

**Propositions of Ph.D. thesis**

**KINETICS AND MECHANISMS OF SOME BIOLOGICALLY  
IMPORTANT REDOX REACTIONS OF  
HYDROGEN SULFIDE**

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## I. INTRODUCTION AND THE AIM OF THE WORK

Hydrogen sulfide ( $\text{H}_2\text{S}$ ) is a toxic gas with characteristic smell of rotten eggs. The major mechanism of sulfide toxicity is via inhibition of mitochondrial respiration by interactions with the cytochrome-c oxidase (CcO) enzyme. However,  $0.7 \mu\text{g/g}$  sulfide<sup>1</sup> concentration was measured in postmortem human brain tissue, confirming the presence of sulfide in the human body. It has been shown that endogenous sulfide is produced via cysteine (Cys) metabolism by reverse transsulfuration pathways. The primary enzymes involved in sulfide production are the pyridoxal phosphate dependent, cystathionin  $\gamma$ -lyase (CSE), cystathionine  $\beta$ -synthase (CBS) and aspartate-/cysteine-aminotransferase (AAT/CAT) in cooperation with 3-mercaptopyruvate sulfurtransferase (3MST). Sulfide catabolism mostly occurs in the mitochondria via oxidative processes driven primarily by the sulfide quinone reductase (SQR) enzyme. Sulfide is oxidized to sulfate or thiosulfate in these processes.

The most notable triggering factor of the proliferating investigations in the field of sulfide biology was the recognition of sulfide's neuromodulator function in 1996. With this discovery, after nitric oxide (NO) and carbon monoxide (CO), sulfide also joined the group of "small signaling molecule", which are often called in the literature as "gasotransmitters".

Despite of the numerous important biological roles of sulfide, its concentrations in physiological samples is still heavily debated. For example, free sulfide concentration values were measured anywhere between  $0.1 \mu\text{M}$  to  $500\text{--}600 \mu\text{M}$  in blood using various sulfide detection methods. Therefore, our goal was to reconcile the chemistry and biological applications of the most commonly used sulfide determination methods and to develop a standardized protocol for making and handling sulfide solutions.

Free sulfide is toxic above  $1 \mu\text{M}$  concentration. Thus, earlier results reporting considerably higher concentrations refer to mobilizable

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<sup>1</sup> Sulfide will be used here after as a generic term to include all the different protonated forms of hydrogen sulfide. Distinction between these species is made only when it is required by the clarity of discussion.

sulfide reserves in biological systems. These sulfide reserves contain relatively large amounts of bonded sulfide, which we proposed to play significant roles in the biological effects of sulfide by acting as a buffer for free sulfide.

Sulfide plays an important role in intercellular signaling processes. Three main mechanisms of the signaling properties have been described in the literature: i) through the formation of persulfides, regulation of thiol-protein functions ii) interactions with metalloenzymes and their redox reactions and iii) cross talk with NO signaling processes.

An important component of sulfide signaling was attributed to protein persulfides, however, even their formation mechanisms remain largely unknown. An early misconception was that the direct reaction of hydrogen sulfide ion ( $\text{HS}^-$ ) with cysteine can produce persulfides. It is now appreciated that sulfide-mediated persulfide formation occurs either via the reactions of oxidized Cys derivatives with sulfide or sulfide oxidation products reacting with Cys thiols. One of the aims of my work is the examination of molecular mechanisms of persulfide formation pathways.

The kinetics and mechanisms of the reactions of disulfides with sulfide to produce persulfide species were investigated. These reactions were initially discredited on biological grounds because it was assumed that they are both kinetically and thermodynamically incompetent in a cellular like environment. In contrast, our mechanistic studies revealed that some of these reactions have potential to occur under biological conditions. We have shown that the kinetic and thermodynamic properties of sulfide-mediated disulfide reduction reactions show large variations, depending on the chemical properties of the corresponding disulfides.

Persulfides can also be generated in reactions of sulfide oxidation products with reduced cysteine thiols (Cys-SH). The oxidation of sulfide in cells is promoted by intracellular reactive oxygen species (ROS). Among these processes we examined the reaction of chloramines with sulfide. Chloramines are produced as secondary antimicrobial agents of the neutrophil oxidant, hypochlorous acid (HOCl).

In biological systems, an antioxidant role was attributed to sulfide, but it is likely that these properties are not mediated through direct scavenging of ROS, but by influencing different enzyme activities. A potential pathway is represented by the reduction of highly oxidizing

metalloenzyme intermediate species. A similar mechanism was confirmed previously with myeloperoxidase (MPO) by our research group. In accordance with these considerations, we wanted to understand how sulfide may neutralize oxidative stress generating effects of oxidized hemoglobin intermediates in atherosclerosis. As a potential pathway, the direct reduction of ferryl-Hb-derivatives by sulfide was investigated.

## II. EXPERIMENTAL METHODS

All chemicals were analytically pure grade reagents and the solutions were prepared by using deionized and ultrafiltered water obtained from a *Milli-Q* system (*Millipore*). Methemoglobin (metHb, Fe<sup>3+</sup>Hb) and oxyhemoglobin (oxyHb, Fe<sup>2+</sup>Hb) were made from human blood by Prof. József Balla's research group.

Sulfide stock solutions were prepared by dissolution of washed Na<sub>2</sub>S·9H<sub>2</sub>O crystals in water under Ar. The concentrations of sulfide stock solutions were assessed by direct spectrophotometry at 230 nm ( $\epsilon_{230\text{nm}} = 7700 \text{ M}^{-1}\text{cm}^{-1}$ ). The pH of these solutions were kept at pH > 9. Sulfide concentrations were always corroborated with an independent measurement using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) method according to the literature where sulfide was reacted with DTNB and the absorbance maximum of the generated 2-nitro-5-thiobenzoate (TNB) was measured at 412 nm ( $\epsilon_{412\text{nm}} = 14100 \text{ M}^{-1}\text{cm}^{-1}$ ). The average of the two obtained concentrations by the two independent methods were used when the difference was less than 5% between the two values. New stock solutions were prepared when the error was higher.

The pH of the aqueous solutions were kept constant with buffers and the appropriate pH were set by carbonate-free sodium hydroxide solutions. The **pH measurements** were performed using *785 DMP Titrino* system which was controlled by a *Tiamo 2.3* software package. A double-junction combination pH glass electrode (*Metrohm 6.255.100*) was used. The electrode was calibrated with potassium hydrogen phthalate (pH 4.008) and sodium tetraborate (pH 9.177), then the read-out of the pH meter was converted to  $-\lg[\text{H}^+]$ . The pH of the solutions were measured both before and after the kinetic and spectrophotometric runs.

The spectra of the reactants and products were recorded on a *Perkin Elmer Lambda 2S UV-visible (UV-Vis) dual beam spectrophotometer*. The concentration of the reactants were calculated from the absorbance, using reported molar absorbance ( $\epsilon$ ) values. The temperature of the reaction mixtures were controlled with a *Techne RB-12, TU-16D* thermostat at  $25.0 \pm 0.1 \text{ }^\circ\text{C}$ .

Kinetic measurements were conducted by simple or sequential **stopped-flow methods** using an *Applied Photophysics SX.18MV* and/or an

*Applied Photophysics SX.20* stopped-flow instrument. Possible unwanted reactions between the reactants and buffers were excluded in preliminary runs by mixing the reactants with buffer prior to the kinetic experiments. The same experimental setup was used for the determination of initial absorbance values in the kinetic runs. The reaction mixtures were thermostated at  $25.0 \pm 0.1$  °C with a *Julabo F12, ED* thermostat. The kinetic curves were fitted by *Micromath Scientist 2.01* and/or *Applied Photophysics Pro-Data Viewer 4.2.0* software packages, using the nonlinear least squares method and suitable mathematical equations. Rate constant values were obtained by averaging at least three parallel kinetic runs.

**Nuclear magnetic resonance spectroscopy** (NMR) experiments were performed on a *Bruker 360 MHz (8.5 T)* NMR spectrometer at  $25.0 \pm 0.5$  °C. We studied the reactants, the intermediates and the final products of the reactions of cystine with sulfide. The  $^1\text{H}$ -NMR spectra of the reactions were recorded after equilibrium conditions were established. The chemical shift values of the peaks in the NMR spectra were referenced to 4,4-dimethyl-4-silapentane-1-sulfonic acid, which was used as an internal standard. The NMR spectra were evaluated with the *MestReNova 8.1.2-11880* software package. For the NMR measurements, the pD of the solutions were kept at constant values with deuterated buffers. The read-out of the pH meter,  $\text{pH}^*$ , was corrected according to the following expression:  $\text{pD} = \text{pH}^* + 0.4$ .

### III. NEW SCIENTIFIC RESULTS

#### **1. The experimental conditions for using hydrogen sulfide as a reagent were optimized**

Sulfide is a reactive molecule, and numerous methods for the determination of sulfide concentrations are known from the literature. Because of its exceptional reactivity, the preparation, storage and handling of sulfide stock solutions are far from being obvious.

##### ***1.1 We explored why orders of magnitude different free sulfide concentrations were reported earlier in biological systems.***

Most of the sulfide detection methods are based on irreversible derivatization, evaporation or precipitation of sulfide. These methods and the applied different experimental conditions shift the equilibria between biomolecule-bound and free sulfide, resulting in overestimated free sulfide levels.

##### ***1.2 We demonstrated that all examined, commercially distributed sulfide chemicals contain polysulfide contaminants in significant amounts.***

##### ***1.3 We developed a method that can be used to prepare and store relatively pure and stable sulfide stock solutions, and proposed a protocol for the determination of sulfide concentrations in aqueous solution.***

To avoid polysulfide contaminations, sulfide stock solutions were prepared by washing the surface of  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  crystals at least three times with deionized water followed by dissolution in deionized water under argon gas. Sulfide stock solutions should be stored in the dark, on ice, under argon in saturated threefold deionized water. Working solutions should be made by dilution of these stock solutions with diethylene triamine pentaacetic acid (DTPA) containing buffer immediately before the experiments are conducted. We proposed two methods for concentration determination of sulfide stock solutions. This method allowed estimation of contaminant concentrations.

##### ***1.4 We proved that the use of tris (hydroxymethyl) aminomethane (TRIS) buffer is advantageous over phosphate buffer salt solutions (PBS), because it contains less metal contaminants that can catalyze sulfide oxidation.***

DTPA is used to chelate trace amounts of metal contaminants to decrease their sulfide oxidation catalyzing capacity. Nevertheless, we proved that DTPA is not suitable in itself for stabilization of sulfide solutions.

***1.5 We provided evidence that the decay of sulfide content of stock solutions is primarily due to evaporation.***

Notwithstanding with earlier literature, which suggested that the concentration decay of sulfide stock solutions is the result of oxidation processes by air oxygen, we demonstrated that the sulfide content of different buffered solutions decreases more rapidly by shifting the pH to the more acidic regions. This observation is consistent with evaporation, rather than oxidation being the primary cause of sulfide loss.

**2. Kinetics and mechanisms of the reduction of disulfide species by hydrogen sulfide**

Earlier, several models were proposed for the molecular mechanisms of persulfides formation. A potential pathway for persulfide generation in biological systems was proposed to be via reduction of disulfide species by sulfide. However, this model was questioned in several grounds by a number of investigators. In order to get a deeper insight into the kinetic and thermodynamic properties of these reactions and their potential biological implications, we investigated the reactions of sulfide with DTNB, cystine and oxidized glutathione (GSSG) as model disulfides.

***2.1 We demonstrated that the reaction of sulfide with DTNB is a multistep equilibrium processes.***

We demonstrated that 2 TNB molecules are produced per DTNB at excess of sulfide over DTNB, suggesting that sulfide fully reduced the disulfide to 2-nitro-5-thiobenzoate thiol (TNB-SH). In contrast, one sulfide generates only one TNB at a high excess of DTNB. These observations were interpreted by a model in which 2-nitro-5-thiobenzoate persulfide (TNB-SSH) is generated in the first reaction step, which is subsequently reduced by the remaining sulfide to form TNB and inorganic polysulfide species.

**2.2 Under pseudo-first-order conditions, i.e. by using one of the reactants in excess, we showed that all the kinetic traces in the sulfide – DTNB reaction fit to a single exponential function (Figure 1). Identical pseudo-first-order rate constants were obtained when the reactions were followed by detecting the formation of TNB and the decay of DTNB.**

We determined the apparent second-order rate constant at pH 7.40 for the sulfide – DTNB reaction from the adequate concentration dependencies of the pseudo-first-order rate constants to be  $(8.9 \pm 0.1) \times 10^2 \text{ M}^{-1}\text{s}^{-1}$ . We corroborated that this apparent second-order rate constant indeed corresponds to the direct reaction of DTNB with sulfide which is the initial step in the proposed kinetic model. The results confirm that all subsequent reaction steps are considerably faster.

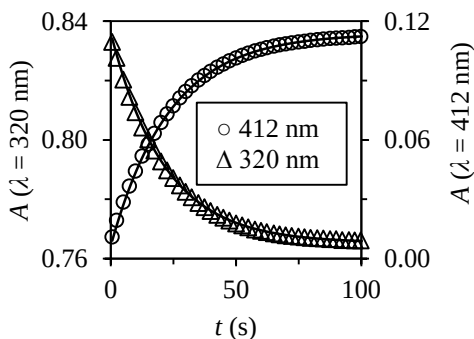
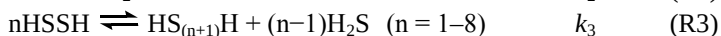
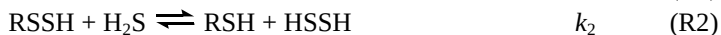


**Figure 1.** Representative stopped-flow kinetic traces ( $\circ$ ,  $\Delta$ ) with corresponding single exponential fits (—) at 320 nm ( $\Delta$ ) and 412 nm ( $\circ$ ).  $[\text{DTNB}] = 5.0 \cdot 10^{-5} \text{ M}$ ,  $[\text{sulfide}]_{\text{tot}} = 5.0 \cdot 10^{-6} \text{ M}$ , 0.10 M phosphate buffer,  $I = 1.00 \text{ M}$ , pH = 7.40,  $T = 25 \text{ }^{\circ}\text{C}$ . ( $k_{\text{obs}}^{320\text{nm}} = (4.68 \pm 0.01) \times 10^{-2} \text{ s}^{-1}$  and  $k_{\text{obs}}^{412\text{nm}} = (4.27 \pm 0.01) \times 10^{-2} \text{ s}^{-1}$ .)

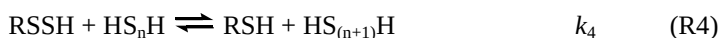
**2.3 We proposed a general kinetic model for the reduction of disulfides by sulfide on the example of DTNB reduction.**

The bimolecular reaction of DTNB with sulfide is the rate determining step in this model (reaction 1), which produces 1 TNB-SSH and 1 TNB molecule. In the second step, TNB-SSH reacts with another sulfide to give TNB and inorganic disulfide (HSSH) (reaction 2). Subsequently, HSSH either disproportionates (reaction 3) or reacts with another TNB-SSH (reaction 4) which eventually yields polysulfide species.

The pH profile of the rate determining step reflects the acid dissociation equilibrium of sulfide, therefore  $\text{HS}^-$  was proposed to be the dominant reactant and the reaction most likely starts with a nucleophilic attack of  $\text{HS}^-$  on DTNB. The pH independent rate constant for this step was calculated to be  $(1.09 \pm 0.01) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ .



and/or



where RSSR, RSSH and RSH stand for disulfides, persulfides and thiols; in this system representing: RSSR = DTNB, RSSH = TNB-SSH, RSH = TNB.

The apparent second-order rate constant for the reaction of TNB-SSH with sulfide was estimated on the basis of kinetic simulations. Simulations only allowed to provide the following range for this value:  $5 \times 10^3 - 5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ .

***2.4 We showed that the reactions of cystine or GSSG with sulfide are described with multistep kinetic models, where the corresponding reduction rates are considerably smaller than in the DTNB-sulfide system.***

According to preliminary spectrophotometric measurements the reactions of cystine and GSSG with sulfide exhibit complex kinetic properties with a characteristic induction period in the kinetic traces suggesting a multi-step reaction mechanism.

***2.5 We demonstrated that the reaction of cystine with sulfide is an equilibrium process with a similar proposed mechanism to the reduction of DTNB by sulfide.***

On the basis of  $^1\text{H}$ -NMR measurements we provided evidence that even at an excess of sulfide the equilibrium is not shifted towards the formation of polysulfide species in the reaction of cystine with sulfide. Cysteine persulfide (Cys-SSH) could be detected upon equilibration of the reaction mixtures, indicating that it is more stable than TNB-SSH. The

equilibrium could be shifted towards the reactant species by adding polysulfide to the reaction mixtures.

***2.6 Our data indicated that the kinetic and thermodynamic properties of the reactions of disulfides with sulfide show large variations, largely depending on the chemical properties of the disulfide. These observations potentiated some sulfide-mediated disulfide reduction reactions in biological systems.***

### **3. Kinetics and mechanisms of the reactions of hydrogen sulfide with amino acid chloramines**

A potential pathway of endogenous polysulfide production is the oxidation of sulfide with ROS. The neutrophil oxidant HOCl was shown to primarily react with protein amin groups to produce chloramine species, which are regarded as secondary oxidizing agents of white blood cells with important roles in the clearance of invading pathogens and in inflammation.

***3.1 We confirmed that polysulfides are the primary products in the reactions of the model chloramines N-chlorotaurine (TauCl) or N-chloroglycine (GlyCl) with an excess of sulfide.***

***3.2 The recorded stopped-flow kinetic traces at excess sulfide over the chloramine derivatives detected 2 reactions. While the rates of the corresponding faster reaction steps were found to be different with TauCl and GlyCl, the slower reaction steps proceeded with similar rates in the two systems.***

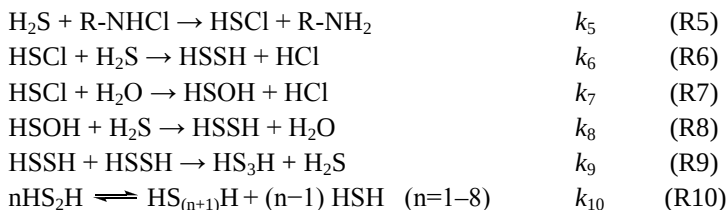
Systematic concentration dependency studies of the rate law revealed that the faster process is first-order for the chloramine and sulfide concentrations. Linear regression analyses of the pseudo-first-order rate constants that were obtained at different sulfide concentrations (at an excess of sulfide) yielded the following apparent second-order rate constants for the faster reactions:  $k_5^{\text{TauCl}} = (5.44 \pm 0.01) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  and  $k_5^{\text{GlyCl}} = (1.13 \pm 0.02) \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ .

The rates of the slower reactions were found to be independent of the sulfide concentration, but their half-lives decreased with the initial concentration of the chloramines. In line with these observations the

apparent second-order rate constants for the second reactions were obtained by fitting this part of the kinetic traces to a second-order kinetic equation:  $k_9^{\text{TauCl}} = (6.5 \pm 0.4) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_9^{\text{GlyCl}} = (5.3 \pm 0.6) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ . Therefore the results strongly suggest that the slower reaction proceeds via a disproportionation reaction of a similar intermediate species that is generated in the reactions of sulfide with TauCl or GlyCl.

### 3.3 We proposed a general kinetic model of the reactions of chloramines with sulfide.

In the model, the first detected reaction corresponds to the bimolecular reactions of the corresponding chloramine species with sulfide. The pH dependency dataset indicated that the deprotonated  $\text{HS}^-$  and the protonated chloramine derivatives are the major reacting species in a wide pH range (reaction 5). The subsequent reaction steps (reaction 6–8) are fast, which is in agreement with literature data on thiol oxidation reactions with chlorinating species. We propose that the slower (under our experimental conditions) detected reaction corresponds to the disproportionation reaction of HSSH eventually yielding the appropriate distribution of polysulfide species and  $\text{H}_2\text{S}$  (reaction 9).



## 4. The reaction of ferryl-Hb derivative with sulfide

Highly reactive ferryl-Hb derivatives (ferryl-Hb derivative,  $\text{Fe}^{4+}\text{Hb}^{++}$ ) are formed in the reactions of the heme groups of human hemoglobin with peroxide species. The structure and chemical properties of these ferryl-Hb derivatives are still not fully understood. Nevertheless, these derivatives cause oxidative stress in atherosclerotic lesions, resulting in complicated atheromas and can lead to rupture as demonstrated by Balla et al. In a collaborative study with Prof József Balla's research group we

found that sulfide is protective against the detrimental properties of oxidized Hb species in atherosclerosis and my work was to investigate the underlying molecular mechanisms of this protective effect.

**4.1 We showed that there is a rapid and significant change in the UV-Vis spectral characteristics of the heme prostetic group upon the reactions of ferryl-Hb species with sulfide.**

Ferryl-Hb derivative was generated in the reaction of metHb with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Ferryl-Hb derivative was reacted with sulfide and the spectral changes in the soret band region of Hb were monitored (Figure 2). An excessive bathochromic shift of the band at 400 nm and the appearance of new bands in the 530 and 580 region were observed upon mixing ferryl-Hb derivative with an excess of sulfide. Based on excessive literature data, the new peak at 620 nm corresponds to the formation of sulfhemoglobin.

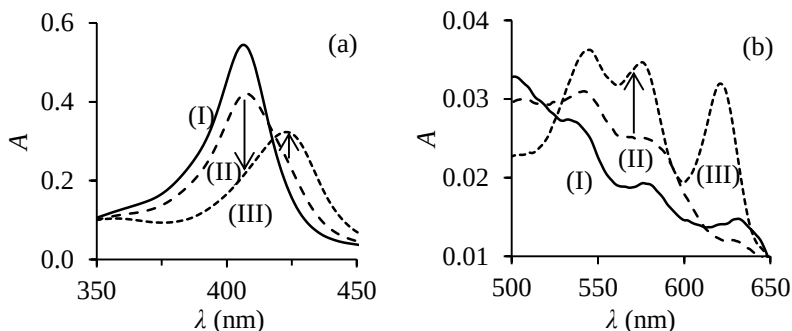


**Figure 2.** UV-Vis spectra of metHb (I), reaction of metHb with  $\text{H}_2\text{O}_2$  after 400 s (II) and the reaction of ferryl-Hb derivatives with sulfide after an additional 60 s (III). (a):  $\lambda = 350\text{--}450\text{ nm}$ , (b):  $\lambda = 500\text{--}650\text{ nm}$ .  $[\text{metHb}] = 4.0 \cdot 10^{-6}\text{ M}$ ,  $[\text{H}_2\text{O}_2] = 8.0 \cdot 10^{-6}\text{ M}$ ,  $[\text{sulfide}]_{\text{tot}} = 1.0 \cdot 10^{-4}\text{ M}$ ,  $2 \cdot 10^{-2}\text{ M}$  phosphate buffer,  $\text{pH} = 7.40$ ,  $T = 25\text{ }^\circ\text{C}$ . (Arrows indicate wavelengths at 406, 425 and 570 nm where the kinetic traces were recorded.)

**4.2 We obtained evidence that the ferryl-Hb derivatives of the heme groups on the alpha and beta chains exhibit different reactivities. In addition, we proved that the same ferryl-Hb derivatives are formed in the reaction of metHb with  $\text{H}_2\text{O}_2$  and in the reaction of oxyHb with  $\text{H}_2\text{O}_2$ .**

The biphasic characters of the kinetic traces that were recorded at 406, 425 and 570 nm for the reactions of ferryl-Hb derivative with sulfide could be described with a double exponential equation providing similar rate constants (within the experimental error) at each wavelength. According to literature results, these reactions represent the different reacting heme groups at the alpha and beta chains of hemoglobin. The obtained pseudo-first-order rate constants for both reactions show linear dependency on the sulfide concentration, resulting the following apparent sulfide independent second-order-rate constants:  $(1.4 \pm 0.1) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  and  $(6.5 \pm 0.2) \times 10^2 \text{ M}^{-1}\text{s}^{-1}$ .

On the basis of literature results, ferryl-Hb derivatives are generated not only by the reaction of metHb with  $\text{H}_2\text{O}_2$ , but also in the reaction of oxyHb with  $\text{H}_2\text{O}_2$  via the following proposed model:



The reactions of the ferryl-Hb derivatives generated in the reactions of oxyHb and sulfide were also investigated at 406, 425 and 570 nm. The measured sulfide concentration dependency and the apparent second-order rate constants were very similar (at all three recorded wavelengths) to the ones that were obtained when metHb was used to generate ferryl-Hb. The calculated values for the apparent second-order rate constants in this systems were:  $(1.7 \pm 0.2) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$  for the faster reaction and  $(7.0 \pm 0.8) \times 10^2 \text{ M}^{-1}\text{s}^{-1}$  for the slower reaction.

#### ***4.3 We demonstrated that the formation of sulfhemoglobin is orders of magnitude faster than the reactions detected below 600 nm.***

The detected reactions at wavelengths over 600 nm are also characterized with two steps that can still be interpreted with the different reactivities of alpha and beta chain heme groups. The rate constants in this region also linearly depend on the sulfide concentration. The following estimates were obtained for the apparent second-order rate constants:  $(1.7 \pm 0.2) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$  and  $(6.5 \pm 0.3) \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ . These values are 2 orders of magnitude faster than the ones that were detected below 600 nm.

**4.4 Preliminary polychromatic UV-Vis titration experiments of ferryl-Hb derivatives with sulfide indicated that the detected reactions below and above 600 nm are not consecutive but parallel reactions. This is an indication that at least two different Ferryl-Hb derivatives are generated in the reactions of Hb with peroxide. The formation of sulfhemoglobin is exclusively due to reactions of the faster reacting ferryl derivatives.**

**4.5 Quantitative analyses based on the corresponding absorbance changes suggested that the two ferryl derivatives are formed at about a 50–50 % ratio.**

#### IV. POSSIBLE APPLICATION OF THE RESULTS

This doctoral work confirmed that optimization of the experimental conditions for using hydrogen sulfide as a reagent is an important prerequisite to obtain reliable results in the fields of hydrogen sulfide chemistry and biology. The work articulated the idea that a sulfide buffer system exists in biological systems, which maintains the concentrations of free sulfide below 1  $\mu\text{M}$  and possibly contributes to sulfide-signaling.

The scientific work provides novel insights into the underlying molecular mechanisms of some of sulfide's biological actions. The obtained kinetic parameters help predicting the biological significance of the studied reactions as well as their products and intermediate species. The results represent important elements of the rigorous chemical approach that is required to reconcile current controversies in sulfide signaling and toxicity.

The thesis provides evidence that sulfide may decrease oxidative stress by reducing highly oxidizing  $\text{Fe}^{4+}$  forms of hemoglobin, which are important deleterious byproducts in complicated atherosclerotic lesions. These results may form the basis of future studies on targeted therapies to develop new pharmaceutical agents for preserving the flexibility of the arteries and enable the prevention of heart attack or stroke.

## V. TUDOMÁNYOS PUBLIKÁCIÓK

### Az értekezés alapját képző közlemények

4. Anita Vasas, István Fábián and Péter Nagy

**Kinetics and mechanism of the reactions of hydrogen sulfide with amino acid chloramines**

Kézirat előkészítés alatt

3. Viktória Jeney, László Potor, Péter Nagy, Emese Tolnai, Anita Vasas, Enikő Balogh, Ágnes Gyetvai, Gábor Méhes, Matthew Whiteman, Mark E. Wood, Sándor Olvasztó, György Balla, József Balla

**Elevated levels of H<sub>2</sub>S inhibit hemoglobin-lipid interactions in atherosclerotic lesions**

*Antioxidants and Redox Signaling*, **2016**, revízió alatt

2. Anita Vasas, Éva Dóka, István Fábián and Péter Nagy

**Kinetic and thermodynamic studies on the disulfide-bond reducing potential of hydrogen sulfide**

*Nitric Oxide – Biology and Chemistry*, **2015**, 46, 93-101

Impakt faktor: 3,521

1. Péter Nagy, Zoltán Pálincás, Attila Nagy, Barna Budai, Imre Tóth, Anita Vasas **Chemical aspects of hydrogen sulfide measurements in physiological samples**

*Biochimica et Biophysica Acta – General Subjects*, **2014**, 1840, 876-891

Impakt faktor: 4,381

### Posztterek

5. Anita Vasas, Éva Dóka István Fábián and Péter Nagy

**Kinetic and thermodynamic studies on the disulfide-bond reducing potential of hydrogen sulfide**

*3rd European Conference on the Biology of Hydrogen Sulfide H<sub>2</sub>S*, 3-6. May 2015., Athens, Greece

4. Péter Nagy, Tobias P. Dick, Romy Greiner, Zoltán Pálinkás, Budai Barna, Anita Vasas, Attila Nagy

**Redox-, coordination- and solution-chemistry of sulfide in relation to some of its biological actions**

*2<sup>nd</sup> European Conference on the Biology of Hydrogen Sulfide*, 8-11. September 2013., University of Exeter, England

3. Anita Vasas, István Fábián and Péter Nagy

**Kinetics and Mechanism of the Reactions of Hydrogen Sulfide with Amino Acid Chloramines**

*Debrecen Colloquium on Inorganic Reaction Mechanisms*, 11-15. June 2013., Debrecen, Hungary

2. Anita Vasas, István Fábián and Péter Nagy

**Kinetics and Mechanism of the Reactions of Hydrogen Sulfide with Amino Acid Chloramines**

*Gordon Research Conferences, Inorganic Reaction Mechanisms*, 3-8. March 2013., Galveston, Texas, USA

1. Anita Vasas, István Fábián and Péter Nagy

**Kinetics and Mechanism of the Reactions of Hydrogen Sulfide with Amino Acid Chloramines**

*First European Conference on the Biology of Hydrogen Sulfide*, 15-18. June 2012., Smolenice, Slovakia

## **Előadások**

4. Vasas Anita, Dóka Éva, Fábián István, Nagy Péter

**A diszulfidok  $H_2S$  általi redukciójának kinetikai és mechanisztikus vizsgálata**

*IV. Interdiszciplinális Doktorandusz Konferencia*, 2015. május 14-15., Pécs, Magyarország

3. Vasas Anita, Fábíán István, Nagy Péter

**A hidrogén-szulfid diszulfidokkal való reakcióinak kinetikája és mechanizmusa**

*MTA Reakciókinetikai és Fotokémiai Munkabizottsági Ülése*, 2014. június 26-27., Siófok, Magyarország

2. Péter Nagy, Zoltán Pálinkás, Attila Nagy, Anita Vasas

**Chemical Aspects of Hydrogen Sulfide Measurements in Physiological Samples**

*WG3 COST Gasotransmitters Meeting*, 18. May 2013., Athens, Greece

1. Vasas Anita, Fábíán István, Nagy Péter

**A hidrogén-szulfid klóraminokkal való reakcióinak kinetikája és mechanizmusa**

*MTA Reakciókinetikai és Fotokémiai Munkabizottsági Ülése*, 2012. október 25-26., Gyöngyöstarján, Magyarország



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Ph.D. List of Publications

Candidate: Anita Varga-Vasas

Neptun ID: F4A6M6

Doctoral School: Doctoral School of Chemistry

### List of publications related to the dissertation

#### Foreign language scientific article(s) in international journal(s) (2)

1. **Vasas, A.**, Dóka, É., Fábán, I., Nagy, P.: Kinetic and thermodynamic studies on the disulfide-bond reducing potential of hydrogen sulfide.

*Nitric Oxide-Biol. Chem.* 46, 93-101, 2015. ISSN: 1089-8603.

DOI: <http://dx.doi.org/10.1016/j.niox.2014.12.003>

IF: 3.521 (2014)

2. Nagy, P., Pálinskás, Z., Nagy, A., Budai, B., Tóth, I., **Vasas, A.**: Chemical aspects of hydrogen sulfide measurements in physiological samples.

*Biochim. Biophys. Acta-Gen. Subj.* 1840 (2), 876-891, 2014. ISSN: 0304-4165.

DOI: <http://dx.doi.org/10.1016/j.bbagen.2013.05.037>

IF: 4.381

**Total IF of journals (all publications): 7,902**

**Total IF of journals (publications related to the dissertation): 7,902**

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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