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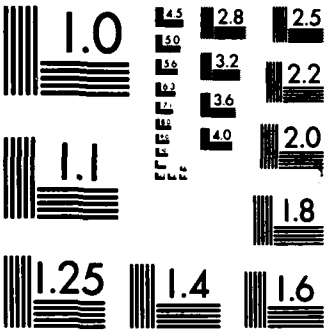

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This research program involves characterization of membrane structure and function in the methanogenic archaebacterium, Methanococcus voltae. The objectives are: to characterize the energy transducing cytoplasmic membrane, to delineate the energetics 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 transductic is being investigated.

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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 methanogensis 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-

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 inititiated 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

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 sytem 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 particulalry 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 sytem. J. Bacteriol., 169: 660-665.

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

4. Presentations of ONR-sponsored research.

Marine Biological Laboratory, Woods Hole, June 1987
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