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Preface

Analysis of thiols

Thiols (RSH) have been of continuing interest for many years because of their important role in several biological processes. Biological (amino)thiols are products of sulfur metabolism. They are ubiquitous and have been extensively studied in various biological systems. Accurate and interference-free measurement of thiols and their metabolites in biological fluids and tissues is of great biological, clinical and pharmacological importance.

Chemically, thiols (RSH), i.e., thioalcohols, are sulfhydryl groups (–SH) containing compounds. Traditionally, thiols are often referred to as mercaptans. Unless masked in proteins, the sulfhydryl group is the most reactive chemical functionality in cells, as it undergoes almost all known chemical reactions which are of immense biological interest: substitution of the SH group, addition, elimination and oxidation to produce S–C, S–metal, S–S or S–O bonds, respectively. The biological activity of thiols largely depends upon the readily oxidizability of the SH group due to numerous oxidation states in which the element sulfur may exist. This behavior distinguishes thiols from other biomolecules with functional groups of comparable nucleophilicity. Because sulfenic and sulfinic acids are unstable and oxidize further, these substances are intermediates of particular importance.

The most peculiar property of the SH group, which determines both its chemistry and biology, is the readily oxidizability and reactivity, unlike disulfides that are much less reactive than thiols. Nevertheless, disulfides contribute to the protein's tertiary structure when cysteine residues are part of the same polypeptide chain, or they contribute to the quaternary structure of multi-unit proteins when cysteine residues belong to different peptide chains. Disulfides are resistant to further oxidation and, under mild oxidizing conditions, they are the major, if not the only detectable product of thiol oxidation. In vivo disulfides exist in several forms, which can be categorized into high molecular mass (HMM) and low molecular mass (LMM) disulfides. HMM disulfides also include mixed disulfides with LMM thiols such as GSH, whereas LMM disulfides are constituted of two LMM thiols (Fig. 1).

LMM thiols, notably cysteine (Cys), cysteinylglycine (CysGly), glutathione (γ-L-glutamyl-L-cysteinylglycine; GSH), homocysteine (Hcy) and their symmetrical and asymmetrical disulfides, play numerous important roles in metabolism and homeostasis. These (amino)thiols act as part of an antioxidant defense network and protect biological systems against oxidative injury. Thus, altered thiol concentrations in different tissues and conditions have been linked to particular pathologic conditions. Importantly, there is increasing evidence of the involvement of thiols in metabolic regulation, signal transduction and regulation of gene expression.

Thiols are metabolically closely related. One of the best known of LMM thiols is GSH. It is the major non-protein thiol which is present in millimolar concentrations within cells, but at much lower concentrations in the extracellular space such as in plasma. GSH acts both as a redox-buffer to prevent oxidative damage and as a detoxifying molecule against endogenous and exogenous electrophiles. Also, by acting as a free radical scavenger, GSH helps in regenerating other antioxidants, e.g., vitamin E and C. GSH is also responsible for the maintenance of protein thiol status within cells. Hcy is an endogenous thiol generated by the demethylation of methionine, an essential amino acid derived from dietary proteins; more than 80% of Hcy is found in plasma. Once formed, Hcy is either irreversibly converted by transsulfuration to Cys or it is remethylated to methionine. Cys and CysGly are precursors of GSH biosynthesis, and CysGly is its catabolite. In particular, Cys is a critical substrate for protein synthesis and the rate-limiting precursor of GSH and taurine synthesis. Interestingly, both Cys and Hcy can act as substrates for the enzymes cystathionine β-synthase and cystathionine γ-lyase to product hydrogen sulphide (H_2S) [1]. This gaseous molecule elicits an array of physiological effects including modulation of blood pressure. The interest of the scientific community in this transmitter molecule is exponentially rising. LMM and HMM thiols are thought to be involved in the transport and storage of nitric oxide (NO) bioactivity.

Several studies have demonstrated that measurement of aminothiol concentrations in biological fluids has emerged as a useful tool for diagnosing and monitoring the presence of human diseases and metabolic disorders related to oxidative stress. Total Hcy and Cys concentrations in biological fluids are also routinely used in the clinical diagnosis of genetic and metabolic diseases.

To elucidate the function of these important amino acids in basic and clinical research, their unequivocal identification and accurate determination in plasma and urine is essential. Because of their extensive inter-conversion between thiols, the simultaneous analysis of thiols and their numerous metabolites in biological samples is very meaningful. This need has generated various analytical assays for aminothiols detection and quantification in research and clinical settings (see for instance Ref. [2]).

There is a bewildering variety of published methods for the determination of thiols and their reaction products in biological fluids and tissues. If this on the one hand reflects the great biological interest in these groups, on the other hand it makes the reported data difficult to interpret. As a matter of fact, the results from one method often do not agree with those obtained from another method [3]. To understand this discrepancy, one must bear in mind that reliable determination of thiols, disulfides and their

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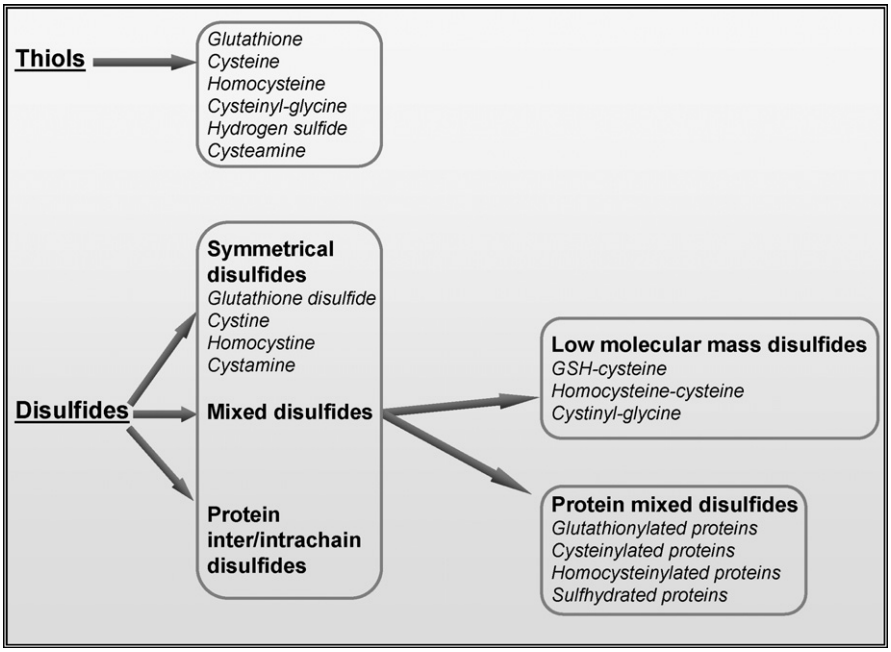

Fig. 1. Main physiological thiols and their disulfides.

reactions products in relevant biological systems is not an easy task from the analytical perspective The following reasons are worth mentioning: (i) Thiols and disulfides have not chromophores and derivatization is an indispensable step in most analytical methods. Derivatization reagents used for their analysis in such methods may react incompletely and variably, may generate by-products including those from the reaction with other electrophilic groups. These circumstances may finally generate additional interfering signals. (ii) Within cells thiols occur at several orders of magnitude higher than their disulfides, whereas extracellular thiol concentrations are conversely rather low. Therefore, lysis of cells such as erythrocytes or incomplete removal of the extracellular milieu can affect thiol and/or disulfide analysis. (iii) Thiols can be easily oxidized during sample manipulation. (iv) Some of the thiols of interest in the biological matrix considered (e.g., GSH in the urine or in plasma, Hcy in blood, Cys in tissues, H_2S in blood and other tissues) may occur at nM to low μM concentrations; this makes their determination somewhat difficult, being their levels close to the LOQ of the applied method. As a whole, analysis of thiols is a challenge even for skilled analysts and analytically oriented biochemists.

Determination of thiols and disulfides is commonly achieved by effective separation techniques of proper derivatives by HPLC and CE in conjunction with various detection methods. Measurement of disulfides in most analytical methods is indirect, because of lack of structural features suitable for use of conventional detectors, and involves reduction of the disulfide bond and derivatization of the SH group. When disulfides are detected, blocking of the SH group is required for a specific analysis. A frequently used approach is fluorescence measurement which requires pre- or post-column derivatization of the SH group with fluorophore. However, there is a number of SH-reactive reagents commercially available that allow UV–vis absorbance detection. Also there are few methods that are able to measure thiols without any derivatization.

This special issue of Journal of Chromatography B presents a series of key reviews and original research articles on the quantitative analysis of thiols and their reaction products in biological matrices. The Guest Editors have tried to select among leading scientists in this area who provided in recent years a plethora of analytical methods and techniques to measure almost the whole thiol family, i.e., both LMM and HMM thiols in relevant biological matrices, notably blood and urine, applying wide a spectrum of analytical techniques such as HPLC, CE, GC, GC–MS and GC–MS/MS, and more recently LC–MS and LC–MS/MS.

In the review articles, the authors focused their attention to analytical and pre-analytical issues associated with measurement of the most important physiological thiols and disulfides. Particular attention was paid to analytical shortcomings, which are generally accepted to be responsible for the great discrepancy with regard to the physiological concentrations reported in the literature so far. In the present special issue, recent advances in analytical technologies and in automation, aiming at developing validated and proven analytical methods for thiols suited for use in basic and clinical research, were also presented and discussed. Reliable analysis of thiols in biological samples is challenging. However, the articles published in this special issue let draw the conclusion that it is possible to develop analytical methods of analytically acceptable accuracy and precision and with the potential for high-throughput analysis. We hope that the papers collected in this special issue of Journal of Chromatography will inform the reader and stimulate further work in this area of research.

We wish to sincerely thank all the authors for their contributions, the colleagues who are experts in the field and served as anonymous referees of the papers, the Editors of the Journal of Chromatography B for having given us the opportunity to edit this special issue, as well as people at the Editorial Office, particularly Mr. Eduard Hovens, for invaluable help. Finally, we would like to express our sincere gratitude to the Thematic Volume Editor, Dr. Dimitrios Tsikas, for his precious and fundamental support during all the phases of the preparation of this special issue.

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