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Review

Stabilising cysteinyl thiol oxidation and nitrosation for proteomic analysis☆

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ABSTRACT

Oxidation and S-nitrosylation of cysteinyl thiols (Cys-SH) to sulfenic (Cys-SOH), sulfinic (Cys-SO_2H), sulfonic acids (Cys-SO_3H), disulphides and S-nitrosothiols are suggested as important post-translational modifications that can activate or deactivate the function of many proteins. Non-enzymatic post-translational modifications to cysteinyl thiols have been implicated in a wide variety of physiological and pathophysiological states but have been difficult to monitor in a physiological setting because of a lack of experimental tools.

The purpose of this review is to bring together the approaches that have been developed for stably trapping cysteine either in its reduced or oxidised forms for enrichment and or subsequent mass spectrometric analysis. These tools are providing insight into potential targets for post-translational modifications to cysteine modification in vivo.

This article is part of a Special Issue entitled: Special Issue: Posttranslational Protein modifications in biology and Medicine.

Contents

Abbreviations: BSO, buthionine sulfoximine; BST, biotin switch technique; Cys-SOH, cysteine sulphenic acid; DCP, 3-(2,4-dioxocyclohexyl) propyl; DTNB, dithionitrobisbenzoic acid; DTT, dithiothreitol; Grx, glutaredoxin; GSH, glutathione; GSR, glutathione reductase; GSSG, glutathione disulphide; HNE, hydroxynonenal; IAA, iodoacetic acid; IAM, iodoacetamide; ICAT, isotope-coded affinity tag; IEF, isoelectric focussing; NEM, N-ethyl maleimide; NO, nitric oxide; PEG, polyethylene glycol; ROS, reactive oxygen species; SMase, sphingomyelinase; SNO, nitrosothiol; Trx, thioredoxin; TrxR, thioredoxin reductase.

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1. Introduction

Cysteine is present less frequently than expected based on random chance within protein sequences, typically constituting around 1% of the total number of amino acids. However, it is more prevalent around active sites of proteins and is highly reactive, suggesting an important biological function [1]. Early studies ascribed a key role for cysteine in protein structure via coordinated, enzyme-catalysed disulphide bond formation. More recently, in addition to enzyme catalysed reactions e.g., prenylation and palmitoylation, a number of other non-enzymatic modifications of cysteine have been reported including acetylation [2], oxidation [3], nitrosylation [4], glutathiolation [5] and Michael additions of oxidised lipids [6]. The improvements in sensitivity of proteomic analyses provide a route to characterise the sites and nature of modifications that are observed. Cysteine's reactivity poses many challenges in analysis of its non-enzymatic modifications as many of them are labile and modifiable under different redox conditions. Therefore the cysteine moiety must be effectively trapped in whichever form it is found biologically, before the system is disrupted for analysis. This has been hampered in the past by a lack of available tools to detect oxidised moieties. The present review focuses on tools that are now available to support the analysis of protein-bound cysteine oxidative modifications and their application to analysis of the redoxome.

2. The biological importance of cysteine

Cysteine residues on proteins play key roles in catalysis and regulation see Fig. 1 adapted from [7]. Indeed, cysteine and arginine mutations contribute to a greater number of genetic diseases than any of the other amino acids [8]. This latter observation was linked to the importance of cysteine in disulphide bond formation and protein stability where cysteinyl mutants can exhibit aberrant structure and function. There are now many examples of thiol-dependent redox regulation of protein function including cell signalling (Fig. 1) and transcription factor activity (Table 1) such as nucleophilic and redox catalysis (e.g., Trx), allosteric regulation (e.g., PTP1B) independent of structural disulphide bond formation (IgG) or metal binding (metallothionen). For such regulatory mechanisms to be effective within and beyond cells there is an absolute requirement for redox homeostasis.

3. The need for and maintenance of cellular redox balance

Endogenous cellular antioxidants inactivate oxidising free radicals and protect aerobic cells from oxidant injury. Cellular antioxidant systems have the capacity to buffer intracellular redox potential by reversible oxidation of redox cysteine residues on proteins such as glutaredoxin (Grx) and thioredoxin

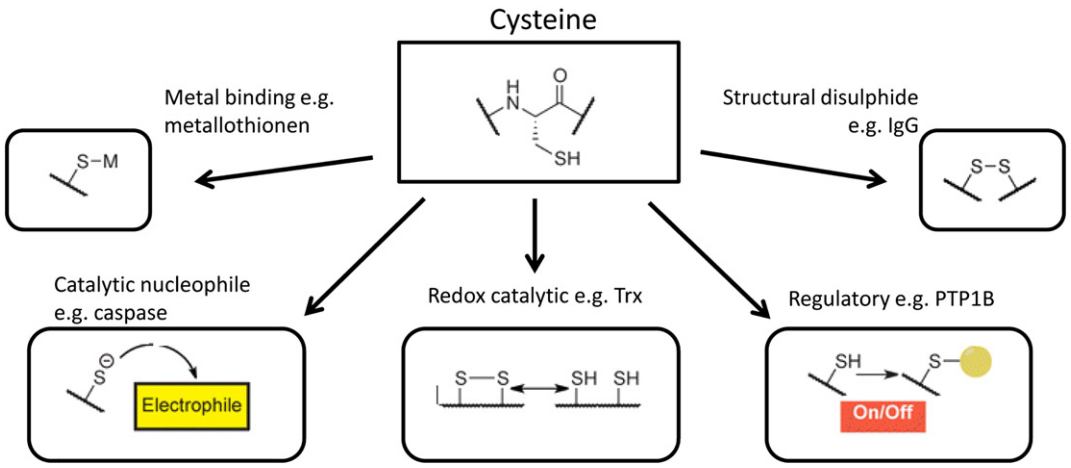

Fig. 1 – Common cysteine-thiol reactions in biological systems (adapted from Pace et al., [7]); thioredoxin (Trx), PTP1B (protein tyrosine phosphatase 1B), IgG (immunoglobulin G).