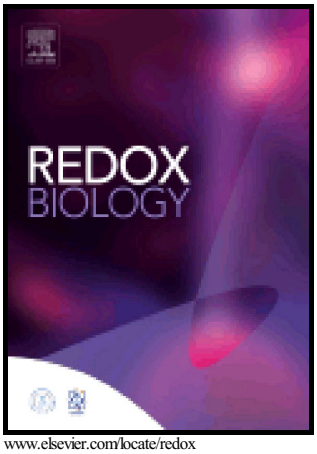

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PII: S2213-2317(18)30465-8
DOI: https://doi.org/10.1016/j.redox.2018.07.019
Reference: REDOX965

To appear in: *Redox Biology*

Received date: 4 June 2018
Revised date: 15 July 2018
Accepted date: 22 July 2018

Cite this article as: Joey De Backer, Jamoliddin Razzokov, Dietmar Hammerschmid, Carl Mensch, Zainab Hafideddine, Naresh Kumar, Geert van Raemdonck, Maksudbek Yusupov, Sabine Van Doorslaer, Christian Johannessen, Frank Sobott, Annemie Bogaerts and Sylvia Dewilde, The effect of reactive oxygen and nitrogen species on the structure of cytoglobin: A potential tumor suppressor, *Redox Biology,* https://doi.org/10.1016/j.redox.2018.07.019

The effect of reactive oxygen and nitrogen species on the structure of cytoglobin: a potential tumor suppressor

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Abstract

Many current anti-cancer therapies rely on increasing the intracellular reactive oxygen and nitrogen species (RONS) contents with the aim to induce irreparable damage, which subsequently results in tumor cell death. A novel tool in cancer therapy is the use of cold atmospheric plasma (CAP), which has been found to be very effective in the treatment of many different cancer cell types *in vitro* as well as *in vivo*, mainly through the vast generation of RONS. One of the key determinants of the cell's fate will be the interaction of RONS, generated by CAP, with important proteins, *i.e.* redox-regulatory proteins. One such protein is cytoglobin (CYGB), a recently discovered globin proposed to be involved in the protection of the cell against oxidative stress. In this study, the effect of plasma-produced RONS on CYGB was investigated through the treatment of CYGB with CAP for different treatment times. Spectroscopic analysis of CYGB showed that although chemical modifications occur, its secondary structure remains intact. Mass spectrometry experiments identified these modifications as oxidations of mainly sulfur-containing and aromatic amino acids. With longer treatment time, the treatment was also found to induce nitration of the heme. Furthermore, the two surface-exposed cysteine residues of CYGB were oxidized upon treatment, leading to the formation of intermolecular disulfide bridges, and potentially also intramolecular disulfide bridges. In addition, molecular dynamics and docking simulations confirmed, and further show, that the formation of an intramolecular disulfide bond, due to oxidative conditions, affects the CYGB 3D structure, thereby opening the access to the heme group, through gate functioning of His_{117}. Altogether, the results obtained in this study (1) show that plasma-produced RONS can extensively oxidize proteins and (2) that the oxidation status of two redox-active cysteines lead to different conformations of CYGB.