Glurho protein: a novel multi domain sulfurtransferase from prokaryotes

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The genome analysis of *X. fastidiosa* revealed a new putative gene which translation result in a protein with three distinct domains: the N-terminal domain similar to monothiolic glutaredoxins, the C-terminal portion similar to rhodaneses, involved in the sulfur (S) and cyanide (CN-) metabolism and a central region similar to biosynthesis sites of Fe-S clusters, therefore this protein was nominated as Glurho. Protein databases searches revealed that *X. fastidiosa* Glurho homologues are only present in prokaryotes, most of which are pathogenic to animals and plants, due to these characteristics Glurho proteins may represent promising targets for therapeutic drugs. Aiming the protein characterization, HED and peroxidase assay were performed to determine glutaredoxin domain activity, but none activity was detected. Relative to rhodanese domain, *in vitro* assays were performed showing that Glurho is able to transfer sulfur to thiolic substrates (GSH and DTT) and detoxify efficiently KCN. We also demonstrated that at least one cysteine residue is essential to rhodanese activity, since the NEM alkylation annihilated enzyme activity. Due to this, the substitution of the cysteine residue from rhodanese domain (Cys¹⁶⁶) by a serine abolished the rhodanese activity as expected, indicating that this residue is essential to the enzyme functionality. Unexpectedly the Cys³³ replacement by serine increased the rhodanese activity in 3-fold, indicating that the Cys³³ may play a regulatory role in enzyme activity. We also investigate the *in vivo* effects of CN tolerance by the heterologous enzyme expression in *E. coli*, which revealed that Glurho is able to protect the bacterial cells against cyanogenic compounds even at high concentrations (1.5 mM). The effect of CN tolerance in both mutants Glurho⁴⁳⁵ and Glurho⁴²⁶⁶ by the heterologous enzyme expression in *E. coli* cells were lethal indicating that the Cys²⁶⁶ is essential to cell survival by detoxifying the CN while the Cys³³ may possess further roles in the enzyme activity. Due to these facts we postulate that Cys³³ of Grx domain may be involved in the Fe-S cluster biosynthesis as described for some Grx similar to the Glurho Grx domain.

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