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Comparison of analytical methods using enzymatic activity, immunoaffinity and selenium-specific mass spectrometric detection for the quantitation of glutathione peroxidase 1

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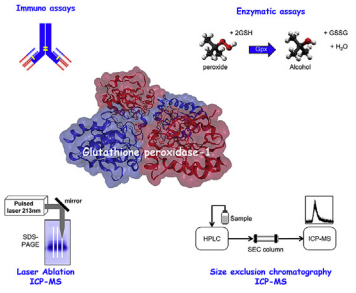
HIGHLIGHTS

- 'Selenium status' can be evaluated by measuring glutathione peroxidase-1 (Gpx1).
- Four major types of analytical methodologies to measure Gpx1 were revisited.
- Enzymatic assay, immunodetection andICP MS analyses of Gpx1 are complementary strategies to evaluate 'selenium status'.

GRAPHICAL ABSTRACT



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ABSTRACT

Glutathione peroxidase 1 (Gpx1), one of the most responsive selenoproteins to the variation of selenium concentration, is often used to evaluate "selenium status" at a cellular or organismal level. The four major types of analytical methodologies to quantify Gpx1 were revisited. They include (*i*) an enzymatic assay, (*ii, iii*) polyacrylamide gel electrophoresis (PAGE) with (*ii*) western blot detection of protein or (*iii*) inductively coupled plasma mass spectrometry (ICP MS) detection of selenium, and (*iv*) size-exclusion chromatography with ICP MS detection. Each of the four methods was optimized for the quantification of Gpx1 with maximum sensitivity. The methods based on the enzymatic and immunodetection offer a much higher sensitivity but their accuracy is compromised by the limited selectivity and limited dynamic range. The advantages, drawbacks and sources of error of each technique are critically discussed and the need for the cross-validation of the results using the different techniques to assure the quality assurance of quantitative analysis is emphasized.

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Abbreviations

Ar	argon
GF-AAS	graphite furnace atomic absorption spectrometry
Gpx1	glutathione peroxidase 1
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
H_2O_2	hydrogen hydroperoxide
HPLC	high-performance liquid chromatography
HRP	Horseradish peroxidase
ICP MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ICP-QQQ-MS	inductively coupled plasma-triple quadrupole-mass spectrometry
IEF	isoelectric focusing
IPG strips	immobilized pH gradient strips
LA-ICP MS	laser ablation-inductively coupled plasma mass spectrometry
LOD	limits of detection
MES	2-(N-morpholino)ethanesulfonic acid
NADPH	β-nicotinamide adenine dinucleotide 2′-phosphate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Se	selenium
Sec	selenocysteine
SEC	size exclusion chromatography
SQ-ICP MS	single quadrupole inductively coupled plasma-mass spectrometry
t-BHP	*tert*-butyl hydroperoxide

1. Introduction

Selenoproteins constitute an essential family of enzymes, in which selenium, in the form of selenocysteine, is critical for the catalytic or redox reactions [1,2]. To date, 25 selenoprotein genes have been identified in human genome (24 in rodents), and half of them have been functionally characterized in antioxidant defense, redox homeostasis, and redox signaling [3–6].

Glutathione peroxidase 1 (Gpx1), the first selenoprotein discovered in mammals, has been the most studied [7]. Gpx1 is the most abundant selenoprotein and is found in cytosol, mitochondria, and, in some cells, in peroxisome intracellular compartments. Gpx1 prevents the accumulation of H_2O_2 in various physiological and pathophysiological conditions, using GSH as a cofactor. This selenoenzyme is also able to reduce lipid hydroperoxides and other soluble hydroperoxides (such as *tert*-butyl hydroperoxide or cumene hydroperoxide). Ubiquitously expressed in animals, Gpx1 has been purified from erythrocytes and is now one of the two commercially available selenoproteins together with the thioredoxin reductases. Gpx1 forms a homo-tetramer of approximately 88 kDa (22 kDa for the monomer) and has a single selenocysteine residue in the catalytic site. In mammals, Gpx1 is part of the glutathione peroxidase family composed of eight members (Gpx1-Gpx8), half of which are selenoproteins (Gpx1-Gpx4; Gpx6 in humans [3]), the others having a catalytic cysteine residue instead of a selenocysteine. Gpxs have critical antioxidant functions in different cell types, intracellular compartments or in the plasma. They also have partially overlapping substrate specificities, as reviewed elsewhere [7]. The function and substrate specificity of Gpxs are often driven by the cellular localization.

The organism's selenium status has to be considered carefully, since the beneficial window of this element is narrow. Selenoprotein expression is highly dependent on selenium levels from the diet and is also affected by pathophysiological conditions [4,8–10]. Gpx1 is one of the most sensitive selenoproteins to selenium level variations [8,9,11,12], and is therefore commonly used as a marker of selenium status in epidemiological studies. Therefore, various analytical methodologies based on the different properties of Gpx1 were developed to quantify this selenoenzyme.

Historically (1967), it is the enzymatic activity of GPx1 that was proposed for its detection in blood samples [13]. Since Gpx1 is able to reduce a wide variety of hydroperoxides (ROOH) to corresponding alcohols (ROH) and water using cellular glutathione as the reducing reagent. Several substrates were used for *in vitro* enzymatic assays, including H_2O_2, *tert*-butyl hydroperoxide and cumene hydroperoxides. These enzymatic assays allowed the routine quantification of Gpx1 in many biological fluids and cellular extracts [8,9,11,12,14].

Alternatively, immunodetection of Gpx1 was widely developed, mostly in biomedical research. It is based on the separation of the Gpx1 from other proteins by gel electrophoresis and the blotting onto the membrane containing a labelled antibody. After the washing of the excess of the antibody, the Gpx1-antibody complex is detected by chemiluminescence with a secondary antibody coupled with horseradish peroxidase (HRP). When a luminol substrate is present, HRP produces blue light that is quantitatively related to the amount of Gpx1-antibody complex. This strategy allowed the relative evaluation of Gpx1 levels in many samples from various organisms [8,9,11,12,14].

The recognition of the importance of selenium in the activity of Gpx1 resulted in the use of inductively coupled plasma mass spectrometric detection (ICP MS) to quantify the Gpx1 separated from other selenoproteins by gel electrophoresis (SDS or IEF) [16,17] or size-exclusion chromatography [15,16] using the intensity of the selenium signal.

The purpose of this study was to optimize the above methods, i.e. (i) enzymatic assay, SDS-gel electrophoresis with (ii) immunoblotting and with (iii) laser ablation – ICP MS detection, and (iv) size-exclusion chromatography – ICP MS for the quantification of selenium in the same preparation of Gpx1 with maximum sensitivity and discuss critically their advantages and limitations.

2. Materials and methods

2.1. Materials

Glutathione peroxidase (Gpx) from bovine erythrocytes, glutathione reductase (GR), L-glutathione reduced (L-GSH), *tert*-butyl hydroperoxide (t-BHP), and β-nicotinamide adenine dinucleotide 2-phosphate reduced (NADPH), were purchased from Sigma-Aldrich. Antibodies were purchased from Abcam (Gpx1, #ab108429) and Sigma (anti-Rabbit IgG (whole molecule) produced in goat coupled with horseradish peroxidase, #A6154); NuPAGE 4-12% bis–Tris polyacrylamide gels, MES SDS running buffer were purchased from Life Technologies.

2.2. GPx enzymatic assay

Gpx activity was measured in an enzymatic-coupled assay as described elsewhere [13]. The reaction mixture was composed of