

Singlet Oxygen Promotes Mixed Disulfides Formation Through Oxidation

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Thiols and disulfide bonds might be potential targets for oxidative modifications mediated by singlet molecular oxygen ($^1\text{O}_2$), a reactive oxygen species. Furthermore, disulfide bonds undergo thiol scrambling in the presence of free thiols and those may be increased by the action of oxidative species, generating possible proteins crosslinks. In this work, we used model peptides and proteins containing disulfide bonds (oxidized Glutathione, (GSSG) and Cystine, (CSSC)), Insulin and Oxytocin and submitted them to oxidation with $^1\text{O}_2$ using a clean chemical source called *N,N'*-bis(2,3-dihydroxypropyl)-1,4-naphthale-nedipropanamide-1,4-endoperoxide (DHPN¹⁶[$^1\text{O}_2$]). After oxidation, disulfide bonds were incubated with free thiols. All the reaction products were detected using liquid chromatography coupled with mass spectrometry (LC-MS/MS) and the modifications were compared with the results using the free amino acid (Cysteine (Cys) and reduced Glutathione (GSH)). Cys and GSH showed rapid consumption in the presence of $^1\text{O}_2$, generating CSSC and GSSG as the major products, respectively. Mass spectrometry analyses of Cys and GSH oxidation products after reaction with $^1\text{O}_2$ revealed that the thiol incorporates $^1\text{O}_2$ generating other oxygenated products: RS-OOH and RS-OOOH, confirming the incorporation of oxygen atom into the thiol sulfur through mechanisms that are still being elucidated. However, the reaction of $^1\text{O}_2$ and CSSC or GSSG did not lead to a significant consumption of parent molecules (<10 %), but generated minor oxidation products through the break of the disulfide bonds, such as RS-OOH and RS-OOOH. Our results also showed that if disulfide bonds are put in contact with free thiols right after singlet oxygen-mediated oxidation, they are more prone to thiol shuffling. Thus, after singlet oxygen oxidation, peptides and proteins containing thiols demonstrated a inter disulfide bonds scrambling, generating new adducts species and other free thiols. The proportions of the shuffle products were different when comparing oxidized and non-oxidized disulfides, suggesting a role of $^1\text{O}_2$ in proteins crosslinks.

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