GELSILOX: A NOVEL STRATEGY FOR THE STUDY OF DYNAMIC ALTERATIONS IN THE THIOL REDOX PROTEOME

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Many physiological and pathological conditions are associated with reactive oxygen and nitrogen species accumulation, a condition termed oxidative stress. Redox proteomics, the systematic and exhaustive characterization of oxidized cysteines, should help to establish the physiological scope of Cys oxidation and give clues to its mechanisms. Redox proteomics still remains a technical challenge, mainly because of the labile nature of some thiol-redox modifications, the lack of tools to directly detect the modified residues and the relatively late development of highly sensitive analytical instruments. Here we present a simple and straightforward method for the analysis of the dynamic redox proteome, *GELSILOX*. This novel method is based on a technology previously developed in our laboratory that allows several treatment steps on proteomes, without sample losses, within concentrating SDS-PAGE gels. Proteomes are subjected to double alkylation to differentially label the oxidation state of Cys, subjected to in-gel digestion, differential $^{18}O/^{16}O$-labeling and IEF fractionation followed by LC-LIT-MS/MS analysis. *GELSILOX* allows the precise quantification of oxidative thiol modifications in several hundreds of peptides using a statistical model developed by our group, determining the exact Cys modified, and the simultaneous differential quantification of the whole proteome in the same experiment. We show the validity of the method with the detection of 341 Cys sites that increase their oxidized state as a response to the treatment with diamide, a thiol-specific oxidant. Using *GELSILOX*, we have been able to identify the two most sensitive Cys sites that are oxidized, as well as 24 expression changes that take place in endothelial cells treated with low H_2O_2 concentrations. The results obtained to date, the simplicity and general applicability of the method suggest that *GELSILOX* may become a powerful tool to identify, quantify, and monitor oxidative thiol modifications *in vitro* and *in vivo,* allowing a simultaneous high-throughput quantification of the whole proteome.