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GLUTAREDOXIN S12: UNIQUE PROPERTIES FOR REDOX SIGNALING

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Cysteines made acidic by the protein environment are generally sensitive to pro-oxidant molecules. Glutathionylation is a post-translational modification which can occur either by GSSG-mediated thiol/disulfide exchange or by the reaction of reduced glutathione (GSH) with oxidized cysteines as sulfenic acids (-SOH). The reverse reaction (deglutathionylation) is strongly stimulated by small disulfide oxidoreductases named glutaredoxins (Grx) that require glutathione (GSH) or thioredoxin reductases for their regeneration.

By using structural analyses coupled with thiol titrations, fluorescence measurements, site-directed mutagenesis and mass spectrometry we have determined the structural and biochemical properties of poplar GrxS12, an atypical chloroplastic Grx possessing a monothiol active site ($_{28}$WCSYS$_{32}$). We show that GrxS12 is able to catalyze deglutathionylation of different substrates including proteins through a monothiol mechanism requiring only the most N-terminal cysteine of the enzyme. The reaction with glutathionylated substrates proceeds by a ping-pong mechanism. The pK_a of GrxS12 catalytic cysteine is very low (3.9) and makes GrxS12 itself sensitive to oxidation by H_2O_2 and to direct glutathionylation by GSSG, GSH plus oxidants and nitrosoglutathione (GSNO). Glutathionylated-GrxS12 (GrxS12-SSG) is temporarily inactive until it is deglutathionylated by GSH in a slow reaction that limits the overall process. Based on the equilibrium between GrxS12 and glutathione ($E_{m(GrxS12\text{-}SSG)}$= -315 mV, pH 7.0), GrxS12-SSG is predicted to accumulate *in vivo* under conditions of mild oxidation of the GSH pool that may occur under stress. Moreover, GrxS12-SSG is predicted to be more stable in chloroplasts in the dark (pH~7.0, K_{ox} 309) than in the light (pH~7.9, K_{ox} 69). These peculiar catalytic and thermodynamic properties could allow GrxS12 to act as a stress-related redox sensor allowing glutathione to play a signaling role through glutathionylation of GrxS12 target proteins.