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# Redox Control of 20S Proteasome Gating

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## Abstract

The proteasome is the primary contributor in intracellular proteolysis. Oxidized or unstructured proteins can be degraded via a ubiquitin- and ATP-independent process by the free 20S proteasome (20SPT). The mechanism by which these proteins enter the catalytic chamber is not understood thus far, although the 20SPT gating conformation is considered to be an important barrier to allowing proteins free entrance. We have previously shown that S-glutathiolation of the 20SPT is a post-translational modification affecting the proteasomal activities. **Aims:** The goal of this work was to investigate the mechanism that regulates 20SPT activity, which includes the identification of the Cys residues prone to S-glutathiolation.

**Results:** Modulation of 20SPT activity by proteasome gating is at least partially due to the S-glutathiolation of specific Cys residues. The gate was open when the 20SPT was S-glutathiolated, whereas following treatment with high concentrations of dithiothreitol, the gate was closed. S-glutathiolated 20SPT was more effective at degrading both oxidized and partially unfolded proteins than its reduced form. Only 2 out of 28 Cys were observed to be S-glutathiolated in the proteasomal  $\alpha 5$  subunit of yeast cells grown to the stationary phase in glucose-containing medium. **Innovation:** We demonstrate a redox post-translational regulatory mechanism controlling 20SPT activity. **Conclusion:** S-glutathiolation is a post-translational modification that triggers gate opening and thereby activates the proteolytic activities of free 20SPT. This process appears to be an important regulatory mechanism to intensify the removal of oxidized or unstructured proteins in stressful situations by a process independent of ubiquitination and ATP consumption. *Antioxid. Redox Signal.* 16, 1183–1194.

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