FUNCTIONAL ADAPTATION OF MERCURIC REDUCTASES FROM THE DEEP BRINE ENVIRONMENT OF ATLANTIS II IN THE RED SEA TO HIGH TEMPERATURE

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The lower convective layer (LCL) of the Atlantis II (ATII) brine pool of the red sea is a unique environment characterized by high salinity of around 4 Molar, temperature of 68ºC, and very high concentrations of heavy metals. We have previously described a metagenome-derived mercuric reductase, ATII-LCL MerA, from the LCL of the ATII brine pool that is thermo-stable at 60ºC and retain more than 70% of its activity after 10 minutes incubation at 70ºC. One of the structural characteristics of this enzyme, that distinguish it from a thermo-sensitive ortholog, is the limited substitutions of amino acids, less than 9%, including the presence of 4 aspartic acids at positions 414 to 417 replacing 4 alanine in the thermo-sensitive MerA. In this work, we identified a metagenome-derived MerA from the ATII-LCL environment, ATII-LCL-NH, that is lacking all the substitutions observed in ATII-LCL MerA. Site-directed mutagenesis replacing alanine 415 and 416 found in ATII-LCL-NH with the corresponding aspartic acids present in ATII-LCL increased the thermo-stability of the enzyme. However, substituting the 4 alanine, 415 to 417, with the corresponding four aspartic acids present in ATII-LCL decreased tremendously the thermal stability of the enzyme. Three-dimensional modeling of the MerA with the substituted Aspartic acids 415/416 revealed newly formed salt-bridge with arginine residue at position 420 and hydrogen bonds that may explain the enhanced thermal stability of this ATII-LCL-NH with the substituted Aspartic 415/416.

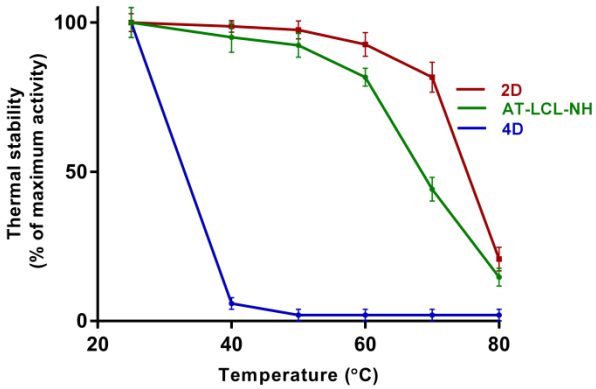

Figure 1 Thermal stability of Atlantis II LCL-NH mercuric reductase (green) and various mutants.