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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A considerable extent of biochemical diversity exists in the metabolic pathways utilized in nature for the biosynthesis of aromatic amino acids and vitamin-like derivatives. The overall objective is to evaluate the biochemical diversity within the archaebacteria for comparison with biochemical diversity already known or now emerging within the eubacteria. This will provide a comprehensive and rigorous sense of what aspects of diversity can be separated at the hierarchical level of the split between archaebacteria and eubacteria and it will provide a sense of which aspects of diversity are unique to each kingdom.

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PRINCIPAL INVESTIGATOR: Roy A. Jensen

CONTRACTOR: University of Florida

CONTRACT TITLE: Biochemical-pathway Diversity in Archaebacteria

START DATE: 1 February 1988

RESEARCH OBJECTIVE: To assess the extent to which the archaebacteria possess unique biochemical features of aromatic amino acid biosynthesis and regulation. The biochemical diversity within the archaebacteria will be compared to the biochemical diversity already known or now emerging within the eubacteria.

PROGRESS (Year 1): Extreme halophiles such as Halobacterium vallismortis possess a prephenate dehydratase enzyme which is subject to allosteric activation by hydrophobic amino acids. This example of metabolic interlock is characteristic of much or all of the Gram-positive lineage of eubacteria. We have extended the enzymological base of information in the extreme halophile lineage, and we have begun the study of organisms within the one of the three methanogen orders (Methanomicrobiales) that is phylogenetically nearest to the Halobacteriales. Within the latter methanogen order, Methanohalophilus mahii (a member of the family Methanosarcinaceae) has been selected for in-depth study.

Prephenate dehydrogenase. The enzyme exhibits a Km value for prephenate of 0.56 mM. It displays substrate ambiguity with respect to pyridine nucleotide requirement, although $NADP^+$ (Km = 0.079 mM) is preferred to NAD^+ (Km = 1.25 mM). It is quite sensitive to feedback inhibition by L-tyrosine, 100% inhibition being obtained at 0.2 mM L-tyrosine when Km levels of prephenate are used. Activity rates measured in the presence of 3.0 M KCl were 8-fold less than when 0.2 M KCl was present in the buffer.

Shikimate dehydrogenase. Like all eubacterial enzymes described to date, shikimate dehydrogenase was specific for NADP. It was incapable of substituting quinate for shikimate. Km values for shikimate and $NADP^+$ were 0.71 mM and 0.50 mM, respectively. Activity rates measured in the presence of 3.0 M KCl were 7-fold less than when 0.2 M KCl was present in the buffer.

Chorismate mutase. The enzyme is very active but possesses a rather low affinity for chorismate. Each of the three aromatic amino acids causes modest activation (in the range of 10%).

DAHP synthase. No activity was detected in spite of an extensive series of assays carried out under alternative conditions.

Other activities not detected. Arogenate dehydrogenase, arogenate dehydratase, and 4-hydroxyphenyllactate dehydrogenase (a new eubacterial enzyme of tyrosine biosynthesis) were not detected. These activities were not necessarily expected to be present.

Prephenate dehydratase. Activity was quite low in the absence of allosteric activators (tyrosine, tryptophan, leucine, methionine and isoleucine). The relative efficiencies of activator molecules were: TYR > LEU = MET > TRP > ILE. Activation by tyrosine was very dramatic (13-fold at 2 mM TYR). Valine was ineffective as an activator. Phenylalanine was an exceedingly potent inhibitor, causing complete inhibition at only 13 μM. Phenylalanine was able to antagonize tyrosine activation quite effectively.

M. mahii may possess one of the most interesting prephenate dehydratases of the "metabolic interlock" class. Tyrosine activation of the M. mahii enzyme (13-fold) is much greater than the <2-fold effect seen with Halobacterium vallismortum, or the 21% increase seen with the Acholeplasma laidlawii enzyme. The cyanobacterial (Synechocystis) enzyme exhibits >6-fold activation by tyrosine but differs from M. mahii in the domination of activation over inhibition in Synechocystis. Tryptophan activates the M. mahii enzyme substantially compared to its potent inhibitory effect in Bacillus subtilis.

Emerging perspective. The character states of aromatic amino acid biosynthesis are generally similar in the extreme halophiles and the methanogen order studied here. In addition to the common possession of the interlock-type of prephenate dehydratase, the enzymological similarities include the curious properties of chorismate mutase and the lack of detectable DAHP synthase. These results support the placement of extreme halophiles within the archaebacterial kingdom (as proposed by Woese), rather than in the eubacterial kingdom (as proposed by Lake).

WORK PLAN (Year 2): In addition to the existing focal point of prephenate dehydratase as a character state of interest, key enzymes of the entire pathway will be examined with respect to the objective of identifying diversity and biochemical novelty that will provide an expanded base of useful character states. For example, we are considering the possibility that absence of DAHP synthase reflects the presence of a different enzyme which utilizes glyceraldehyde-3-P instead of erythrose-4-P as substrate. Such an enzyme is present in the eukaryote lineage (higher plants), a result recently obtained in our laboratory. Since folate cofactors are used in extreme halophiles but not in other archaebacteria, we will attempt to demonstrate the expected presence of PABA synthase in extreme halophiles and its expected absence in the nearest-neighbor methanogens. We will study prephenate dehydratase in a progression of organisms as listed in the original proposal. It is clear that the interlock-type of prephenate dehydratase is not a conservative feature of the extreme halophiles, but is more broadly distributed. We will determine the hierarchical distribution of this character state in the archaebacteria.

Detailed enzymological characterization of the prephenate dehydratases from major archaebacterial groupings will be carried out. These will include evaluation of possible complexes or multifunctionality, kinetic studies, and analysis of possible molecular-weight interconversions mediated by allosteric effectors. The effects of extreme *in vitro* conditions (e.g., high salt, high temperature) will be determined in relationship to the *in vivo* ambient conditions of growth associated with any given archaebacterium. The results anticipated will provide a basis for molecular-genetic approaches expected to be in place by year 3.

We will also employ our expertise with the aromatic pathway to gain insight into gene-enzyme relationships in selected archaebacterial systems. For example, Konisky (Univ. of Illinois) has reported 1,2,4-triazole-3-alanine-resistant mutants of Methanococcus voltae which excrete not only histidine, but tyrosine and phenylalanine as well. We are collaborating with Dr. Konisky in order to decipher the molecular basis for these putative regulatory mutants.

INVENTIONS (Year 1): None.

PUBLICATIONS AND REPORTS: It is the nature of this work that we screen a wide variety of character states in a broad assemblage of organisms. The perspective gained then dictates back-tracking to the most interesting enzymes in the most interesting organisms. This generates in-depth characterizations of key organisms in a phylogenetic distribution and provides a solid basis for overview interpretations. Year 1 has been the time for collection of the skeleton of information that will be the basis for focused studies in progress and for the publication of results.

Some of the results with archaebacteria will be presented at the GORDON CONFERENCE ON POPULATION BIOLOGY AND EVOLUTION OF MICROORGANISMS (July 24-28, 1989) in a talk entitled "Evolution of Metabolic Pathways".

TRAINING ACTIVITIES: Dr. Raj Bhatnagar, a citizen of India, has carried out initial studies. Since his return to India, Dr. Xia Tianhui, from China, has worked on this project.

AWARDS/FELLOWSHIPS: None.

DEFENSE LOGISTICS AGENCY

Inter-Office Memorandum

IN REPLY REFER TO DTIC-W (L. Mason/47967/lkm)

28 JUN 1989

SUBJECT: DTIC Family Day - 1989

TO: All DTIC Employees

1. It's almost here! DTIC's fifth annual Family Day.

2. This IOM is to ask for your assistance. We are in need of personnel to assist us with the various duties on Family Day.

3. If you would like to assist in the areas listed below, please sign your name beside the duty and return to Ms. Leslie Mason, 5C401, or to your Family Day representative by 14 Jul 89.

Cooking_____ Clean-up_____

Set-up_____ Photographs_____

Games_____ Child Care _____

WILLIAM M. THOMPSON
Acting Deputy Administrator