more accessible to \(\text{OH}^-\).

We reported earlier that HPE-IAM is the most suitable agent, among 25 electrophilic alkylating agents, for determining and quantifying polysulfides, because much less polysulfide degradation was observed with HPE-IAM compared with other reagents such as MBB and NEM and even IAM \[2,16,21\]. In fact, polysulfide stabilization with HPE-IAM is totally unexpected, because such an effect is apparently contradictory to the potential electrophilic reaction of HPE-IAM with polysulfides. Nevertheless, provided that there is some structural similarity between HPE-IAM and tyrosine, which we now understand is the case, it is logical to expect that the hydrolysis-promoting effect of IAM may be counteracted by the HPE residue of the same compound.

Another interesting point is that this decomposition does not involve the usual redox processes that are affected by standard endogenous reductive reactions, which were recently found to be mediated by thioredoxin and thioredoxin reductase, for example \[22\]. Alkaline hydrolysis occurs simply by hydroxylation of particular sulfide residues by \(\text{OH}^-\) that is formed in excess at alkaline pH, independent of oxidation by redox-active species such as reactive oxygen species (ROS). In this context, we found here that hydroxylation of polysulfur residues was antagonized by tyrosine and HPE-IAM, with this hydroxylation being completely distinct from the redox-dependent reaction mediated by ROS and endogenously expressed counteracting reducing systems. We should emphasize that this polysulfide stabilization maintained by hydroxyphenyl is a completely new mode of regulation of polysulfide homeostasis or metabolism, and may be a novel physiological process or may become a specific target for pharmacological RSS modulation. Additional investigation of polysulfide and RSS metabolism in vivo and even in humans is therefore now warranted.

Also, the protective effect of tyrosine and HPE against polysulfide degradation has a great benefit for the reactive sulfur metabolomics analysis that we are developing \[2–4\]. Because a serious drawback of alkylation labeling analysis in LC-MS/MS measurement has been alkaline decomposition that was enhanced by electrophilic alkylating agents and dimedone, we can now develop the most specific, reliable, and useful approach to the sulfur metabolomics analysis by using the polysulfide stabilization effect of tyrosine and its related molecules. We expect that the result will be that artificial polysulfide decomposition can be minimized during the processing of biological samples for investigation of the sulfur metabonomics. Likewise, instability of RSS may somehow prevent clarification of the physiological and regulatory functions of RSS with various

Fig. 3. Protective effects of tyrosine on instability of GS-S-SG and GS-SS-SG caused by alkylating reagents. We incubated 1 \(\mu\)M GS-S-SG (A, B) or GS-SS-SG (C, D) in 100 mM phosphate buffer for the indicated times at pH 7.5 (A, C) or pH 8.0 (B, D) in the presence or absence of HPE-IAM, IAM, NEM, MBB, or tyrosine (Tyr) (1 mM each) at 37°C. Graphs show mean ± s.d. of intact GS-S-SG or GS-SS-SG concentrations as quantified via LC-ESI-MS/MS. Each value was obtained from triple determinations. Statistical significance was determined by Student’s \(t\)-test. *\(P < 0.05\). (E) Dose-dependent profile of tyrosine and various compounds containing hydroxyl moieties in terms of its effect on GS-SS-GS stability. GS-SS-SG (10 \(\mu\)M) was incubated with 0.25, 1, 5, 10 mM IAM, HPE-IAM, tyrosine, or other hydroxypHENyl-containing compounds (e.g., 4-acetamidophenol, glycerol, sucrose, methanol, or ethanol) in 100 mM phosphate buffer (pH 8.0) for 90 min at 37°C. Statistical significance was determined by Student’s \(t\)-test. *\(P < 0.05\) (vs. the control at 90 min). (F) Molecular structures of chemicals used in Fig. 3E.
proteins and enzymes such as protein disulfide isomerase or clarification of the plasticity and reversibility of many protein structures and functions. Therefore, our discovery of the stabilization of polysulfides that was improved by this effect of tyrosine and its related molecules may also be of great benefit for gaining a better understanding of the chemical biology and physiology of reactive polysulfides and RSS.

Finally, we confirmed that the reaction of dimedone and sulfenyl moiety may be caused artifactually without oxidative modification of cysteine thiols, as we noted recently [16]. This reaction may even be prevented by the protective activity and polysulfide stability conferred by tyrosine and HPE-IAM, as reported here. All these beneficial effects of hydroxyphenyls on polysulfides can therefore be exploited to develop a precise sulfur metabolomics analysis, which we are now pursing.

5. Conclusion

We here report that the simple amino acid tyrosine and its related hydroxyphenyl-containing compounds such as HPE-IAM have a unique polysulfide-sustaining effect, which may contribute to the regulation of physiological functions of polysulfide-containing derivatives and RSS. This effect may also be involved in redox signaling, as affected even by ROS as well as the most canonical cell signal transduction related to cellular phosphorylation.

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Author contributions

H.A.H., A.T., T.I., A.N., P.N., J.M.F, H.I., H.M., and T.A., conceptualization and data curation; H.A.H., A.T., T.I., A.N., T.M., and M.M., investigation and methodology; P.N., and T.A., grant acquisition. T.A., supervision and writing the original draft. H.A.H., A.N., S.F., R.T., T.S., H.M., H.I., and T.A., review and editing of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.redox.2019.101096.
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