J Biochem Tech (2010) 2(5):S9-S10
ISSN: 0974-2328

Determination of GSH and GSSG in blood plasma of patients by HPLC-ED

Ondrej Zitka, Natalia Cernei, Vojtech Adam, Ales Horna, Rene Kizek*

Received: 25 October 2010 / Received in revised form: 13 August 2011, Accepted: 25 August 2011, Published: 25 October 2011

Abstract

Thanks to many chemicals and physical stresses generated by outside environment there still is unpredictable danger of damaging of organism by reactive oxygen species (ROS). Biological harmfulness of ROS is generally given by the subsequent oxidation of essential cellular structures. Organism is defending against ROS by synthesis of small molecules which can neutralize radicals. The most suitable existing organic molecule widespread in whole organism which can decrease effect of ROS is glutathione (GSH). Therefore we interested in changes of GSH levels by oncological patients. We optimized fast and robust method for analysis blood plasma of patients by employment of HPLC with coulometric detection. Our results based on hunderts of analyzed samples shows significant oxidation damage by disbalance between GSH/GSSG ratio.

Keywords: reactive oxygen species, glutathione, HPLC with electrochemical detection.

Introduction

If oxidative stress in form of ROS is occuring in the organism mechanisms which can stabilize homeostasis are employed. One of well described mechanism is stabilisation of homeostasis by synthesis of tripeptide glutathione (GSH) which consists of aminoacids glutamine, cysteine and glycine (Obr. 1). It can be find in tissues and body liquids of animals and also in plants. Neutralization of ROS by GSH is possible by cascade of reactions which are called as Glutathione-acorbate cycle (Noctor and Foyer 1998). On the end of cycle oxidized form of glutathione (GSSG) and reduced form of previous danger radical are produced. Therefore observing of concentration levels between both forms of glutathione GSH and GSSG might be very useful for examination of state of oxidation damaging of whole organism. Concentration of GSH and GSSG in real samples as blood or tobacco cells extract have to be analyzed by various methods as Capilary electrophoresis with fluorescenc detection after selective derivatization (Maeso et al. 2005; Zhang et al. 2005). This kind of approach is very sensitive especially if laser induced florescence (LIF) detector is used. Moreover if direct detection after separation step must be preferred electrochemical detector is the most sensitive an selective (Hiraku et al., 2002). Both methods of detection LIF for electrophoresis or electrochemical detection by HPLC has unlimited benefits in field of miniaturization in the near future. Anyway gluthation is are reactive compound which free level in biological matrix could be strongly affected by bonding to other proteins (Fratelli et al. 2002) and thus this problematic is firther disputed (Rossi et al. 2002).

Ondrej Zitka, Natalia Cernei, Vojtech Adam, Rene Kizek*

Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

Ales Horna

Radanal Ltd., Okruzni 613, CZ-530 03, Pardubice, Czech Republic

*Tel: +420 545 133 350, Fax: +420 545 212 044
E-mail: kizek@sci.muni.cz

Materials and Methods

Aim of our work was to optimize method which might be useful for determination of GSH and GSSG. These compounds of interests are involved in gluthation-ascorbate cycle and thus we asume that could be good representing in determination of oxidative damage of organism.

We analyzed more than 7 hundreds patients blood plasma. Samples of blood serum of cancer patients were denatured in 5% trifluoroacetic acid and after centryfugation supernatant were injected in chromatographic system. For purpose of separation and detection of GSH and GSSG gradient chromatographic system was employed. System consisted of two chromatographic pumps, autosampler and multichannel coulometric electrochemical detector Coularray.

HPLC-ED system consists of two chromatographic pumps Model 582 ESA (ESA Inc., Chelmsford, MA) (working range 0.001-9.999 ml min^{-1}) and chromatographic column with reverse phase Zorbax eclipse AAA C18 (150 × 4.6; 3,5 µm particles, Agilent

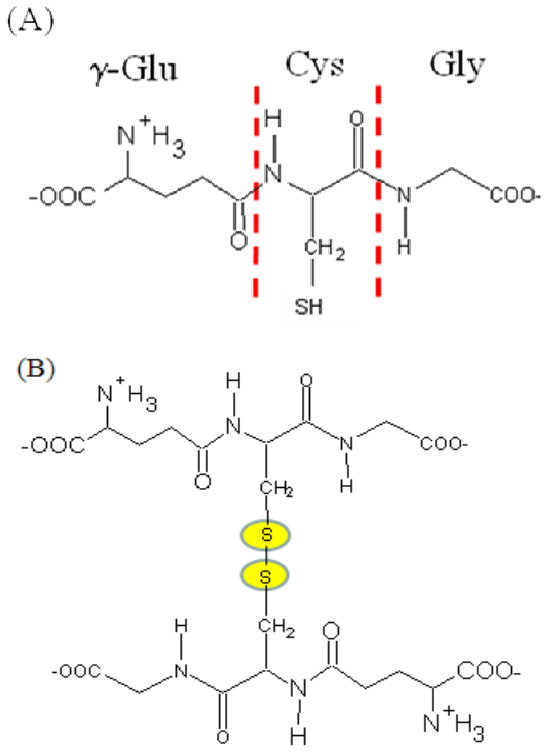

Figure 1: Structure of glutathione reduced form (A) and gluthatione – oxidized form (B).

Technologies, USA) and twelve-channel CoulArray electrochemical detector (Model 5600A, ESA, USA). Detector consists of three flow analytical chambers (Model 6210, ESA, USA). Each chamber contains four analytical cells. One analytical cell contains two referent (hydrogen-paladium), and two counter and one porous graphite working electrodes. Electrochemical detector is situated in control module which is thermostated. Sample (20 µl) was injected by autosampler (Model 542, ESA, USA), which has thermostated space for column. Column was termostated at 30°C. Flow rate of mobile phase was 1 ml min^{-1}. Mobile phase consists of A: trifluoroacetic acid (80 mM) a B: 100% Met-OH. Compounds were eluted by following linear increasing gradient: 0-2 min (3%B), 2->4 min (10%B), 6->10 min (40%B), 10-12 min (98%B). Detection was carried out at applied potential 900mV.

Results and discussion

There was some scientific interest with regards to exclusively option of bond between GSH and cis-Pt in treated organism (Ishikawa and Aliosman, 1993). Later these types of interaction were studied on theoretical computing (Zimmermann et al. 2005), and so by real in vitro experiment (Zitka et al. 2007). But for better understanding of behaving of glutathiones in live cells we tried to study only contents of them in cancer patients. We developed fast and rapid method for separation and electrochemical detection of GSH and GSSG. Time of one analysis was 20 minutes. Required sensitivity of detection of glutathiones is due to fact that thiol group is strongly electroactive at applied potential 900mV.

Conclusion

Our developed method was useful for analysis more than 7 hundreds

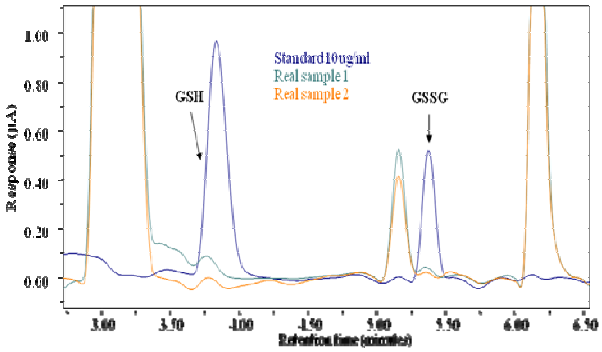

Figure 2: Chromatograms of standard of GSH and GSSG in overlay with two real samples obtained from blood plasma of two patients

of patients. We observed that there was significant different level between concentrations of GSH and GSSG.

Acknowledgements

The work has been supported by GA ČR 522/09/0239, MSMT 6215712402 and 1M06030.

References

Fratelli M, Demol H, Puype M et al (2002) Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. Proceedings of the National Academy of Sciences of the United States of America 99:3505-3510

Hiraku Y, Murata M, Kawanishi S (2002) Determination of intracellular glutathione and thiols by high performance liquid chromatography with a gold electrode at the femtomole level: comparison with a spectroscopic assay. Biochimica Et Biophysica Acta-General Subjects 1570:47-52

Ishikawa T, Aliosman F (1993) Glutathione-associated cis-diamminedichloroplatinum(ii) metabolism and atp-dependent efflux from leukemia-cells - molecular characterization of glutathione-platinum complex and its biological significance. Journal of Biological Chemistry 268:20116-20125

Maeso N, Garcia-Martinez D, Ruperez FJ et al. (2005) Capillary electrophoresis of glutathione to monitor oxidative stress and response to antioxidant treatments in an animal model. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 822:61-69

Noctor G, Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49:249-279

Rossi R, Milzani A, Dalle-Donne I et al (2002) Blood glutathione disulfide: In vivo factor or in vitro artifact? Clinical Chemistry 48:742-753

Zhang JY, Hu ZD. Chen XG (2005) Quantification of glutathione and glutathione disulfide in human plasma and tobacco leaves by capillary electrophoresis with laser-induced fluorescence detection. Talanta 65:986-990

Zimmermann T, Zeizinger M, Burda JV (2005) Cisplatin interaction with cysteine and methionine, a theoretical DFT study. Journal of Inorganic Biochemistry 99:2184-2196

Zitka O, Huska D, Krizkova S et al (2007) An investigation of glutathione-platinum(II) interactions by means of the flow injection analysis using glassy carbon electrode. Sensors 7:1256-1270