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Chemical Function of Substituted Amino Acids in Glyceraldehyde-3-phosphate Dehydrogenase

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The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

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13. ABSTRACT (*Maximum 200 words*)

An investigation of the amino acids responsible for the thermostability of the *T. aquaticus* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was inititated. The gene encoding this enzyme was isolated from a genomic library and sequenced. The gene encoded a functional GAPDH by virtue of its ability to rescue a mutant bacterial strain whose own GAPDH gene was deleted. The heat-stability of the isolated enzyme is impressive, i.e., it retains 100% of its activity after 2 hours at 90°C while requiring 100°C to lose 50% of its activity. Defining the conditions for crystallization as well as the physicochemical properties of the purified GAPDH are currently ongoing.

14. SUBJECT TERMS
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Statement of the Problem Studied

The three-dimensional structure of the glycolytic enzyme, glyceraldehyde-3-phophate dehydrogenase (GAPDH) from *Thermus aquaticus* is to be determined and directly compared to the available structures of homologous but more thermolabile enzymes. The purpose of this comparative analysis is to determine the critical domains responsible for enzyme thermostability. It has been proposed that internal and surface ionic salt bridges that interact across adjacent segments function to stabilize the enzyme against thermal denaturation. Their presence will be confirmed by structural analysis and their role in thermostabilization will be directly tested by removing the critical domains by site-directed mutagenesis.

Summary of the most Important Results

To solve the above problem, the gene encoding GAPDH from *T. aquaticus* was isolated and ultimately sequenced in spite of its high G+C content (Hecht et al., 1989). To demonstrate that it encoded a functional GAPDH, after an upstream terminator region of an adjacent gene was removed, the GAPDH gene was then shown to rescue a novel bacterial strain whose host GAPDH gene was itself deleted. With this new strain, we are now able to prepare and purify to homogeneity 200 mg of *T. aquaticus* GAPDH per 4 liters of culture. The heat-stability of the isolated enzyme is impressive, i.e., it retains 100% of its activity after 2 hours at 90°C while requiring 100°C to lose 50% of its activity. Defining the conditions for crystallization as well as the physicochemical properties of the purified GAPDH are currently ongoing.

Publication List

Hecht, R. M., Garza, A., Lee, Y.-H., Miller, M. D. and Pisegna, M. A. (1989). Nucleotide sequence of the glyceraldehyde-3-phosphate dehydrogenase gene from *Thermus aquaticus* YT1. Nucleic Acids Res. 17, 10123.

Huang, X.-Y., Barrios, L.A.M., Vonkhorporn, P., Honda, S. Albertson, D. G., and Hecht, R. M. (1989). Genomic organization of the glyceraldehyde-3-phosphate dehydrogenase gene family of *Caenorhabditis elegans*. J. Mol. Biol. 206, 411-424.

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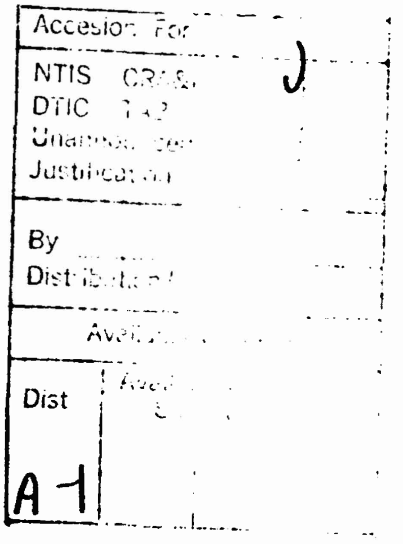

Report of Inventions

None