World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:18, No:09, 2024

Identification of the Putative Interactome of Escherichia coli Glutaredoxin 2 by Affinity Chromatography

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Abstract : The glutaredoxin (Grx) and thioredoxin (Trx) systems keep the intracellular environment reduced in almost all organisms. In Escherichia coli (E. coli), the Grx system relies on NADPH+ to reduce GSH reductase (GR), the latter reducing oxidized diglutathione to glutathione (GSH) which in turn reduces cytosolic Grxs, the electron donors for different intracellular substrates. In the Trx system, GR and GSH are replaced by Trx reductase (TrxR). Three of the Grxs of E. coli (Grx1, 2, 3) are reduced by GSH, while Grx4 is likely reduced by TrxR. Trx1 and Grx1 from E. coli may reduce ribonucleotide reductase Ia to ensure a constant supply of deoxyribonucleotides for the synthesis of DNA. The role of the other three Grxs is relatively unknown, especially for Grx2 that may amount up to 1 % of total cellular protein in the stationary phase of growth. The protein is known as a potent antioxidant, but no specific functions have been attributed to it. Herein, affinity chromatography of cellular extracts on immobilized Grx2, followed by MS analysis of the resulting eluates, was employed to identify protein ligands that could provide insights into the biological role of Grx2. Ionic, strong non-covalent, and covalent (disulfide) interactions with relevant proteins were detected. As a means of verification, the identified ligands were subjected to in silico docking with monothiol Grx2. In other experiments, protein extracts from E. coli cells lacking the gene for Grx2 (grxB) were compared to those of wild type. Taken together, the two approaches suggest that Grx2 is involved in protein synthesis, nucleotide metabolism, DNA damage repair, stress responses, and various metabolic processes. Grx2 appears as a versatile protein that may participate in a wide range of biological pathways beyond its known general antioxidant function.

Keywords : Escherichia coli, glutaredoxin 2, interactome, thiol-disulfide oxidoreductase
Conference Title : ICPPI 2024 : International Conference on Protein-Protein Interactions
Conference Location : Rome, Italy
Conference Dates : September 12-13, 2024