Heterologous Expression and Enzymological Activity of a Mouse and a C452S-mutant QSOX (Quiescin-Sulfhydryl Oxidase)

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The flavoprotein quiescin-sulfhydryl oxidase (QSOX) inserts disulfide bonds into unfolded, reduced proteins with the concomitant reduction of oxygen to hydrogen peroxide. It presents three CxxC motifs that, in recombinant human QSOX1 are crucial for efficient catalysis. This study reports the heterologous expression and enzymological activity of both recombinant (recQSOX) and a C452S mutant (rec C452S) mouse short isoform QSOX. Through megaprimer technique, the C452S mutant, in the FAD-proximal CxxC, was constructed from the recombinant sequence. The two constructs were cloned into pET 32a+ vector and expressed in AD494 *E. coli*. After metal affinity chromatography, the two recombinant proteins were assayed. UV-visible spectra of recQSOX and recC452S were identical in the visible range. Sulfhydryl oxidase activity of the recQSOX was determined by the homovanilic acid-HRP method, using 24 or 48 nM enzyme. Results showed that, while recQSOX presented an activity of 26 nmol $H_2O_2.min^{-1}.mg^{-1}$, it was completely absent in the recC452S QSOX. These data confirm that the FAD-proximal CxxC motif is essential for sulfhydryl oxidase activity, and may be a useful tool for the study of QSOX roles.

Keywords: Quiescin-sulfhydryl oxidase; Short isoform; thiol proteins, Heterologous expression; Enzymological activity;