Crystal Structure and Catalytic Regulation of an *S*-Formylglutathione Hydrolase

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S-formylglutathione hydrolase from *Arabidopsis thaliana (At*SFGH) shows hydrolytic activity toward xenobiotic carboxyl-esters and glutathione thioesters [1]. *At*SFGH has the characteristics of a cysteine hydrolase, being sensitive to inactivation by thiol alkylating agents while insensitive to conventional serine hydrolase inhibitors. However, when the crystallographic structure of *At*SFGH was determined, although a conserved cysteine (Cys59) implicated in catalysis was identified in the active site, a serine-histidine-aspartic acid catalytic triad was also observed. The importance of the serine residue in catalysis was subsequently demonstrated using a combination of site-directed mutagenesis and covalent modification using a fluorophosphono biotinylated suicide substrate. Mutation of the Cys59 to serine had no effect on carboxyesterase activity. However, Cys59 could reversibly regulate the activity of *At*SFGH through the formation of mixed disulfides, with glutathione or a range of synthetic functionalised thiols, leading to temporary inactivation of the enzyme. We conclude that while Cys59 does not play a direct role in the catalytic mechanism, its conservation and location close to the active site serine confers a specific regulatory function in which the *At*SFGH can be reversibly inactivated by *S*-glutathionylation in the course of catalysis and under oxidative conditions.

[1] Kordic S., Cummins I., Edwards R., *Arch Biochem Biophys*., 2002, **399**, 232.

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