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Mechanistic Basis for Biological Polymer Stability,Electron Transfer and Molecular Sensing in Extreme Environments

Matthew Posewitz
COLORADO SCHOOL OF MINES

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Air Force Research Laboratory
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14. ABSTRACT

Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions (Hg2+) and to catalyze their reduction to a more volatile, less toxic elemental form. Biochemical and structural characterization of MerA from the thermophilic crenarchaeon Metallosphaera sedula demonstrated that this is a thermostable enzyme that remains active after extended incubation at 97C. At 37C, the NADPH oxidation-linked Hg2+ reduction specific activity was found to be 1.9 mol/minmg, increasing to 3.1 mol/minmg at 70C. M. sedula MerA crystals were obtained and the structure was solved to 1.6 , representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from M. sedula to mesophillic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. Biochemical and structural characterization of enolase from Chloroflexus aurantiacus, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria established a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was 80C, close to the measured thermal stability of the protein which was

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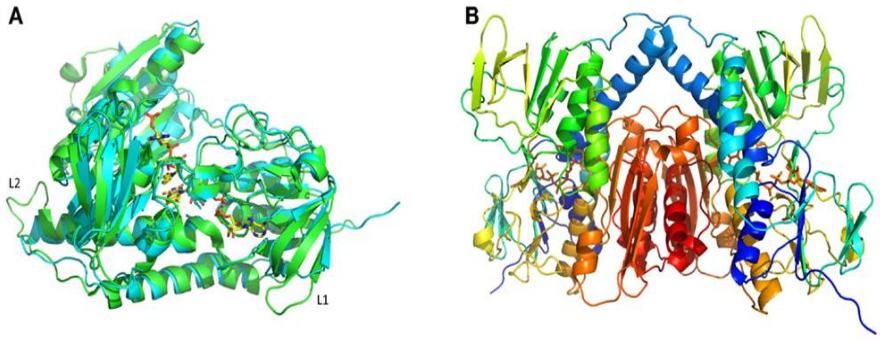
Mechanistic Basis for Biological Polymer Stability,Electron Transfer and Molecular Sensing in Extreme Environments

FA9550-14-1-0147

Matthew C. Posewitz (Colorado School of Mines)
John W. Peters (Montana State University)
Patrick C. Hallenbeck, Donald Veverka (U.S. Air Force Academy)

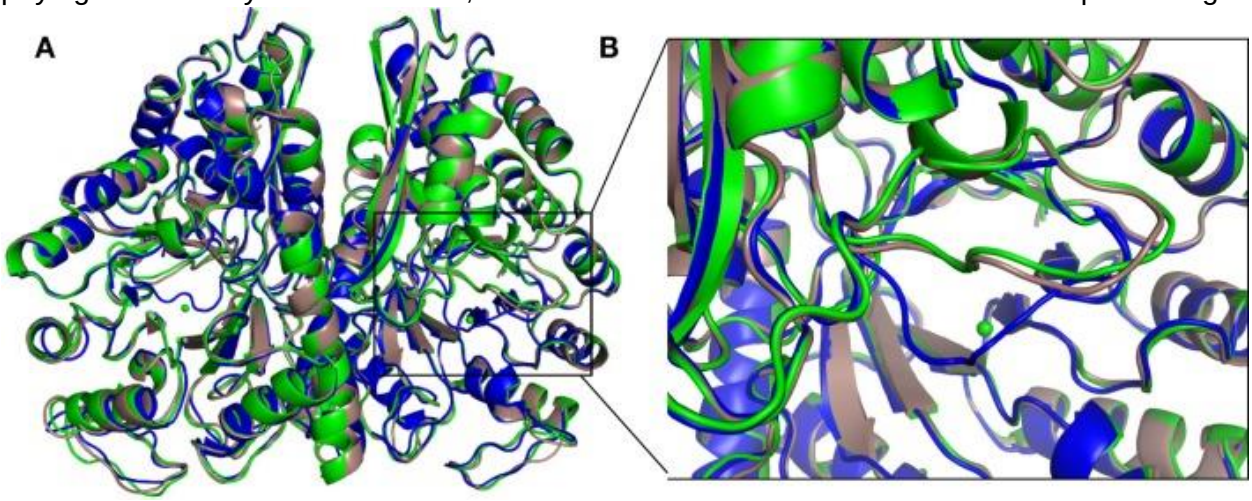
Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Four specific projects were pursued as summarized below (biochemical characterizations and structural analysis of two thermophilic enzymes, electron transfer reactions in cyanobacteria, and the first description of a thermophilic microbial fuel cell.

Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions (Hg^{2+}) and to catalyze their reduction to a more volatile, less toxic elemental form. Here, we perfromed biochemical and structural characterization of MerA from the thermophilic crenarchaeon *Metallosphaera sedula*. MerA from *M. sedula* is a thermostable enzyme, and remains active after extended incubation at 97°C. At 37°C, the NADPH oxidation-linked Hg^{2+} reduction specific activity was found to be 1.9 µmol/min·mg, increasing to 3.1 µmol/min·mg at 70°C. *M. sedula* MerA crystals were obtained and the structure was solved to 1.6 Å, representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from *M. sedula* to mesophillic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme.

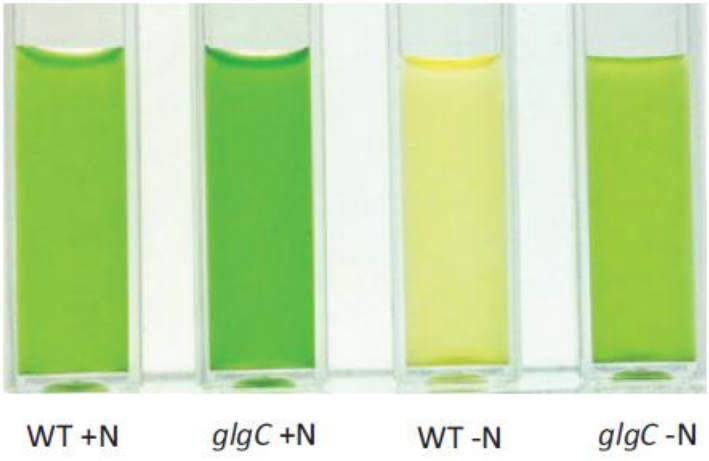


Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. We report the purification and biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria. The protein was purified as a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was 80°C, close to the measured thermal stability of the protein which was determined to be 75°C, while the pH optimum for enzyme activity was 6.5. The specific activities of purified enolase determined at 25 and 80°C were 147 and 300 U mg^{-1} of protein, respectively. K_m values for the 2-phosphoglycerate/phosphoenolpyruvate reaction determined at 25 and 80°C were 0.16 and 0.03 mM, respectively. The K_m values for Mg^{2+} binding at these temperatures were 2.5 and 1.9 mM, respectively. When compared to enolase from mesophiles, the biochemical and structural properties of enolase from *C. aurantiacus* are consistent with this being thermally adapted. These data are consistent with the results of our

phylogenetic analysis of enolase, which reveal that enolase has a thermophilic origin.



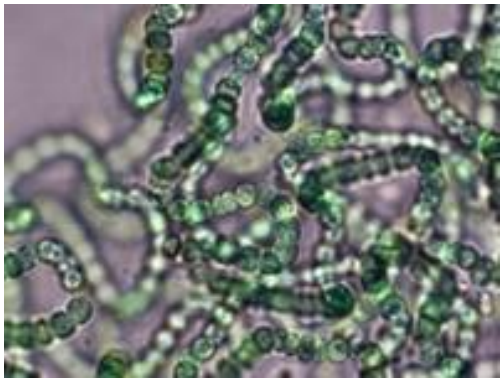
Cyanobacterial glycogen-deficient mutants display impaired degradation of light-harvesting phycobilisomes under nitrogen-limiting growth conditions and secrete a suite of organic acids as a putative reductant-spilling mechanism. This genetic background, therefore, represents an important platform to better understand the complex relationships between light harvesting, photosynthetic electron transport, carbon fixation, and carbon/nitrogen metabolisms. In this study, we conducted a comprehensive analysis of the dynamics of photosynthesis as a function of reductant sink manipulation in a glycogen-deficient glgC mutant of Synechococcus sp. strain PCC 7002. The glgC mutant showed increased susceptibility to photoinhibition during the initial phase of nitrogen deprivation. However, after extended periods of nitrogen deprivation, glgC mutant cells maintained higher levels of photosynthetic activity than the wild type, supporting continuous organic acid secretion in the absence of biomass accumulation. In contrast to the wild type, the glgC mutant maintained efficient energy transfer from phycobilisomes to photosystem II (PSII) reaction centers, had an elevated PSII/PSI ratio as a result of reduced PSII degradation, and retained a nitrogen-replete-type ultrastructure, including an extensive thylakoid membrane network, after prolonged nitrogen deprivation. Together, these results suggest that multiple global signals for nitrogen deprivation are not activated in the glgC mutant, allowing the maintenance of active photosynthetic complexes under conditions where photosynthesis would normally be abolished.



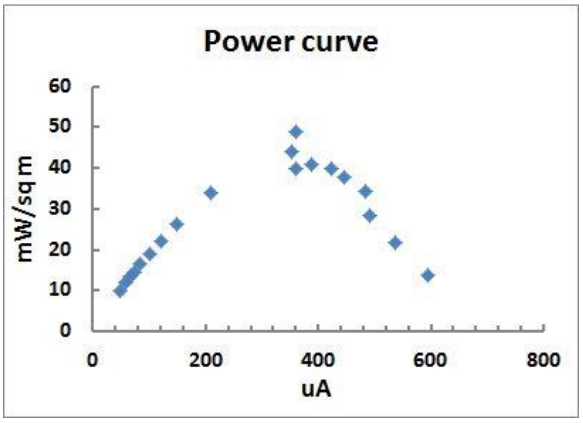
Many previous studies have shown that several different kinds of mesophilic organisms are capable of interacting with inorganic electrodes and giving up electrons derived from organic materials. This has led to research into the use of microbial fuel cells (MFC) for waste treatment and power generation, or to the use of microbial electrocatalysis for the electrosynthesis of chemicals and fuels, basically producing compounds of interest from electrically driven CO_2 fixation. Many different types of extremophiles are known that are robust and resistant to heat or

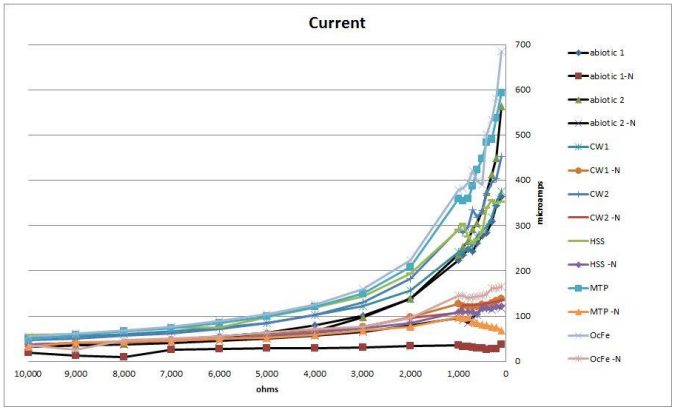
pH extremes. These organisms have evolved unique metabolic adaptations to their environments and may have unique sensing capabilities.

The goals of the work carried out at USAFA were to discover and characterize electrically active extremophiles using both defined, characterized extremophiles and enriched environmental samples. These could potentially be used in the long term to develop robust miniaturized threat detection systems, for remote power generation and in general for developing systems with increased capabilities for organic-inorganic electrical connectivity.



A variety of enriched samples taken from thermophilic environments (hot springs) in Colorado and New Mexico, were tested. These consisted mostly of assemblages of green algae and cyanobacteria with associated bacteria. In some cases the number of organisms was restrained enough to allow the determination of genome sequences. Interestingly, one sample (OC1) was enriched for a novel acidobacterium previously only observed in Octopus Hot Springs in Yellowstone. In another case (MTP1), a unique (above the genus level) thermophilic cyanobacterium could be described. All environmental enrichments were tested in thermophilic MFCs and this last enrichment in particular showed relatively (compared to control and other cultures) high currents and gave a power curve that demonstrated real connectivity with the electrode.





These initial results are encouraging and represent one of the first demonstrations of activity in a thermophilic MFC. Future research will focus on determining the role in electron transfer of the organisms in this biofilm as well as the molecular mechanism(s) involved.

Publications for the one year of funding:

Artz, J.H., White, S.N., Zadvorny, O.A., Fugate, C.J., Hicks, D., Gauss, G.H., Posewitz, M.C., Boyd, E.S., and Peters, J.W. (2015) Biochemical and Structural Properties of a Thermostable Mercuric Ion Reductase from *Metallosphaera sedula*. *Frontiers in Bioengineering and Biotechnology* Jul 13;3:97. doi: 10.3389/fbioe.2015.00097.

Zadvorny O.E., Boyd, E.S., Posewitz, M.C., Zorin, N.A., and Peters, J.W. (2015) Biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*: Evidence for a thermophilic origin. *Frontiers in Bioengineering and Biotechnology* Jun 1;3:74. doi: 10.3389/fbioe.2015.00074.

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Krishnan, A., Kumaraswamy, G.K., Vinyard, D.J., Gu, H., Ananyev, G., Posewitz, M.C., and Dismukes, G.C. (2015) Metabolic and photosynthetic consequences of blocking starch biosynthesis in the green alga *Chlamydomonas reinhardtii sta6* mutant. *Plant Journal* **81**, 947-960.

D'Adamo, S., and Posewitz, M.C. (2015) Hydrogenase evolution and function in eukaryotic algae. In: Low-Oxygen Stress in Plants; Plant Cell Monographs, M. Roegner (Ed.), De Gruyter, Berlin, in press. pp. 147-174.

Hallenbeck, P.C., Grogger, M., Veverka, D. (2014) Recent Advances in Microbial Electrocatalysis. *Electrocatalysis* **5**, 319–329.

Hallenbeck, P.C., Grogger, M., Mraz, M., Veverka, D. (2015) Draft Genome of a thermophilic photoheterotrophic Chloracidobacterium thermophilum strain OC1 from Ojo Caliente, New Mexico, submitted to *Genome Announcements*.

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Matthew Posewitz

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The AFOSR Program Manager currently assigned to the award

Hugh DeLong

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Abstract

Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions (Hg2+) and to catalyze their reduction to a more volatile, less toxic elemental form. Biochemical and structural characterization of MerA from the thermophilic crenarchaeon Metallosphaera sedula demonstrated that this is a thermostable enzyme that remains active after extended incubation at 97°C. At 37°C, the NADPH oxidation-linked Hg2+ reduction specific activity was found to be 1.9 µmol/min·mg, increasing to 3.1 µmol/min·mg at 70°C. M. sedula MerA crystals were obtained and the structure was solved to 1.6 Å, representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from M. sedula to mesophillic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. Biochemical and structural characterization of enolase from Chloroflexus aurantiacus, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria established a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was 80°C, close to the measured thermal stability of the protein which was

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Artz, J.H., White, S.N., Zadvorny, O.A., Fugate, C.J., Hicks, D., Gauss, G.H., Posewitz, M.C., Boyd, E.S., and Peters, J.W. (2015) Biochemical and Structural Properties of a Thermostable Mercuric Ion Reductase from Metallosphaera sedula. Frontiers in Bioengineering and Biotechnology Jul 13;3:97. doi: 10.3389/fbioe.2015.00097.

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Patrick Bradshaw to Hugh DeLong

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Laboratory Task Manager

Program Officer

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Equipment/Facilities			
Supplies			
Total			

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