Biologically relevant mechanisms of cysteine polysulfidation

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Summary of doctoral (PhD) thesis

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2019

1. Introduction

The topic of my PhD research was the mechanistic investigation of signaling pathways mediated by hydrogen sulfide. For a long time, hydrogen sulfide was considered as a noxious gas due to the inhibition of mitochondrial energetics via blocking *cytochrome–c–oxidase*. Surprisingly, then it was found to be produced in vivo via enzymatic and non enzymatic pathways and accumulating evidence indicated its regulating role regarding biochemical pathways. These discoveries corroborated that sulfide plays a key role in numerous physiological and patophysiological processes due to mediating intracellular signaling pathways, resulting that now sulfide is regarded as a gasotransmitter, like NO or CO.

During my research work I studied the per- and polysulfidation reactions of cysteine residues, focusing on their detection, which is still quite challenging. A comprehensive study was focused on the recent detection techniques, considering their advantages, disadvantages and limitations. The impact of the nature of the applied electrophile agent and experimental conditions like concentration and incubation time was thorougly studied with respect to three commonly used, cell permeable alkylating agents, and these parameters were found to have a significant effect on the detected speciation due to shifting the dynamic redox equilibrium reactions of reactive sulfur species as well as due to cleavage of longer chain polysulfides.

Polysulfides as signaling molecules are also produced during the cross-talk of signaling events mediated by sulfide and nitric–oxide, due to the formation and decomposition of a hybrid S/N intermediate called nitrosopersulfide ($SSNO^-$). Experiments were carried out focusing on the chemical characterization of this species, as well as studying its decomposition reactions and its biochemical effects on several biologically relevant models.

Kinetic data suggested that nitrosopersulfide is resistent towards the reducing capacity of the thioredoxin and glutahione dependent eznymatic systems and can function as a sustained polysulfide donor, inducing slow per- and polysulfidation reactions, thus playing a key role in signaling processes. Experiments on reduced glutathione, human serum albumine and HEK293 cells corroborated the delayed polysulfidating effects of $SSNO^-$ via a slow production of HS_x^- during its decomposition.

Flow cytometry experiments indicated that *transient receptor potential ankyrin 1* (TRPA1) receptors are also activated by inorganic polysulfides, which are released during the slow decomposition of nitrosopersulfide.

Regarding the results mentioned above, I believe that my doctoral research work contributed to the deeper understanding of the molecular mechanisms of sulfide mediated signaling pathways.

2. Objectives

During my doctoral research work I aimed to study the molecular mechanisms of signaling pathways mediated by hydrogen sulfide, focusing on the polysulfidation reactions of cysteine thiols according to the following points:

1., A detailed analysis was carried out, focusing on the recently applied polysulfide and thiol detection techniques, which are based on an electrophilic labeling of reactive sulfur species. Advantages, disadvantages and limitations of these protocols were investigated in a comprehensive study, sheding light on the importance of the applied experimental conditions (nature of the alkylating agent, concentration, incubation time) in order to have adequate readouts reflecting the real speciation.

2., I also aimed to investigate whether irreversible and specific alkylation of sulfenic acid modifications on Cys residues by dimedone may shift intracellular redox equilibria.

3., Another goal of my doctoral research was to investigate the cross-talk of signaling pathways mediated by hydrogen sulfide and nitric oxide due to studying the effects of a main intermediate called nitrosopersulfide ($SSNO^-$), which is produced during the chemical interaction between the two signaling molecules. $SSNO^-$ induced sustained per- and polysulfidaton was studied on cysteine thiols of reduced glutathion, human serum albumin, HEK293 cells and TRPA1 ion channels, in order to have a deeper insight into the molecular mechanisms of signaling pathways mediated by both H_2S and NO.

3. Materials and methods

3.1.Materials

Pure, analytical grade reagents were purchased from leading chemical companies (Sigma-Aldrich, Cayman Chemical, VWR Chemicals, Santa Cruz Biotechnology, Thermo Fisher Scientific, Nacalai Tesque, Wako Pure Chemical Industries, Invitrogen). Defined polysulfide salts were provided by Dojindo Laboratories. Recombinant TrxR1 and TRP14 proteins were kindly provided by our collaborator, Prof. Elias Arnér from the Karolinska Institute.

Sulfide stock solutions were prepared as previously described in the literature, polysulfide stock solutions were prepared in ultrapure water (Millipore) or in the indicated buffer by dissolving technical potassium polysulfide (K_2S_x; a mixture of different chain length polysulfides), sodium disulfide (Na_2S_2), sodium trisulfide (Na_2S_3) or sodium tetrasulfide (Na_2S_4) salts.

Nitrosopersulfide stock solutions were prepared by the reaction of 1 mM S–nitroso–N–acetyl–DL–penicillamine (SNAP) or S–nitrosoglutatione (GSNO) with 10 mM sulfide. All solutions were prepared and diluted fresh immediately before use in experiments.

3.2.Methods

3.2.1. UV-Visible (UV-Vis) spectrophotometry

Concentrations were determined by measuring the absorbance at the corresponding wavelength with a BioTek Powerwave XS plate reader, then the observed absorbance and molar absorbance (ε) values were used to calculate the exact values. Ultra pure water or the indicated buffer was used as reference. Kinetic experiments were carried out with the BioTek Powerwave XS plate reader or an Agilent Cary 8454 diode array spectrophotometer. With respect to the methylene blue method, cold cyanolysis, Bradford and bicinkoninic acid (BCA) assays, exact concentrations were calculated from the absorbance values using previously recorded calibration curves.

3.2.2. LC/MS–MS

The speciation of inorganic polysulfides was analysed without any previous separation, after the direct infusion of the diluted solutions into the electrospray (ESI) ionization source of the mass spectrometer with a SYNAPT G2-Si ESI-TOF-MS instrument, or following a previous alkylation, UHPLC-MS/MS method was used with a Waters Acquity UPLC system coupled to a Xevo TQ-S detector.

LC-ESI-MS/MS analyses of polysulfidated CySH és GSH adducts were performed with a Nexera UHPLC system (Shimadzu) coupled to a triple quadrupole (Q) LCMS-8050 mass spectrometer.

After tripsine digestion and trichloroacetic acid precipitation, dimedone labeled polysulfidated samples were subjected to LC/MS-MS analysis on a Thermo Ultimate 3000 HPLC system, coupled to a Thermo LTQ-XL linear iontrap mass spectrometer.

Polysulfidated GSH derivatives that were generated upon $SSNO^-$ decomposition were analysed with UPLC-MS/MS method with a Waters Acquity UPLC system coupled to a Xevo TQ-S qvadrupole mass spectrometer (Waters).

LC-ESI-MS/MS analyses of polysulfidated and oxidated, then dimedone labeled CysSH derivatives were performed on a Nexera UHPLC system, (Shimadzu) coupled with a triple quadrupole (Q) LCMS-8050 mass spectrometer (Shimadzu).

Analytes were identified by tandem mass spectrometry, their quantitative analysis was performed in SRM mode.

3.2.3. Protein assays

PMSA

Purified human recombinant GAPDH was desalted to remove reductants. Desalted protein samples were incubated with Na_2S_2 or Na_2S_3, excess inorganic polysulfides were removed by a second desalting step. Protein quantification was performed by the bicinkonininic acid assay to normalize the total protein content of each sample. Sulfhydryl groups were labeled with biotin–PEG36–maleimide, then the labeled samples were subsequently incubated with various electrophile agents (IAM, MBB,

NEM, DTNB), and finally heated in the presence or absence of 2–mercaptoethanol. Samples were stored at -20 °C, then SDS-PAGE and Coomassie staining was performed to determine the extent of GAPDH polysulfidation and to assess the ability of electrophiles to cleave dialkyl polysulfur chains.

Preparation of human serum albumin samples for ProPerDP assay

Following the prereduction and quantitation of the protein, HSA was treated with previously prepared and purified $SSNO^-$ and control solutions at room temperature, protected from light. Aliquots were taken from the reaction mixtures at 0, 20, 40 and 60 minutes and incubated with iodoacetyl–PEG–biotin (IAB) in order to stabilize polysulfide derivatives. Excess IAB was removed by desalting and ultafiltration. Biotinylated proteins were pulled down by streptavidin coated magnetic beads, then the beads were separated from the solution with a magnetic particle separator. Then, supernatant was placed into a clean tube and beads were washed with TBST in order to eliminate non-specific adhesion. Beads were then resuspended and incubated with TCEP, followed by magnetic separation. Finally, beads were boiled in SDS-PAGE loading buffer to remove all bound material. Samples were stored at -20 °C until SDS–PAGE and western blotting.

SDS-PAGE

Samples containing ~50 ng protein were run on nonreducing 12% polyacrylamide gels at 120 V for at least 90 minutes. Gels were visualized via Coomassie Brilliant Blue staining or western blotting, followed by BCIP-NBT staining.

Coomassie Brilliant Blue staining

Following SDS–PAGE, gels were fixed in a 40% ethanol–10% acetic acid solution, then incubated in QC Colloidal Coomassie staining solution. Gels were then washed several times with deionized water in order to eliminate the background signal and visualize the bands.

Western blotting

Following SDS-PAGE gel electrophoresis, plasma samples were transferred to polyvinylidene difluoride membranes, which were then blocked overnight. Membranes were then incubated in solutions of the primary and secondary antibodies. Between the two incubation steps, membranes were washed with TBST in order to eliminate non-specific adhesion. Bands were visualized by 5-bromo-4-chloro-3-indolyl phosphate–nitro blue tetrazolium, then quantitative analysis of the bands was performed by using Image J software (National Institutes of Health).

3.2.4. Cell cultures and related experiments

HEK293 cells and their treatment

HEK293 cells were purchased from the American Type Culture Collection (ATCC) and were stably transfected by Prof. Elias Arnér's group at the Karolinska Institute. Cells were kept in Dulbecco′s-Modified Eagle Medium (DMEM), complemented with fetal bovine serum, penicilline and streptomycine. Cells were washed with DMEM and stained with the sulfane sulfur specific fluorescent probe SSP4. Excess probe was removed by washing twice with DMEM, then cells were incubated with purified $SSNO^-$/control and fluorescence was recorded with a microscope.

TRPA1 expressing and TRPA1 deficient CHO cells and their treatment

Intracellular Ca^{2+} influx was measured, which reflects to receptor activation. In order to corroborate that the detected increase in $[Ca^{2+}]$ levels was specific to TRPA1 activation, control experiments were performed on TRPA1 deficient cells.
TRPA1 expressing CHO cells were prepared and kindly provided by Dr. Zoltán Sándor at the Department of Pharmacology and Pharmacotherapy of the University of Pécs.
Cells were kept in Dulbecco′s-Modified Eagle Medium (DMEM), complemented with fetal bovine serum albumin, L-glutamine, non-essential amino acid solution, penicilline and streptomycine. Before treatment, the medium was gently removed from cells and

trypsin solution was applied. For each sample, approximately 10^4 TRPA1-expressing CHO cells were resuspended in cell culture medium, then incubated with the calcium sensitive fluorscent stain Fluo-4 AM. Then, cells were suspended in extracellular solution (ECS) and the appropriate volumes of $SSNO^-$ and control solutions were added to the cell suspensions and samples were analysed by flow cytometry. Fluo-4 AM was excited by a 488 nm laser, fluorescence was detected at 504 nm. Mean green fluorescence of the samples was compared to base fluorescence of dye-loaded control cells. As positive control, cell groups were reacted with the selective TRPA1 receptor agonist mustard-oil.

4. Results and discussion

4.1. Detection of reactive sulfur species in biological samples

Direct infusion ESI-MS detection of dissolved sodium trisulfide, sodium tetrasulfide and mixed potassium polysulfide salts all resulted in similar speciation patterns. The most abundant species was found to be the trisulfide radical anion ($S_3^{\bullet-}$), while disulfide ($S_2^{\bullet-}$) and tetrasulfide radical anions ($S_4^{\bullet-}$) were also observed, and mass peaks indicative of the presence thiosulfate radical anions ($S_2O_3^{\bullet-}$) and protonated thiosulfate anions, as well as HSO_4^- were found.

However, the presence of the polysulfide radical species can be the result of the homolytic cleavage or $1e^-$ artifact oxidation of open chain polysulfides in the source of the mass spectrometer. Therefore, we propose that under the applied conditions, experimental data regarding polysulfide speciation should be interpreted with caution.

With respect to the derivatization of inorganic polysulfides, the impact of two alkylating agents: iodoacetamide (IAM) and N-ethylmaleimide (NEM) at different concentrations was examined, using UHPLC separation and MS-MS detection. Under the applied conditions, NEM-derivatized polysulfides could be separated with increasing number of sulfurs from S1 to S4, while IAM-derivatized polysulfides were detected with a number of sulfurs from S1 to S7. In consistence with the direct infusion

experiments, the speciation pattern looked very similar, regardless of the initial source of inorganic polysulfides, however the applied concentrations of the agents had an impact on the apparent speciation of derivatized polysulfides.
This observation could be interpreted with different alkylation rates and the different mode of alkylation.

In order to study the differencies with respect to these two alkylating agents, polysulfide solutions were at first fully reacted with a 10-fold molar excess of IAM, and then with NEM. Atfer the addition of NEM, IAM-derivatized S1–S3 species were still observed but in a lower abundance, however no longer chain polysulfides were detected. Surprisingly, after NEM addition, hybrid S1–S3 adducts were observed with one carboxamidomethyl moiety from IAM and one succinimide moiety from NEM. This suggests that NEM had reacted with the polysulfide chain due to an attack on a midchain sulfur atom, leading to the cleavage of the chain.

A few possible models were proposed to explain these observations and one possible model, the shift of hydrolysis equilibria was further studied. Hence, this model is relevant with respect to the detection of sulfenic acids, thus the shift by specific sulfenic acid capturing tools could result in eventual capturing of cysteine residues that were originally present as polysulfides. This potential caveat of sulfenylation measurement protocols was addressed in experimental models as well.
Following the speciation study of inorganic polysulfides, cysteine and GSH polysulfidation was investigated. These derivatives were previously demonstrated to play a major role in polysulfidation-regulated redox signaling events by triggering polysulfidation on functional and regulatory protein cysteine residues via transpersulfidation reactions.
Their detection is based on an initial electrophilic labeling, therefore we investigated whether alkylation of these species in simple aqueous solutions can change their speciation.
Using a recently developed quantitative MS method we examined how incubation time and alkylating agent concentration affect speciation of cysteine and GSH polysulfides using the hydroxyphenyl derivative of iodoacetamide (HPE–IAM), monobromobimane (MBB) and N-ethylmaleimide (NEM) as representative compounds.

Our results suggest that the concentration of HPE–IAM had only a little effect on the speciation of cysteine and GSH persulfides. Time resolved measurements corroborated that HPE–IAM can not cleave dialkyl polysulfide chains efficiently.

In a similar experiment with NEM, a strikingly different distribution was detected. Our observations corroborated that NEM favours the formation of alkylated cysteine thiols. Surprisingly, neither concentration, nor incubation time had much effect on the detected concentration of the NEM derivatized GSH persulfide species, which was more abundant than the corresponding NEM-alkylated cysteine persulfides. This observation indicates that rates and effectiveness of derivatization will much depend on the nature of the detectable species as well.

When these experiments were repeated with monobromobimane (MBB), the concentration and incubation time had little effect on the MBB-derivatized cysteine and GSH persulfide levels, but the detected concentrations of the trisulfide species increased with increasing MBB concentration after 3 h incubation with the akylating agent. At higher MBB concentrations, the time-resolved decrease in the trisulfide adducts indicated that like NEM, MBB can also cleave longer polysulfide chains.

Alkylation induced polysulfide cleavage was further investigated using *glyceraldehyde 3-phosphate dehydrogenase* (GAPDH) and a recently developed protein polysulfide detection method (PMSA), which builds on the NEM induced cleavage of longer dialkyl polysulfide chains. Our results indicated that the nature of the alkylating agent made a difference in the observed speciation pattern: NEM was the most efficient in cleaving the already alkylated Cys-polysulfide residues on the protein, followed by MBB, and IAM had only a slight cleaving effect. Another outcome is that NEM and 2-mercaptoethanol or DTNB treatments showed a similar pattern, which suggests that NEM cleaved all polysulfide chains on the protein.

4.2. Dimedone based detection of cysteine polysulfenic acids

The potential of alkylation induced shift of hydrolysis equilibria raises an important issue regarding the common detection of protein sulfenic acids, since specific trapping of these species would pull the equilibria towards the formation of the hydrolytic products, leading to increased sulfenic acid levels. In other words, a

proportion of the detected protein sulfenic acid pool in biological systems could in fact represent protein per- and polysulfides instead.

In addition, beside hydrolysis, the oxidation of cysteine-persulfides also leads to intermediate formation of perthiosulfenic acid species, which was corroborated by theoretical calculations, as well as experimental results.

These assumptions were evaluated in a relevant biological context, by incubating dimedone with isolated GAPDH, pretreated with inorganic polysulfides or hydrogen-peroxide. Surprisingly, more dimedone labelled cysteine and cysteine polysulfide derivatives were detected in samples, where the protein was treated with inorganic polysulfides, however this treatment is not expected to directly provide cysteine-sulfenic acid.

Our observations suggest that a fraction of the previously detected sulfenic acid pool, which used dimedone-based molecules as capturing agents, could in fact represent polysulfide species.

4.3. Cross-talk between hydrogen sulfide and nitric oxide mediated signaling pahtways

With respect to the cross-talk between sulfide and NO mediated signaling processes, I aimed to study the chemical and biological effects of nitrosopersulfide ($SSNO^-$), which is an intermediate generated during the chemical interaction between the two gasotransmitters and their metabolites.

Spectral and kinetic studies corroborated that nitrosopersulfide, prepared by the reaction of the nitric-oxide donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) and excess sulfide exerts the same spectral characteristics like the previously identified PNP^+SSNO^- and it is indeed a relatively long lived intermediate of the NO-sulfide interaction.

Studying the selective reduction of inorganic polysulfides generated during $SSNO^-$ preparation, the widely used *tris-(2-carboxyethyl)phosphine* (TCEP) did not show sufficient selectivity, while a reduction column working with immobilized *D,L-dithiotreitol* (DTT) was not found to selectivelyreduce polysulfides beside $SSNO^-$.

Moreover, $SSNO^-$ was found to exhibit unexpected resistance towards the thioredoxin (Trx) and glutathione (GSH) dependent enzymatic machineries, which are responsible

for the reduction of intracellular disulfides. This discovery carries biological relevance as well, since it suggests that by escaping from the reducing capacity of these main NADPH dependent reducing machineries, $SSNO^-$ can function as a sulfane sulfur trafficking molecule, generating inorganic polysulfides upon decomposition. Inorganic polysulfides play a key role in signaling pahtways via the generation of polysulfidated cysteine posttranslational modifications. The presence of $SSNO^-$ released inorganic polysulfides was corroborated by LC/MS analysis as well.

$SSNO^-$ induced delayed polysulfidation was studied on several biologically relevant models. The time dependent formation of polysulfidated thiol species were detected by LC–MS following the reaction of $SSNO^-$ with reduced glutathione, corroborating the sustained effect of $SSNO^-$.

When a thiol protein, *human serum albumin* was treated with purified $SSNO^-$, increased amount of protein-persulfides were identified using the ProPerDP method. The time dependent formation of human serum albumin persulfide (HSA-SSH) was found to be consistent with the decreasing amount of nitrosopersulfide, while no detectable amount of HSA-SSH was found in control samples, which indicated that the enzymatic reduction of in situ generated inorganic polysulfides was effective.

The sulfane sulfur donor properties of nitrosopersulfide were studied in a cellular system as well. Increased fluorescent signal was detected in HEK293 cells upon $SSNO^-$ treatment, previously stained with the sulfane sulfur probe SSP4. The time resolved appearance of the signal indicated the formation of sulfane sulfur containing species like per- and polysulfides, suggesting that $SSNO^-$ exhibits delayed intracellular per- and polysulfidation effects.

The effect of $SSNO^-$ on *transient receptor potential ankyrin 1* (TRPA1) ion channels was studied by treating TRPA1 expressing and TRPA1 deficient CHO cells with purified $SSNO^-$ and control solutions. In the control study, after a rapid activation, a time resolved decrease in activity was observed, which is attributed to the transient activation by the in situ generated inorganic polysulfide byproducts. However, upon $SSNO^-$ treatment, at distinct timepoints, increasing Ca^{2+} influx was detected, suggesting that SSNO exerts a sustained effect regarding the activation of these ion channels most likely via a slow release of the activating inorganic polysulfide species. TRPA1

deficient cells did not show any reaction, corroborating that the detected Ca^{2+} influx was specific to the activation of the ion channels.

5. Summary

1. Concerning the solution chemistry of inorganic polysulfides and the PMSA assay of GAPDH, our results indicate that under the applied experimental conditions, their speciation did not depend on their initial source due to their dynamic redox equilibrium reactions, which take place after dissolution of various polysulfide salts.

2. The result of our PMSA experiments on GAPDH suggested that the thiol residues of recombinant GADPH were partially polysulfidated.

3. When derivatization was used during the detection protocol of cysteine-polysulfur species – which is necessary in order to form stable modifications of these unstable and reactive species – we found that the nature of the applied alkylating agent made a difference in the final detected speciation of inorganic polysulfides.

- Studying the alkylation of inorganic, cysteine, glutathione and GAPDH polysulfides, our observations suggested that the widely used, strong electrophile N–ethylmaleimide (NEM) cleaves polysulfide chains due to the attack of midchain sulfur atoms
- This cleavage can take place after a previous alkylaton step, which means that both the incubation time and the concentration of NEM are crucial with respect to the speciation of the detected alkylated species, because the extent of polysulfide cleavage increased with increasing incubation time and NEM concentration
- Studying the polysulfidation reactions and the NEM induced cleavage of the polysulfidated chains of GAPDH, our observations suggested that given enough time, NEM cleaved all polysulfide chains on the protein

- On the contrary, the application of another widely used alkylation agent, iodoacetamide (IAM) and its hydroxyphenyl analogue (HPE–IAM) was not found to lead to polysulfide cleavage under the applied conditions, making them ideal agents for the detection of longer chain polysulfides.
- Monobromobimane (MBB), another frequently used alkylating agent in biology, also induced polysulfide cleavage but to a lesser degree compared to NEM.
- During the polysulfidation and alkylation study of low molecular weight thiols, glutathione derivatives were found to be more stable than the corresponding cysteine derivatives, and the NEM induced cleavage of these occured to a lesser extent.

4. When the alkylation induced polysulfide cleavage was studied, an important issue was discovered regarding the dimedone based detection of sulfenic acids, suggesting that a certain part of the previously identified sulfenic acid pool in biological systems may represent polysulfide species

5. When the chemical and kinetic characterization of the reaction mixture, prepared by the reaction of S–nitroso–N–acetyl–DL–penicillamine with excess sulfide was performed, a species was identified. Our experiments corroborated that this species exhibited the same spectral characteristics which were previously attributed to nitrosopersulfide by Seel and coworkers, as well as Wedmann and his colleagues.

6. When the selective reduction of in situ generated polysulfide byproducts was studied, several problems were observed with respect to the selectivity or the efficiency of the commercially available reducing agents (TCEP, DTT).
Two biological reducing machineries, the thioredoxin and glutathione systems were found to have no effect on the stability and decomposition of nitrosopersulfide, providing a selective and efficient way for the elimination of inorganic polysulfide byproducts in $SSNO^-$ preparations.
7. Our LC/MS experiments corroborated that the slow and continuos decomposition of $SSNO^-$ induces the formation of inorganic polysulfides. Hence we concluded that by escaping from the reductive capacity of the previously mentioned enzymatic systems,

nitrosopersulfide could function as a long range sulfane sulfur trafficking molecule, inducing delayed polysulfidation reactions on cysteine thiols.

8. $SSNO^-$ induced polysulfidation was studied on several biologically relevant models:

- On reduced glutathione, S1-S7 glutathione–polysulfur species were detected upon $SSNO^-$ treatment by LC/MS analysis and the delayed polysulfidating effect of $SSNO^-$ was also corroborated by their time dependent formation
- When human serum albumin was treated with $SSNO^-$, a time dependent formation of human serum albumin–persulfide (HSA–SSH) was observed by the ProPerDP method and the increasing amount of HSA–SSH was found to be consistent with the decreasing concentration of nitrosopersulfide, corroborating its sustained persulfidation character.
- When HEK293 cells, previously stained with the sulfane sulfur specific SSP4 probe were treated with nitrosopersulfide, an increasing fluorescent signal was detected reflecting the intracellular function of sulfane sulfur species.
- The sustained effect of nitrosopersulfide due to the continuous release of inorganic polysulfides was also studied with respect to the activation of TRPA1 ion channels. Upon $SSNO^-$ treatment, a time dependent, increase in the activation was observed, compared to when cells were treated with a bolus of inorganic polysulfides. The treatment of TRPA1 deficient cells did not lead to any Ca^{2+} influx, suggesting that the detected signal is indeed attributed to the activation of these ion channels.

9. With respect to each model, our experiments were carried out with a „control solution" as well, in order to represent the presence of sulfide and inorganic polysulfides, which are generated as byproducts during $SSNO^-$ preparation, but can not be completely abolished from the reaction mixture. With this solution, only transient effects were observed, indicating that these reactive species, can only induce short term activation of TRPA channels in contrast with nitrosopersulfide, which induced sustained effects due to the slow release of its decomposition products.

6. Documented publications related to the present doctoral (PhD) thesis

6.1. International publications related to the present doctoral (PhD) thesis

1. David E. Heppner, Milena Hristova, Tomoaki Ida, Ana Mijuskovic, Christopher M. Dustin, Virág Bogdándi, Jon M. Fukuto, Tobias P. Dick, Péter Nagy, Jianing Li, Takaaki Akaike, Albert van der Vliet

Cysteine Perthiosulfenic Acid (Cys-SSOH): A Novel Intermediate in Thiol-Based Redox Signaling (co-author publication in a referred international journal)

Redox Biology, 14, 379-385

Published in 2018, IF: 7,126

2. Virág Bogdándi, Péter Nagy

Dojindo Products in Mechanistic Studies of Reactive Sulfur Species (first author publication in an international journal)

Dojin News, 2018, 164, 1–4

ISSN: 0385–1516

Published in 2018

3. Virág Bogdándi, Tomoaki Ida, Thomas R. Sutton, Christopher Bianco, Tamás Ditrói, Grielof Koster, Hillary Henthorn, Magda Minnion, John P.Toscano, Albert van der Vliet, Michael D. Pluth, Martin Feelisch, Jon M. Fukuto, Takaaki Akaike, Péter Nagy

Speciation of Reactive Sulfur Species and Their Reactions with Alkylating Agents: Do we have any clue about what is present inside the cell? (first author publication in a referred international journal)

British Journal of Pharmacology, 176, 646-670

Published in 2019, IF: 6,810

4. Christopher Bianco, Takaaki Akaike, Tomoaki Ida, Péter Nagy, Virág Bogdándi, John Toscano, Yoshito Kumagai, Catherine Henderson, Robert Goddu, Joseph Lin, Jon Fukuto

The reaction of hydrogen sulfide with disulfides: formation of a stable trisulfide and implications for biological systems (co-author publication in a referred international journal)

British Journal of Pharmacology, 176, 671-683

Published in 2019, IF: 6,810

5. Virág Bogdándi, István Zoárd Bátai, Magda Minnion, Zoltán Sándor, Erika Pintér, Martin Feelisch, Péter Nagy:
Nitrosopersulfide (SSNO) is a potential protein Cys polysulfidating agent with sustained and long-range effects (conference abstract in a referred, international journal)
Free Radical Biology and Medicine Volume 120, Supplement 1, S128
Published in 2018

6. Virág Bogdándi, István Zoárd Bátai, Magda Minnion, Zoltán Sándor, Erika Pintér, Martin Feelisch, Péter Nagy:
Nitrosopersulfide (SSNO) is a potential protein Cys polysulfidating agent with sustained and long-range effects
manuscript under submission

6.2. Conference presentations, poster presentations related to the present doctoral (PhD) thesis

1. Marián Grman, Miriam M. Cortese-Krott, Virág Bogdándi, Martin Feelisch, Karol Ondriaš, Péter Nagy
Polysulfides as Intermediate Species and Products of Nitrosopersulfide Synthesis and Decomposition (poster)
4th International Conference on the Biology of Hydrogen Sulfide
June 2016, Naples, Italy

2. Bogdándi Virág, Nagy Péter, Miriam Cortese–Krott, Marian Grman, Martin Feelisch, Ming Xian
Kénhidrogén által vezérelt jelátviteli folyamatok molekuláris mechanizmusai
XIII. Pro Scientia Aranyérmesek Konferenciája
November 2016, Pécs

3. Virág Bogdándi, David E. Heppner, Milena Hristova, Tomoaki Ida, Ana Mijuskovic, Christopher M. Dustin, Jon M. Fukuto, Tobias P. Dick, Péter Nagy, Jianing Li, Takaaki Akaike, and Albert van der Vliet
Cysteine Perthiosulfenic Acid (Cys-SSOH): A Novel Intermediate in Thiol-Based Redox Signaling
S–BIO 2017 Plant and Human Sulfur Biology Conference 2017
September 2017, Balatonfüred

4. Bogdándi Virág, Bátai István Zoárd, Sándor Zoltán, Sághy Éva, Pintér Erika, Nagy Péter
A kénhidrogén redox jelátviteli folyamatokban betöltött szerepének biológiai vizsgálata
Doktoranduszok a Klinikai Kutatásokban
October 2017, Pécs

5. Bogdándi Virág, David E. Heppner, Milena Hristova, Tomoaki Ida, Ana Mijuskovic, Christopher M. Dustin, Jon M. Fukuto, Tobias P. Dick, Péter Nagy, Jianing Li, Takaaki Akaike, and Albert van der Vliet
Cisztein–pertioszulfénsav (Cys-SSOH): A tiolfehérjéket érintő redox jelátvitel egy újabban azonosított köztiterméke
A Magyar Tudományos Akadémia Reakciókinetikai és Fotokémiai Munkabizottságának Ülése
November 2017, Budapest

6. Virág Bogdándi, István Zoárd Bátai, Magda Minnion, Zoltán Sándor, Erika Pintér, Martin Feelisch and Péter Nagy
Nitrosopersulfide ($SSNO^-$) is a potential protein Cys polysulfidating agent with sustained and long range effects
7th Interdisciplinary Doctoral Conference
May 2018, Pécs

7. Bogdándi Virág, David E. Heppner, Milena Hristova, Tomoaki Ida, Ana Mijuskovic, Christopher M. Dustin, Jon M. Fukuto, Tobias P. Dick, Péter Nagy, Jianing Li, Takaaki Akaike, and Albert van der Vliet
Cysteine Perthiolsulfenic Acid (Cys-SSOH): A Novel Intermediate in Thiol-Based Redox Signaling (poster)
7th Interdisciplinary Doctoral Conference
May 2018, Pécs

8. Bogdándi Virág
Nitrozoperszulfid biológiai hatásának vizsgálata TRPA1 receptorok aktivációjának tanulmányozásával
Intézményi ÚNKP Konferencia
May 2018, Pécs

9. Bogdándi Virág, Nagy Péter
Reaktív kénszármazékok alkilálási reakciói – avagy mit detektálunk sejten belül?
A Magyar Tudományos Akadémia Reakciókinetikai és Fotokémiai Munkabizottságának Ülése
May 2018, Balatonalmádi

10. Virág Bogdándi, István Zoárd Bátai, Magda Minnion, Zoltán Sándor, Erika Pintér, Martin Feelisch and Péter Nagy
Nitrosopersulfide ($SSNO^-$) is a potential protein Cys polysulfidating agent with sustained and long range effects (poster)
19th SFRRI Biennial Meeting
June 2018, Lisbon, Portugal

11. Virág Bogdándi, Tomoaki Ida, Thomas R Sutton, Christopher Bianco, Tamás Ditrói, Grielof Koster, Hillary A Henthorn, Magda Minnion, JohnPToscano, Albert van der Vliet, Michael D Pluth, Martin Feelisch, Jon M Fukuto, Takaaki Akaike, Péter Nagy
Speciation of Reactive Sulfur Species and their reactions with alkylating agents: Do we have any clue about what is present in the cell?
King's College London Redox Biology & Medicine Symposium
September 2018, London, United Kingdom

12. Virág Bogdándi, Tomoaki Ida, Thomas R Sutton, Christopher Bianco, Tamás Ditrói, Grielof Koster, Hillary A Henthorn, Magda Minnion, JohnPToscano, Albert van der Vliet, Michael D Pluth, Martin Feelisch, Jon M Fukuto, Takaaki Akaike, Péter Nagy
Speciation of Reactive Sulfur Species and their reactions with alkylating agents: Do we have any clue about what is present in the cell?
Medical Conference for PhD Students and Experts of Clinical Sciences
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13. Virág Bogdándi, István Zoárd Bátai, Magda Minnion, Zoltán Sándor, Erika Pintér, Martin Feelisch and Péter Nagy
Nitrosopersulfide ($SSNO^-$) is a potential protein Cys polysulfidating agent with sustained and long range effects (poszter)
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14. Bogdándi Virág, Bátai István Zoárd, Magda Minnion, Sándor Zoltán, Pintér Erika, Martin Feelisch, Nagy Péter
Nitrozoperszulfid: egy újabban azonosított szulfán kén donor molekula, elnyújtott és széleskörű fehérje per- és poliszulfidációs hatással
Az MTA Reakciókinetikai és Fotokémiai Munkabizottság, a Koordinációs Kémiai Munkabizottság és a VEAB Kémiai Szakbizottság közös Tudományos Ülése
November 2018, Veszprém

15. Bogdándi Virág
Kénhidrogén által vezérelt jelátviteli folyamatok molekuláris mechanizmusai
A Magyar Kémikusok Egyesülete CHINOIN munkahelyi csoportjának rendezvénye
May 2019, Budapest

16. Bogdándi Virág
Cisztein származékok poliszulfidációjának biológiailag releváns mechanizmusai
Az MTA Reakciókinetikai és Fotokémiai Munkabizottságának Tudományos Ülése
May 2019, Balatonalmádi

17. Bogdándi Virág, Bátai István Zoárd, Magda Minnion, Sándor Zoltán, Pintér Erika, Martin Feelisch, Nagy Péter
Nitrozoperszulfid: egy újabban azonosított szulfán kén donor molekula, elnyújtott és széleskörű fehérje per- és poliszulfidációs hatással (poster)
MKE Vegyészkonferencia
June 2019, Eger