Poster Presentation

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The thioredoxin system from thermosipho africanus: structure and function.

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The thioredoxin system is a ubiquitous oxidoreductase system that consists of the enzyme thioredoxin reductase (TrxR), the protein thioredoxin (Trx) and the cofactor nicotinamide adenine dinucleotide phosphate (NADPH). The system has been comprehensively studied from many organisms, such as Escherichia coli (E. coli); however, structural and functional analysis of this system from thermophilic bacteria has not been as extensive.

Thermosipho africanus (T. africanus) is a thermophilic bacterium that was first isolated from a marine hydrothermal area in Djibouti, Africa [1]. Strain TCF52B was isolated from a high-temperature oil reservoir in the North Sea [2]. Analysis of the complete genome sequence of T. africanus strain TCF52B, suggested the presence of two putative Trxs (TaTrx1 and TaTrx2) and a TrxR (TaTrxR) as components of its thioredoxin system. In this study, TaTrx1 and TaTrxR have been successfully cloned, overexpressed and purified and characterized using biophysical techniques, biochemical assays and X-ray crystallography.

Our studies have indicated, not surprisingly, that TaTrx1 and TaTrxR are far more stable than the thioredoxin system components of E. coli. These two proteins were able to withstand both higher temperatures and higher concentrations of guanidine hydrochloride before denaturing. Consistent with these results, kinetic assays indicated that TaTrxR had a higher optimal temperature (70 °C) for activity, compared to E. coli TrxR (EcTrxR, 55 °C). Furthermore, TaTrxR was found to be catalytically more efficient at its optimal temperature than at room temperature (7 X) or at 10 °C (255 X); a trend not observed with EcTrxR.

To understand and identify the differences that may contribute to these results, X-ray crystallography was used to determine the structure of TaTrx1 and TaTrxR. A search of current Trx crystal structures indicated that the highest amino acid sequence identity of TaTrx1, to a known crystal structure, was only 26 %, which was too low for molecular replacement to be used to solve the phase problem. Further analysis of the TaTrx1 amino acid sequence indicated that there were six sulfur atoms; therefore, the phase problem was effectively solved using S-SAD [3]. The crystal structure of TaTrxR was successfully solved using molecular replacement, since TaTrxR shared greater than 30 % amino acid sequence identity with known TrxR crystal structures.

In this presentation, an analysis of the structure and function of the thioredoxin system of T. africanus will be discussed and compared to that of E. coli. This analysis includes results obtained from Circular Dichroism, insulin precipitation assays, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) assays and crystal structures.

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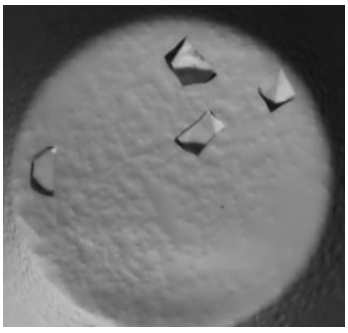

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