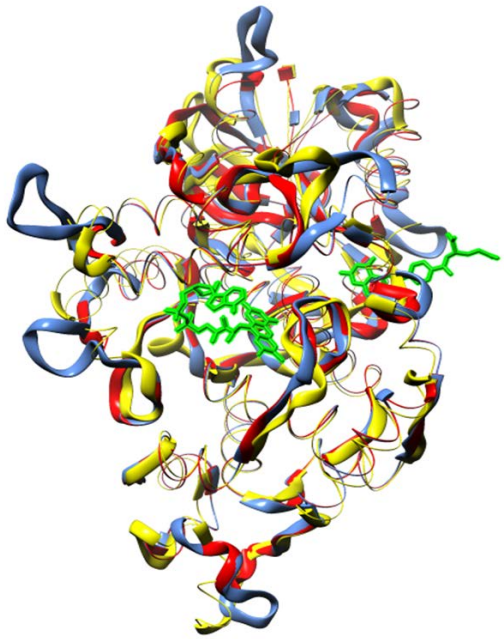
EXTREMOPHILE DNA REPAIR. D. T. Barnard[1], R. A. McBride[1], M. S. A. Maynard[1], Y. M. Gindt[2], and R. J. Stanley*[1], [1]Department of Chemistry, Temple University, 1901 N 13th Street, 250B Beury Hall, Philadelphia, Pennsylvania 19122, United States, [2]Department of Chemistry and Biochemistry, Montclair State University, 1 Normal Avenue, Montclair, New Jersey 07043, United States.

Introduction: Extremophiles provide an interesting platform to explore biological functions that can occur under harsh conditions. As compared to life forms living under "normal" conditions (mesophiles like ourselves) these organisms can be found living in hot water springs (thermophiles) or on arctic ice floes (psychrophiles). Thus the study of extremophilic proteins highlight evolutionary adaptations that allow them to flourish and thus are relevant to exploring how life may exist on other planets[1]. Terrestrially, DNA repair is crucial for life to exist; when UV damage of DNA occurs, thymine dimers (cyclobutane pyrimidine dimers or CPDs) are created [2]. Since evolution is a fundamental requirement for life as we define it, genetic repair mechanisms, whether acting on DNA or hitherto undiscovered extraterrestrial hereditary molecules, must obey universal physical laws to perform this function. Thus we have chosen the light-driven CPD repair protein DNA photolyase (PL) derived from extremophiles to better understand the limitations of repair under extreme conditions.

PL is a monomeric DNA repair enzyme that has been isolated in mesophiles, hyperthermophiles, and most recently psychrophiles. Photolyase binds the CPD substrate in a light-independent manner and repairs it via photoinduced electron transfer (PET). The photoinduced electron transfer process is exponentially dependent on temperature [3]. In order to understand how Nature has adapted this enzyme to function over a temperature range >100C, a characterization of temperature-dependent repair is necessary.

We have characterized DNA repair by PL isolated from the hyperthermoacidophile archaeon *Sulfolobus solfataricus*, which is found in highly acidic and hot water environments (*Ss*PL). In addition to measuring its temperature-dependent turnover, different osmolytes, small molecules having cytoprotective properties[4], were tested as well. The effects of temperature, viscosity, and ionic strength have been examined to better understand their roles in substrate binding as well as the catalytic repair step. Previous work by our group has elucidated the thermodynamics of substrate binding and its turnover for *Ss*PL over a relatively narrow range of environmental parameters and compared them to the mesophilic photolyase from *E. coli* (*Ec*PL) [5]. Here we present structural stability and catalytic DNA repair data on *Ss*PL and a mutant of *Ss*PL (Q39L), which lacks the second antenna FAD cofactor, in varying solvent conditions that directly correlate to the cellular conditions found in archaeal hyperthermophiles. Steady state fluorescence measurements were used to measure the effect of solvent conditions on protein denaturation, as well as protein stability in the presence of a variety of osmolytes. Catalytic activity was measured using steady state UV/vis absorption and fluorescence spectroscopies.

In contrast to the hyperthermophile, the cold-adapted photolyase from psychrophilic bacterium *Colwellia psychrerythraea* (*Cp*PL) is more difficult to overexpress because of its extreme sensitivity to its thermal environment. We have optimized a method to release protein from inclusion bodies enabling us to isolate *Cp*PL. Homology models of CpPL suggest that it is far more flexible than its warmer cousins.



Homology model of CpPL (blue) based on StPL (red) and EcPL (yellow). CpPL has more loops suggesting greater flexibility

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