

GLUTAREDOXIN 2 DETHIOLIC ACTIVITY UPON THE S-GLUTATHIONYLATED 20S PROTEASOME

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Glutaredoxin is a glutathione-dependent disulfide oxidoreductase directly involved in the reduction of glutathione mixed-disulfides and in cellular antioxidant defense. Five glutaredoxin isoforms are present in the yeast *Saccharomyces cerevisiae*. Glutaredoxin2 is the most abundant isoform among those and it exhibits a multiple subcellular localization. During oxidative challenge, the 20S proteasome Cys-residues are natively S-glutathionylated and this post-translational modification promotes significant alteration on two site-specific proteasomal activities by an allosteric mechanism. The goal of the present work was to explore the dethiolase effect of Grx2 upon the S-glutathionylated proteasome. According to western blotting analyses, Grx2 was able to dethiolate the proteasome core, restoring its compromised activities accessed by fluorimetric assay. In addition, we show that in order to reduce the core, Grx2 enters the latent 20S proteasome chamber. Once inside the catalytic chamber, Grx2 was able to internally dethiolate the proteasome and, subsequently it is degraded by the core particle as confirmed by SDS-PAGE and Mass Spectrometry analyses. The finding that an enzymatic mechanism is able to promote redox modulation of the proteasomal activity gives new insights to its functional role coupled to intracellular redox regulation.