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Through this DOE-sponsored program Konisky has studied the evolution and molecular biology of microbes that live in extreme environments. The emphasis of this work has been the determination of the structural features of thermophilic enzymes that allow them to function optimally at near 100 °C.

The laboratory has focused on a comparative study of adenylate kinase (ADK), an enzyme that functions to interconvert adenine nucleotides. Because of the close phylogenetic relatedness of members of the Methanococci, differences in the structure of their ADKs will be dominated by structural features that reflect contributions to their optimal temperature for activity, rather than differences due to phylogenetic divergence. We have cloned, sequenced and modeled the secondary structure for several methanococcal ADKs. Using molecular modeling threading approaches that are based on the solved structure for the porcine ADK, we have also proposed a general low resolution three dimensional structure for each of the methanococcal enzymes. These analyses have allowed us to propose structural features that confer hyperthermoeactivity to those enzymes functioning in the hyperthermophilic members of the Methanococci. Using protein engineering methodologies, we have tested our hypotheses by examining the effects of selective structural changes on thermoactivity.

Despite possessing between 68-81% sequence identity, the methanococcal AKs had significantly different stability against thermal denaturation, with melting points ranging from 69-103°C. The construction of several chimerical AKs by linking regions of the MVO and MJ A AKs demonstrated the importance of cooperative interactions between amino- and carboxyl-terminal regions in influencing thermostability. Addition of MJ A terminal fragments to the MVO AK increased thermal stability approximately 20°C while maintaining 88% of the mesophilic sequence. Further analysis using structural models suggested that hydrophobic interactions are largely responsible for determining the thermostability of the methanococcal AKs. Construction of chimerical enzyme also demonstrated a distinct separation between thermostability and enzymatic temperature optima, suggesting that overall protein flexibility and stability are not dependently linked.

Sequence comparisons and model building of highly related archaeal adenylate kinases has allowed the prediction of interactions responsible for the large temperature variation in temperatures for of optimal catalytic activity and temperature stability. The tertiary structure for these ADK have been predicted by using homology modeling to further investigate the potential of specific interactions on thermal stability and activity.
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In a collaboration with Prof. G.N. Phillips laboratory at Rice University, we are using X-ray crystallographic analysis to solve the structure of each ADK. This analysis should be very informative in suggesting those details of structure that contribute to the thermal properties of these enzymes.

**Relevant Publications**


