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CHARACTERIZATION OF METHANOGEN MEMBRANE FUNCTION(U)
ILLINOIS UNIV AT URBANA DEPT OF MICROBIOLOGY
J KONTSKY ET AL 24 AUG 87 N00014-86-K-0224

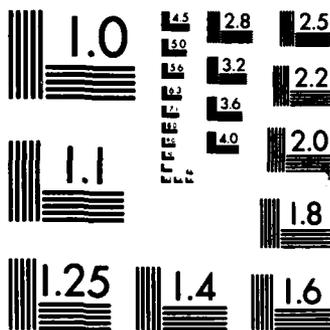
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<p>This research program involves characterization of membrane structure and function in the methanogenic archaeobacterium, <u>Methanococcus voltae</u>. The objectives are: to characterize the energy transducing cytoplasmic membrane, to delineate the energetics of coenzyme M transport, to genetically dissect the coenzyme M transport system, to characterize sodium pumps, and to initiate studies in membrane molecular biology. The role of the methanogen vanadate-sensitive membrane-associated ATPase in energy transduction is being investigated. ←</p>					
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PROGRESS REPORT ON CONTRACT NO014-86-K-0224

PRINCIPAL INVESTIGATOR: Dr. Jordan Konisky

TITLE: CHARACTERIZATION OF METHANOGEN MEMBRANE FUNCTION

1. Project goals

This research program involves characterization of membrane structure and function in the methanogenic archaebacterium, Methanococcus voltae. The objectives are: to characterize the energy transducing cytoplasmic membrane, to delineate the energetics of coenzyme M transport, to genetically dissect the coenzyme M transport system, to characterize sodium pumps, and to initiate studies in membrane molecular biology. The role of the methanogen vanadate-sensitive membrane-associated ATPase in energy transduction is being investigated.

2. Progress and plans for year 2.

a. Discovery and partial characterization of a novel methanogen ATPase.

Progress year 1. The mechanism of coupling between methane formation and ATP synthesis in methanogens has been a topic of controversy. The concomitant decrease in ATP formation and membrane potential caused by the addition of protonophores to Methanosarcina barkeri has led to the conclusion that ATP synthesis is driven by a chemiosmotic gradient of protons generated by the reactions of methanogenesis. An earlier report that methanogenesis from H_2 and CO_2 as well as ATP synthesis can proceed in Methanobacterium thermoautotrophicum in the absence of a measurable membrane potential has recently been clarified in studies using protoplasts of this methanogen. It has been shown that ATP synthesis, methanogenesis, and the membrane potential decrease in parallel in protoplasts treated with the protonophore, 3,5-di-tert-butyl-4-hydroxybenzylidenemalononitrile (SF-6847). The inability of the ionophore to reach the internal membranes in whole cells has been proposed to explain this difference in sensitivity.

Based on evidence that electron transfer-driven ATP synthesis in M. voltae is not dependent on a proton electrochemical gradient, a molecular scheme has been proposed in which ATP synthesis is coupled directly to electron transfer. Furthermore, the M. voltae ATPase is considered not to function physiologically as an ATP synthase, but rather in electrogenic sodium-translocation. It is, therefore, apparent that more detailed characterization of M. voltae ATPases is critical to a more detailed elucidation of this organisms energy metabolism. This past year we have made significant progress in the initial characterization of such ATPases.

Membrane-bound ATPase activity was detected in the M. voltae. The ATPase was inhibited by vanadate, a characteristic inhibitor of E_1E_2 ATPases. The enzyme activity was also inhibited by diethylstilbesterol. However, it was insensitive to DCCD, ouabain and oligomycin. The enzyme displayed a high preference for ATP as substrate, was dependent on Mg^{2+} and had a pH optimum of approximately 7.5. The enzyme was completely solubilized with 2% Triton X-

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100. The enzyme was insensitive to oxygen and was stabilized by ATP. There was absence of homology with the Escherichia coli F_0F_1 ATPase at the level of DNA and protein. These results strongly indicate that the methanogen enzyme is an E_1E type ATPase and our finding represents the first description of such an ATPase in the archaebacteria.

b. Characterization of bromoethanesulphonate resistant mutants of Methanococcus voltae: evidence of a coenzyme M transport system.

Plans year 2. We intend to further characterize this membrane protein. An critical confirmation of the ATPase type requires involve a direct demonstration of the phosphorylated intermediate. Membranes will be phosphorylated with radioactive ATP and the solubilized proteins analyzed for the presence of the phosphoryl group on nondenaturing polyacrylamide gels. Modification of a membrane component in the absence of, but not in the presence of vanadate would define the ATPase. The enzyme will then be purified, cleaved with proteases and the amino acid sequence of its active site determined. The primary interest here is to compare this sequence with that of other characterized similar enzymes. Such a determination has obvious evolutionary implications. We also intend to survey other methanogens, especially the methanococcal group, for the presence of a vanadate-sensitive ATPase.

We intend to proceed to the purification of this ATPase. The goal is to determine its N-terminal amino acid sequence and to use that sequence to generate a DNA oligonucleotide that would serve as a hybridization marker which would be used to clone the structural gene. Alternatively, antibody will be produced and the gene cloned making use of appropriate expression vectors and screening for clones which react with the antibody. Isolation of the gene would lead to its sequence which in turn would lead to studies of its molecular biology.

b. Characterization of methyl CoM uptake into M. voltae.

Progress year 1. Mutants which are resistant to BES (2-bromoethanesulfonic), an analogue of methyl-CoM, a cofactor in methanogenesis have been reported for several methanogens. In the case of the Methanosarcina mutants it was been shown that methane formation from $CH_3-S-CoM$ in extracts prepared from sensitive and resistant strains are equally sensitive to BES suggesting that the mechanism of resistance involved a change in the cell envelope leading to decreased permeability to BES. A similar conclusion can be drawn from a report that a BES resistant mutant of M. voltae is defective in uptake of coenzyme M and that coenzyme M itself can protect cells from the action of BES. These results suggest that in M. voltae BES is taken up by a uptake system which it shares with coenzyme M.

In studies to generate suitable genetic markers, we have isolated and initiated studies to characterizes M. voltae mutants which are resistant to BES. The mutants displayed reduced ability to accumulate (^{35}S)BES relative to the sensitive parental strain. BES inhibited methane production from $CH_3-S-CoM$ in cell-free cell extracts prepared from wild type sensitive or resistant strains. BES uptake required the presence of both CO_2 and H_2 and was inhibited by N-Ethyl maleimide and several reagents known to disrupt energy metabolism. The mutants showed normal uptake of isoleucine and were not cross

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resistant to either azaserine or 5-methyltryptophan and, thus, are neither defective in general energy-dependent substrate transport nor envelope permeability. Both HS-CoM or CH_3^- -S-CoM prevented the uptake of BES and protected cells from inhibition by it. We propose that M. voltae has an energy-dependent carrier-mediated uptake system for HS-CoM and CH_3^- -S-CoM which can also mediate uptake of BES.

Plans year 2. Using radioactive methyl CoM, we are now characterizing the methyl-CoM transport system directly. The goal is to determine the nature of the energetic driving force and to learn the details of the energy coupling steps. The nature of the coupling ion is particularly relevant and we are in the process of defining it. We will also characterize our collection of BES resistant mutants in an attempt to identify whether any of them manifest altered membrane proteins.

3. Publications.

Santoro, N. and J. Konisky (1987). Characterization of bromoethanesulfonate mutants of *Methanococcus voltae*: Evidence of a coenzyme transport system. *J. Bacteriol.*, 169: 660-665.

Dharmavaram, R. and J. Konisky (1987). Identification of a vanadate-sensitive membrane-bound ATPase in the Archaeobacterium, *Methanococcus voltae*. *J. Bacteriol.*, in press

4. Presentations of ONR-sponsored research.

Marine Biological Laboratory, Woods Hole, June 1987
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5. Graduate students and postdoctoral supported

Rita Dharmavaram (female, India)
Nicholas Santoro (male, USA)
Michael Dybas (male, USA)

AFFRONI, Lewis F.
George Washington University
Department of Microbiology
2300 I ST NW
Washington, DC 20037

BALLOU, C. E.
Department of Biochemistry
University of California
Berkeley, CA 94720

BLAKEMORE, R. P.
University of New Hampshire
Dept of Microbiology
Durham, New Hampshire 03824

CLARK, Douglas S.
Department of Chemical Engineering
University of California, Berkeley
Berkeley, California 94720

COLWELL, Rita
Department of Microbiology
University of Maryland
College Park, MD 20742

DANIELS, Lacy
University of Iowa
Department of Microbiology
Iowa City, IA 52242

DENNIS, Patrick P.
Department of Biochemistry
University of British Columbia
2146 Health Sciences Mall
Vancouver, B.C. V6T 1W5

DOYLE, R. J.
Department of Microbiology &
University of Louisville HSC
Louisville, KY 40292

FELBECK, Horst
Marine Biology Research Division
Scripps Institution of Oceanography
University of California - San Diego
La Jolla, CA 92093

FERRY, James G.
Department of Anaerobic Microbiology
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061

FUJIOKA, Roger S.
The University of Hawaii
Water Resources Research Center
Honolulu, HI 96822

GIESE, R. W.
Northeastern Univ
Section of Medicinal Chemistry
360 Huntington Ave
Boston, MA 02115

GUNSALUS, Robert P.
Department of Microbiology
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024

GUPTA, Ramesh
Southern Illinois University
Dept of Chemistry & Biochemistry
Carbondale, IL 62901

JANNASCH, Holger W.
Woods Hole Oceanographic Institution
Woods Hole, MA 02543

KONISKY, Jordan
Department of Microbiology
University of Illinois
809 Sout Wright Street
Champaign, IL 61820

LIDSTROM, Mary E.
California Institute of Technology
Department of Biology
Pasadena, CA 91125

MITCHELL, Ralph
Division of Applied Sciences
Harvard University
125 Pierce Hall
Cambridge MA 02138

MUSCATINE, Leonard
Department of Biology
University of California, Los Angeles
Los Angeles, California 90024

NAGLE, David P.
Department of Botany & Microbiology
University of Oklahoma
Norman, OK 73019

OLSEN, Gary J.
Indiana University
Department of Biology
Jordan Hall 138
Bloomington, Indiana 47405

FACE, Norman R.
Department of Biology
Indiana University
Bloomington, IN 47405

REEVE, John N.
Department of Microbiology
Ohio State University
484 West 12th Avenue
Columbus, OH 43210-1292

WHITE, David C.
Institute for Applied Microbiology
University of Tennessee
10515 Research Drive, Suite 300
Knoxville, TN 37932-2567

WOESE, Carl R.
Genetics Department, 515 Morrill Hall
University of Illinois
505 S. Goodwin Avenue
Urbana, IL 61801

WOLFE, Ralph S.
131 Burrill Hall
University of Illinois
407 S. Goodwin
Urbana, IL 61801

ZINDER, Stephen H.
Department of Microbiology
Stocking Hall
Cornell University
Ithaca, NY 14853

END

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