Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Chemical aspects of hydrogen sulfide measurements in physiological samples ☆

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ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 23 May 2013
Accepted 26 May 2013
Available online xxxx

Keywords:
Hydrogen sulfide
Methylene blue
Monobromobimane
Sulfide selective electrode
Gas chromatography
Fluorescent probe
Protonation equilibrium
Redox chemistry
Coordination chemistry
Blood sulfide pool
Tissue sulfide measurement

ABSTRACT

Background: Owing to recent discoveries of many hydrogen sulfide-mediated physiological processes, sulfide biology is in the focus of scientific research. However, the promiscuous chemical properties of sulfide pose complications for biological studies, which led to accumulation of controversial observations in the literature.
Scope of review: We intend to provide an overview of fundamental thermodynamic and kinetic features of sulfide redox- and coordination-chemical reactions and protonation equilibria in relation to its biological functions. In light of these chemical properties we review the strengths and limitations of the most commonly used sulfide detection methods and recently developed fluorescent probes. We also give a personal perspective on blood and tissue sulfide measurements based on proposed biomolecule–sulfide interactions and point out important chemical aspects of handling sulfide reagent solutions.
Major conclusions: The diverse chemistries of sulfide detection methods resulted in orders of magnitude differences in measured physiological sulfide levels. Investigations that were aimed to dissect the underlying molecular reasons responsible for these controversies made the important recognition that there are large sulfide reserves in biological systems. These sulfide pools are tightly regulated in a dynamic manner and they are likely to play a major role in regulation of endogenous-sulfide-mediated biological functions and avoiding toxic side effects.
General significance: Working with sulfide is challenging, because it requires considerable amounts of chemical knowledge to adequately handle reagent sulfide solutions and interpret biological observations. Therefore, we propose that a rigorous chemical approach could aid the reconciliation of the increasing number of controversies in sulfide biology. This article is part of a Special Issue entitled Current methods to study reactive oxygen species — pros and cons.

1. Introduction

The discoveries that hydrogen sulfide[1] is produced endogenously [1] and that it is a potential neuromodulator [2] introduced a new era for sulfide biology, with exponentially increasing attention to its in vivo actions [3–9]. It is generated in virtually all studied organs during transsulfuration processes by *cystathionine-γ-lyase* (CSE) and *cystathionine-β-synthase* (CBS) [10] and via *3-mercaptopyruvate sulfurtransferase*-mediated (3MST) cysteine metabolism (see Scheme 1) [11]. On the other hand, sulfide catabolism is not well understood, but a major role for mitochondrial oxidation pathways is reported [12,13]. It is now well documented that sulfide is a modulator of pivotal physiological and pathophysiological functions in the gastrointestinal tract [14], brain [3], kidney [15] and vasculature [4] and its role is emerging in other organs too. Its physiological actions include regulation of inflammation [16–18], blood pressure [19], metabolic syndrome [20], energy production [21] and oxidative stress [5,22,23].

However, the promiscuous chemical properties of sulfide make it difficult to measure its physiological concentrations and to handle it as a reagent [24–26]. This resulted in huge discrepancies in reported sulfide levels in virtually all studied tissues and physiological fluids (see Tables S1 and S2) as well as in its biological functions. Therefore, major efforts are devoted to explain the increasing number of controversies that are accumulating in the sulfide literature. It is now accepted that significant amounts of "persulfide", "acid labile" and "alkaline labile" sulfide pools are available in biological systems [24–28]. In addition,

Abbreviations: 3MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine beta-synthase; CCO, cytochrome c oxidase; CSE, cystathionine gamma-lyase; Cys, L-cysteine; DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); DTPA, diethylenetriamine-pentaacetic-acid; DTT, D,L-dithiotreitol; GC, gas chromatography; GSH, glutathione; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid; HSOH, sulfenic acid; MB, methylene blue method; MBB, monobromobimane method; PBS, phosphate buffered saline; RSOH, sulfenic acid derivative; RSSH, persulfide; ROS, reactive oxygen species; SDB, sulfide dibimane; TRIS, tris(hydroxymethyl)aminomethane

☆ This article is part of a Special Issue entitled Current methods to study reactive oxygen species — pros and cons.

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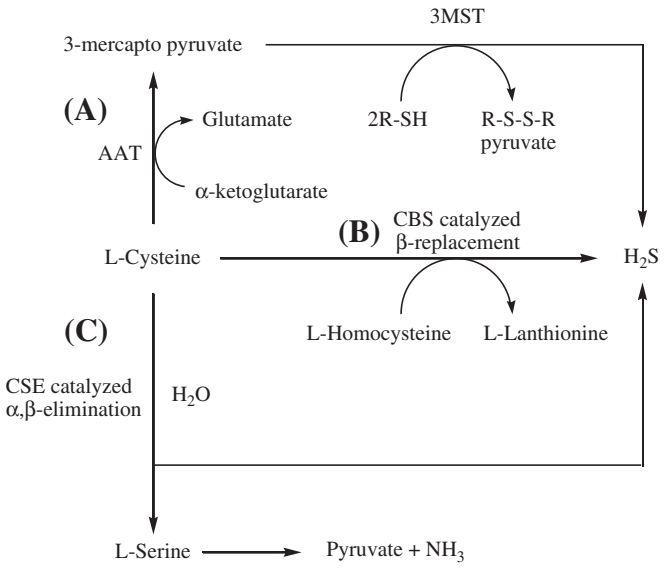
[1] From now on we will use the term sulfide to refer to the sum of its different protonated forms that exist in solution, i.e. H_2S, HS^- and S^{2-}.

0304-4165/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbagen.2013.05.037



Scheme 1. Proposed pathways for sulfide generation by cysteine metabolism via three different enzyme catalyzed pathways. (a) Aspartate/cysteine aminotransferase (AAT) catalyzes the transamination reaction between cysteine and α-ketoglutarate to form 3-mercaptopyruvate, from which the sulfur is transferred to an active site Cys residue of 3-mercaptopyruvate sulfurtransferase (3MST) to give a persulfide derivative. 3MST persulfide is reduced by thiols to give sulfide. (b) Among the sulfide producing catalytic reactions of cystathionine β-synthase (CBS), β-replacement between cysteine and homocysteine was proposed to be the most kinetically favorable under physiological conditions. (c) Sulfide production via cysteine metabolism by cystathionine γ-lyase (CSE) is most efficient by an α,β-elimination reaction, which generates pyruvate and ammonia (via serine) beside sulfide.

physiological sulfide concentrations were determined with a plethora of different techniques, (reviewed in [23–25,27,29]), which operate under very different experimental conditions and therefore liberate sulfide from these pools with different efficacies. Furthermore, polysulfides (that are the dominant sulfide oxidation products in aqueous solutions) are reported to be responsible for some of the observed biological actions of sulfide that are governed via protein sulfhydration reactions [30,31]. Although these recognitions provide some explanations, in order to adequately reconcile controversial biological observations, a better understanding of the chemical properties of sulfide is needed.

In this review we discuss the methodologies that are most frequently used to measure physiological sulfide levels and provide a summary of recently developed fluorescent probes from a rigorous chemical perspective. In addition, we discuss the chemical reactions of sulfide that are most likely to play important roles in its detection and biological actions and give practical advice on how to handle reagent sulfide solutions.

2. Solution chemistry of sulfide

Sulfur is a chalcogen element in group 16 of the periodic table, positioned right below oxygen with an electron configuration of $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^4$. This configuration corresponds to 6 valence electrons and a vacant 3d orbital, which is the reason why sulfur can obtain oxidation states anywhere between −2 to +6. The oxidation state of the sulfide sulfur is −2 and therefore it is a reductant species that cannot be reduced further. The structure of H_2S is similar to that of H_2O, but the two molecules have very different chemical and physical properties. H_2S does not form H-bonds, therefore it is a gas at ambient conditions, is toxic at relatively high concentrations and has a distinct odor. H_2S is heavier than air and dissolves readily in water (solubility ~100 mM at 25 °C) [32]. Due to the strong nucleophilic character of its sulfur center sulfide engages in many different chemical reactions. The most well studied reactions that have already been shown (or proposed) to be important in its biological actions are: 1) reduction of reactive oxygen species (ROS) and disulfide bonds and 2) coordination to metal centers. In addition, its role in electrophile sulfhydration via nucleophilic addition is emerging [33].

2.1. Protonation equilibria

Sulfide solutions are mostly prepared via dissolving sulfide salts or via bubbling H_2S gas into aqueous media. Different sulfide salts of heavy metals with soft characters e.g. PbS and Ag_2S are sparingly soluble (for example at pH 7 solubility of HS^- in 1 mM Pb^{2+} is 6×10^{-20} M and of Ag_2S on an electrode surface is 6×10^{-15} M, respectively), while NaHS and Na_2S are very soluble in water. Therefore, the latter two are often used in biological studies to make reagent sulfide solutions. The aqueous solutions that are made by dissolving these salts are often called H_2S donors and some investigators even measured the rate of sulfide release by these molecules (in Ref. [34] a slow sulfide release was suggested, but Refs. [35,36] showed that H_2S forms upon crystal dissolution). From a chemical perspective the dissolution of these salts is accompanied by dissociation to give solvated Na^+ and HS^- or S^{2-} ions (with solvation shells that may consist of several layers of water molecules) and therefore this is the actual process that introduces sulfide into the solution. These anions are Brönsted bases (S^{2-} is an especially strong one) therefore, upon dissolution acid base reactions with water (e.g. $S^{2-} + H_2O \rightleftharpoons HS^- + OH^-$) take place, which can shift the pH of (even buffered) aqueous solutions. However, under similar conditions (pH, temperature, pressure, etc.), bubbling pure H_2S gas or dissolving high purity sulfide salts in well buffered aqueous solutions results in a similar distribution of HS^- and H_2S. On the other hand, partitioning of sulfide in its different protonation states (i.e. speciation) strongly depends on the applied conditions (especially on the pH). In water solution of sulfide the following protonation equilibria exist:

$$H_2S \rightleftharpoons HS^- + H^+ \quad pK_a^{H_2S} = 7.05 [37] \tag{1}$$

$$HS^- \rightleftharpoons S^{2-} + H^+ \quad pK_a^{HS-} > 15 \tag{2}$$

$$K_a^{H_2S} = [HS^-][H^+]/[H_2S] \tag{3}$$

$$K_a^{HS-} = [S^{2-}][H^+]/[HS^-]. \tag{4}$$

The above equations (Eqs. (1)–(4)) determine the relative ratios of its protonation forms (might be called protonation isomers) at the actual pH, where the pK_a values change with temperature, pressure and ionic strength (the ionic strength is determined by the total concentrations of solvated ions including the buffer). Several values were reported for pK_a^{HS-} in the literature in the range of $13 > pK_a^{HS-} > 19$. However, it is challenging (if not practically impossible) to measure pK_a values in this range, because the concentration of OH^- is already 1 M at pH 14. Fig. 1 shows the speciation of sulfide as a function of pH using $pK_a^{H_2S} = 7.05$ and $pK_a^{HS-} = 15$. Under these conditions at pH 7.4 the percent distribution of H_2S:HS^-:S^{2-} will be 30:70:0.000002, respectively. Although, this shows that the equilibrium concentration of S^{2-} under physiological conditions is very low, it does not necessarily mean that it cannot be the actual reactive species (for an example see the detection of sulfide with sulfide selective electrodes, Section 3.3).

Due to the shift in $pK_a^{H_2S}$ by increasing the temperature from 20 °C to 37 °C, it has been demonstrated that the estimated amount of dissolved H_2S drops by as much as 30% (and the concentration of HS^- increases

Please cite this article as: P. Nagy, et al., Chemical aspects of hydrogen sulfide measurements in physiological samples, Biochimica et Biophysica Acta (2013), http://dx.doi.org/10.1016/j.bbagen.2013.05.037