DOE/ER/13241--T2

RICE UNIVERSITY

Office Of The Vice Provost
For Research and Graduate Studies

October 7, 1997

Contracts Office
University of Illinois

13241

RE: DOE DEFG02-84ER~~13247~~



Dear Contracts Officer:

This letter represents the final technical report for the above refereneced DOE grant.

Summary of work accomplished under the award

Adenylate kinase has been isolated from four related methanogenic members of the Archaea. For each the optimum temperature for enzyme activity was similar to the temperature for optimal microbial growth and was approximately 30 °C for *Methanococcus voltae*, 70 °C for *Methanococcus thermolithotrophicus*, 80 °C for *Methanococcus igneus* and 80-90 °C for *Methanococcus jannaschii*. The enzymes were sensitive to the adenylate kinase inhibitor, $Ap_5A$ [$P^1$,$P^5$ - di (adenosine-5') pentaphosphate], a property that was exploited to purify the enzymes by CIBACRON Blue affinity chromatography. The enzymes had an estimated molecular weight (approximately 23-25 kDa) in the range common for adenylate kinases. Each of the enzymes had a region of amino acid sequence close to its N-terminus that was similar to the canonical P-loop sequence reported for all adenylate kinases. However, the methanogen sequences lacked a lysine residue that has previously been found to be invariant in adenylate kinases including an enzyme isolated from the Archeon, *Sulfolobus acidocaldarius*. If verified as a nucleotide binding domain, the methanogen sequence would represent a novel nucleotide binding motif. There was no correlation between amino acid abundance and the optimal temperature for enzyme activity.

The adenylate kinase genes (adkA) were cloned from four closely related methanogenic members of the Archaea: the mesophile *Methanococcus voltae* (*Mv*), the thermophile *M. thermolithotrophicus* (*Mt*), and the hyperthermophiles *M. jannaschii* (*Mj*) , and *M. igneus* (*Mi*). All four genes encode a protein of 192 amino acids (aa), and the four enzymes were closely related, with 68-81% aa identity in pairwise comparisons. It is anticipated that the enzyme set will provide the basis for studies that can establish the structural basis for ADK thermal stability. *Mj* and *Mi* contained a gene homologous to *M. vannielii sec*Y upstream of adkA, while *Mv* and *Mt* contained an unidentified, yet conserved, upstream open reading frame (ORF). The *Mt*, *Mj*, and *Mi*, but not *Mv*, contained an unidentified, yet highly conserved, ORF directly downstream of adkA. Based on their size, predicted secondary structure and phylogenetic relation to bacterial and eukaryotic adenylate kinases (ADK), it was concluded that the archaeal adkA genes encoded a

unique class of ADK, and suggested that Euryarchaeotal and Crenarchaeotal branches of the Archaea contain separate subclasses of the enzyme.

The adenylate kinase (*adk*A) genes were cloned from four related methanogens: the mesophile *Methanococcus voltae*, the thermophile *Methanococcus thermolithotrophicus*, and the hyperthermophiles *Methanococcus jannaschii*, and *Methanococcus igneus*. All four genes encode a protein of 192 amino acids, and the four enzymes were closely related, with 68-81% amino acid identity in pairwise comparisons. The genome organization in the *adkA* region differed among the four methanococci. *M. jannaschii* and *M. igneus* contained a gene homologous to *M. vannielii secY* upstream of *adkA*, while *M. voltae* and *M. thermolithotrophicus* contained an unidentified, yet conserved upstream open reading frame (ORF). *M. thermolithotrophicus*, *M. jannaschii*, and *M. igneus*, but not *M. voltae*, contained an unidentified, yet highly conserved, ORF directly downstream of *adkA*. Codon usage of the *M. voltae adk*A genes revealed a preference for C in the wobble position of NNY (Y = U or C) codon, despite the fact that the methanococcal genomes are ca. 70% (A+T). This codon preference was not observed in the thermophiles. Significant similarity was observed between the archaeal enzymes and eukaryotic cytosolic adenylate kinases, and the methanococcal genes corresponded well with eukaryotic type 1 adenylate kinase with regard to size and predicted secondary structure. Phylogenetic analysis revealed that the archaeal *adk* genes encoded a unique class of adenylate kinase, and suggested that Euryarchaeotal and Crenarchaeotal branches of the Archaea contain separate subclasses of the enzyme. An analysis of amino acid substitutions in regions of predicted secondary structure led to the conclusion that alterations in protein flexibility and hydrophobicity provide the main driving force in achieving thermal stability.

Highly related adenylate kinases (AKs) from four closely related methanogenic members of the Archaea; the mesophile *Methanococcus voltae* [MVO], the thermophile *M. thermolithotrophicus* [MTH], and the extremethermophilies *M. igneus* [MIG], and *M. jannaschii* [MJA] were physically characterized for their resistance to thermal denaturation. 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 MJA AKs demonstrated the importance of cooperative interactions between amino- and carboxyl- terminal regions in influencing thermostability. Addition of MJA terminal fragments to the MVO AK increased thermal stability approximately 20°C while maintaining 88% of the mesophilic sequence. Further analysis using structural models and site-specific mutagenesis 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.

Publications

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Sincerely

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Jordan Konisky

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