QSOX1b Accelerates in vitro Fibrillar Aggregation of Fibronectin

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The formation of disulfide bonds in folding/refolding of proteins is essential for the activity and stability of many proteins inside/outside of the cells. Three classes of enzymes mainly can oxidize disulfides by redox chemistry: ERO1, PDI, and ERV/QSOX. QSOX1b is a secreted protein that is catalyst of disulfide bond formation, and facile formation of disulfide bonds in unfolded reduced proteins. It is speculated that this protein is able to facilitate protein oxidation/folding within the secretory pathway, as well as the reoxidation/refolding of proteins in extracellular matrix. Plasmatic FN was purified and reduced in presence DTT. Enzymatic kinetic was performed using reduced FN (redFN) and mQSOX1b recombinant protein, by continuous fluorescence assay in denaturant (urea) and no denaturant conditions. The kinetic product was subjected to SDS-PAGE. Our results showed that mQSOX1b protein was able oxidize \textit{in vitro} the redFN, and the $K_M$ values revealed it was ±601nM in the absence and ±281nM in the presence of urea. Denaturing conditions were used and compared because these conditions provide the stability of the redFN molecule, which form fibrillar aggregates through the thiols and adhesive site exposure. The electrophoretic mobility of kinetic product revealed FN multimers larger than 450kDa, which not entered into 4% acrylamide gel even after 5 hours under constant current of 30mA. These multimers formation is accelerated in presence of mQSOX1b, and are undone at presence of DTT. It is known that multimeric fibrillar matrix of FN is essential for several cell mechanisms (such as migration, proliferation and differentiation), so it is possible that the extracellular QSOX1b would be a facilitator of this \textit{in vivo} event. Thus, mQSOX1b has catalytic activity upon plasmatic redFN \textit{in vitro} facilitating formation of FN multimeric fibrillar aggregate. Adhesive and migration properties of these aggregates are being evaluated in cell culture.

Keys words: protein folding, QSOX, ECM.
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