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International Symposium on Topics in Microbial
Diversity, Metabolism, and Physiology

Final Report for May 22 and 23 1992

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July 1993

Prepared for

The U.S. Department of Energy--Chicago Operations Office
Agreement NO. DEFG02-92ER20062

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MASTER

Abstract:

Funds were provided to partially support an International Symposium on Topics in Microbial Diversity, Metabolism, and Physiology which was held on the University of Illinois campus on May 22 and 23, 1992. The funds were used to support travel costs and other expenses of invited speakers.

Narrative:

The symposium brought together an internationally recognized group of microbiologists working in several important areas of microbiology. A sampling of topics presented and discussed included: microbial electron transport schemes, a description of novel microbes inhabiting extreme environments, molecular evolution, enzyme structure and catalysis, novel coenzymes and metabolites, bioluminescence and microbial cell surface structure.

The wide range of topics with an emphasis on a common theme of microbial biochemical diversity attracted 204 participants of which 12 were from outside the U.S., of the remainder, were off-campus attendees. In addition to scheduled lectures, 35 posters were presented. The program evoked much discussion among all the attendees.

A summary of each lecture is provided below. We also include a copy of the symposium program

Energetics of *Methanococcus voltae*

Jordan Konisky
University of Illinois

We provide experimental support for the proposal that ATP production in *Methanococcus voltae*, a methanogenic member of the archaea, is based in an energetic system in which sodium ions, not protons, are the coupling ion. We show that when grown at a pH of 6.0, 7.1 or 8.2, *M. voltae* maintains a $\Delta\Psi$ of approximately -150 mV. These cells maintain a ΔpH of -6, -12, -12mV respectively, which is not favorable to the inward movement of protons. In contrast, the cells maintain a ΔpNa of +4.7, +31, +64 mV, respectively. While the protonophore, 3,3',4', 5-tetrachlorosalicylanilide (TCS), inhibits ATP formation in cells grown at pH6.5, neither ATP formation nor growth is inhibited in cells growing in medium of pH8.2. We show that when grown at pH8.2, cells synthesize ATP in the absence of a favorably oriented $\Delta\mu\text{H}^+/\text{F}$. Whether grown at pH6.5 or pH8.2, *M. voltae* extrudes Na^+ via a primary pump whose activity does not depend on a $\Delta\mu\text{H}^+/\text{F}$. Addition of protons to cells leads to the harmaline-sensitive efflux of Na^+ and vice versa, indicating the presence of a Na^+/H^+ antiporter activity and, thus, a second mechanism for the translocation of Na^+ across the cell membrane. *M. voltae* contains a membrane component which is immunologically related to the H^+ -translocating ATP synthase of the archaea, *Sulfolobus acidocaldarius*. Since we demonstrated that ATP production can be driven by an artificially imposed membrane potential only in the presence of sodium ions, we propose that ATP production in *M. voltae* is mediated by a Na^+ -translocating ATP synthase whose function is coupled to a DmNa^+/F which is generated through a primary Na^+ pump.

Biochemistry of Acetate-Derived Methanogenesis

James G. Ferry
Virginia Polytechnic Institute & State University

The enzymology of acetate conversion to CH₄ and CO₂ was studied in *Methanosarcina thermophila*. The amino acid sequence for acetate kinase and phosphotransacetylase was deduced from the genes encoding the enzymes; PTA has been hyperexpressed in *Escherichia coli*. The Ni/Fe-S component of the CODH complex showed three low-temperature EPR signals distinguishable by the redox potentials and g values; at least one of the signals was attributable to a 4Fe-4S center(s). The as-isolated Co/Fe-S component contained factor III in the base-off form and had a E_m for the Co^{+1/+2} redox couple of -512 mV. The amino acid sequence of the ferredoxin was deduced from the gene and contained cysteine clusters characteristic of [2[4Fe-4S] bacterial-like ferredoxins and is in agreement with the EPR spectra. The purified carbonic anhydrase was a 80,000-Da homodimer and contained an N-terminal amino acid sequence with high identity to the zinc-binding site of human carbonic anhydrase isozymes.

Bioenergetics of Methanogenesis

Gerhard Gottschalk
Gorg-August-Universität Göttingen, Germany

Methanogens derive their metabolic energy from the conversion of a number of simple substrates, such as H₂-CO₂, methanol, methylamines or acetate. The biochemical pathways leading to the formation of methane have been largely elucidated in recent years. Also, the reactions which are coupled to energy conservation have been identified. Using inside out vesicles of *Methanosarcina* strain Gö1 it was shown that the reduction of the heterodisulfide either by H₂ or by reduced coenzyme F₄₂₀ is coupled to the translocation of protons. Ratios of two protons translocated and 0.5 ATP synthesized per 2 electrons transferred to the heterodisulfide were found. In addition, the methyl-tetrahydromethanopterin:coenzyme M methyltransferase is coupled to energy conservation. It could be demonstrated that this methyl group transfer is Na⁺-dependent and is associated with the translocation of Na⁺ across the cytoplasmic membrane. The ion gradients established by these two reactions are then employed for ATP synthesis.

Regulation of Cell Wall Peptidoglycan Metabolism in *Escherichia coli*

Edward E. Ishiguro
University of Victoria

Amino acid deprivation of *Escherichia coli* results in the rapid accumulation of guanosine 5'-diphosphate 3'-diphosphate (ppGpp) in *relA*⁺ bacteria but not in *relA* mutants; ppGpp is a putative nutritional stress signal compound which apparently inhibits various energy-consuming metabolic processes in starved bacteria in what has been termed the stringent response. The synthesis of phospholipids and the activities of both the peptidoglycan biosynthetic and hydrolase systems are among the metabolic processes which are inhibited during the stringent response, and this accounts for the well known penicillin tolerance exhibited by amino acid-deprived *relA*⁺ bacteria. Penicillin tolerance during amino acid deprivation can be suppressed by introducing a mutation in *relA* or by treatment with inhibitors of the stringent response, e.g., chloramphenicol. Recent *in vivo* and *in vitro* studies indicate that the activities of both the membrane-bound penicillin-binding proteins that catalyze peptidoglycan

polymerization and the peptidoglycan hydrolases responsible for the characteristic bacteriolysis exhibit an obligatory dependence on ongoing phospholipid synthesis. It is therefore proposed that the penicillin tolerance of amino acid-deprived *E. coli* is based on an initial inhibition of phospholipid synthesis which in turn results in the inhibition of peptidoglycan metabolism. It is further speculated that the inhibition of phospholipid synthesis may be a global strategy to inhibit other membrane-associated metabolic events in starving bacteria, e.g., initiation of DNA replication and transport.

Cobalamin (vitamin B₁₂)

Jorge C. Escalante-Semerena
University of Wisconsin-Madison

Cobalamin (vitamin B₁₂) is a unique cyclic tetrapyrrole. It contains a cobalt atom chelated by the pyrroles, and an unusual nucleotide as the lower ligand. With some exceptions, the cobalamin biosynthetic (*cob*) genes of *Salmonella typhimurium* are organized as a large operon (~22 kb) divided into three regions; *cobI* functions, synthesize the tetrapyrrole macrocycle; *cobII* functions synthesize the base 5,6-dimethylbenzimidazole (DMB) of the nucleotide; *cobIII* functions assemble the nucleotide loop joining the base to the macrocycle. The *cobII* region is comprised of four complementation groups, *cobIVLM*, of which *cobM* is thought to be a *cis*-acting site. The *cobII* functions are involved only in the synthesis of the imidazole ring. The *cobIII* region is comprised of three open reading frames designated *cobUST* (J. Roth, Univ. of Utah). Mutants with lesions in only two of these loci (*cobU,S*) have been isolated. Why no *cobT* mutants have been isolated is unknown. The regulation of *cob* gene expression is positively controlled by the *poc* (propanediol, cobalamin) gene product in the presence of its effector 1,2-propanediol. The *Poc* protein also controls the expression of the neighboring, divergently transcribed propanediol utilization (*pdu*) genes, *poc* mutants have been isolated, and work in progress to analyze the mechanism of action of this protein.

Metal Oxide and Oxyanion Reduction by *Rhodobacter sphaeroides* 2.4.1

Samuel Kaplan
The University of Texas Medical School at Houston

Employing the facultative photosynthetic bacterium, *Rhodobacter sphaeroides*, we have identified high level resistance to a variety of metal oxides and oxyanions when cultures are grown either chemoheterotrophically or photoheterotrophically. Employing tellurite as a representative oxyanion we have followed resistance levels in a large number of representative members of the *Proteobacteria*, as well as numerous representative strains of *R. sphaeroides*. Under normal growth conditions resistance to different compounds can be attributed to three basic mechanisms: 1) reduction of the oxide or oxyanion to the free metal which is deposited in the cell membrane, 2) an apparent lack of uptake of the oxide or oxyanion, and 3) efflux of the toxic compound. Those metal oxides and oxyanions which are reduced to the free metal either chemoheterotrophically or photoheterotrophically also display high levels of H₂ evolution when cells are grown photosynthetically in the presence of the oxyanion. We have further determined that the levels of resistance to these compounds is directly related to the ability of the organism to generate reducing equivalents which can be used to reduce the oxyanion to the free metal. Thus, organic substrates which are highly oxidized or the presence of other exogenous electron acceptors, e.g. CO₂ DMSO lead to lower MIC levels for the various oxides or oxyanions. Finally, by employing a series of mutant strains of *R. sphaeroides* we have determined that an intact pathway for CO₂ fixation, and normal reaction center (RC) function are essential for high level resistance under photoheterotrophic growth. Chemoheterotrophically, an

intact b/c₁ complex is critical for oxyanion reduction. Surprisingly, the presence of the RC-H polypeptide also appears to be necessary for high level resistance to tellurite in aerobically grown cells.

Role of the *Rhizobium meliloti* Exopolysaccharide in Root Nodule Invasion

John A. Leigh
University of Washington

Rhizobium meliloti induces the formation of alfalfa root nodules and invades the nodules through infection threads to become a nitrogen-fixing endosymbiont. Current studies focus on chemical signals that induce developmental steps nodule formation and invasion. Genetic and phenotypic analyses of *R. meliloti* *exo* mutants that are deficient in the production of exopolysaccharide (EPS) have suggested that the *R. meliloti* EPS, succinoglycan, is a signal for infection thread development and nodule invasion. the EPS signal appeared to be specific, since a mutant that produced a chemically altered form of succinoglycan failed to invade nodules. Furthermore, we have examined the properties of succinoglycan that rescued the nodule invasion defect of *exo* mutants. Of seven fractions that varied only in repeat unit multiplicity and the degree of modification by carboxylated substituents, only a tetrameric form with a relatively high degree of modification induced nodule invasion. EPSs of different repeat unit structure from other species failed to induce nodule invasion. Interestingly, *R. meliloti* produced a second EPS under certain conditions, and genetic studies showed that the EPS also induced nodule invasion.

Virulence Determinants of Oral Pathogens

Barry McBride
University of British Columbia

The oral cavity compiles a number of unique ecosystems each with its own characteristic flora. Numbered amongst the many species that populate these micro-environments are organisms capable of initiating oral and systemic disease. Many of these pathogens can be found in the gingival sulcus; the area between the tooth and the gingiva. In periodontal disease the sulcus becomes enlarged and thus provides a home for a large population of bacteria. The heterogeneous gingiva population undergoes a developmental phase in which there is a population shift from predominantly facultative, gram-positive organism to anaerobic and gram-negative organisms. Indeed in the latter are the pathogens.

Treponema denticola is considered one of the important etiologic agents of periodontal disease. It accumulates in large numbers, invades tissue and produces putative virulence determinants. Included are adhesions which mediate binding to target tissues, agents which induce a cellular response leading to lysis and a cell bound protease. The protease degrades a number of matrix proteins and allows the organism to degrade and pass through basement membrane. Adherence is mediated at least in part by a 53 kD protein which exists in its native state as an oligomer forming an hexagonal array in the outer sheath of the organism.

Genetics and physiology of *Methanobacterium thermoautotrophicum*

David P. Nagle, Jr.
University of Oklahoma, Norman, OK

Our long-term goal is to understand the metabolic machinery of the archaeobacterium, *Methanobacterium thermoautotrophicum* using genetic and biochemical approaches. The results will be used in genetic manipulations

for exploitation of methanogens. *M. thermoautotrophicum* Marbur is a thermophilic autotroph in which metabolic and inhibition studies were straightforward, and resistant mutants could be isolated. We reported transformation of *M. thermoautotrophicum* to base analog resistance. Results with the method have been variable, and not always successful. Thus, we tried to determine specific biological factors involved in this variability and found that the nutritional state of the recipient cells was critical. We are pursuing the use of electroporation (electrotransformation) to improve our transformation system.

Pure and pyrimidine pathways in this organism were determined. Base analog-resistant mutants, cross-resistances to different analogs and enzyme levels in extracts permitted the deduction of pathways which are similar to those found in familiar organisms such as *E. coli* and yeast. Lumazine (2,4-pteridinedione) was a strong inhibitor of methanogenesis. Although methanogens lack folic acid, they use pteridines, flavins, and deazaflavins in central roles. The mechanism of inhibition of methanogenesis by central roles. The mechanism of inhibition of methanogenesis by this pteridine analog may be informative. Preliminary data indicate that lumazine affects the methyl-S-CoM reductase or the CoM-S-S-HTP reductase. We isolated a formate auxotroph of *M. thermoautotrophicum* which suggested a novel anabolic role for formate which is not yet understood. A new formate dehydrogenase, presumably an anabolic enzyme, has been purified 200-fold from the organism.

Selenoenzyme and seleno-tRNA biosynthesis

Thressa C. Stadtman
NIH, Bethesda, MD

A brief discussion of the selenoenzymes and seleno-tRNAs that have been isolated from *Methanococcus vannielii* and studied in my laboratory was presented at the beginning of the lecture. These are a selenium-dependent formate dehydrogenase and a hydrogenase that contain selenocysteine and certain tRNAs that contain 2-selenouridine residues in the "wobble position" of their anticodons.

Both seleno-tRNA formation and specific incorporation of selenocysteine into proteins are defective in *Salmonella* and *E. coli selD* mutants. The lesion involves the failure to synthesize an essential reactive selenium donor. This compound, formed from ATP and selenide, has been shown ³¹P NMR to contain selenium bonded directly to phosphorus. The products of the reaction are selenophosphate, AMP and orthophosphate, 1:1:1. The enzyme that catalyzes this 37 kDa protein that contains 7 cysteine residues, two of which are located at positions 17 and 19 in the sequence -Ala-Gly-Cys-Gly-Cys-Lys-Ile- [Leinfelder *et al.*, PNAS 87, 543 (1990)]. The enzyme is inactivated by alkylation and by site directed mutagenesis it has been shown that Cys-17 is essential for activity. Replacement of Cys-19 with serine had no effect on activity of the enzyme. Since all cysteine residues in wild type and mutant forms of the enzyme are titratable without prior reduction the mechanism of reaction with selenide does not involve cleavage of a disulfide in the enzyme. Demonstration of AMP exchange with ATP in the absence of selenide indicates that the initial step in the reaction sequence involves formation of an E-pyrophosphate intermediate. An essential basic group, either Lys at position 20 or the Arg replacement (but no Gln) may be the site of the pyrophosphate attachment. The isolated ⁷⁵Se-labeled selenophosphate produced by selenophosphate synthetase, complemented the *Salmonella selD* mutant enzyme preparation for synthesis of 2-selenouridine from 2-thiouridine in tRNAs. Selenophosphate is an oxygen-labile relatively unstable compound.

Enzymes Involved in Methanogenesis from Carbon Dioxide

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 Biologie der Philipps-Universität and Max-Planck-Institut
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 Marburg, GERMANY

The reduction of CO₂ to methane proceeds via carrier bound C₁-units. The following intermediates have been identified: *N*-formylmethanofuran, N⁵-formyltetrahydromethanopterin, N⁵, N¹⁰-methenyl-tetrahydromethanopterin, N⁵, N¹⁰-methylenetetrahydromethanopterin, N⁵-methyltetrahydromethanopterin, and coenzyme M. The 7 enzymes catalyzing the interconversion of these carrier-bound C₁-units have been isolated and characterized. The presentation concentrates on the properties of formylmethanofuran dehydrogenase which mediates the first step in methanogenesis, the reversible reduction of CO₂ plus methanofuran (MFR) to N⁵-formylmethanofuran (CHO-MFR).

The formylmethanofuran dehydrogenase from *Methanobacterium thermoautotrophicum* and from *Methanosarcina barkeri* are shown to be molybdenum-iron-sulfur proteins. In both enzymes the molybdenum is bound to molybdopterin guanine dinucleotide. The enzyme from *M. thermoautotrophicum* also contains molybdopterin. Evidence is presented that in *Methanobacterium wolfei* two formylmethanofuran dehydrogenases exist, one of which contains tungsten rather than molybdenum. The molecular and kinetic properties of the two isoenzymes are compared.

Methanopterins

Godfried Vogels
 University of Nijmegen

Methanopterin (MPT) contains as common structural elements a pterin group connected by an ethyl bridge to substituted tetrahydroxypentitylaniline. Starting from the 5-OH position of the pentose, the side chain extends with ribose bound via a phosphate ester to α-hydroxyglutarate and additional amino acids. Though the presence of MPT is, with a single exception (*Archaeoglobus*), limited to the methanogenic bacteria, various analogs are encountered among methanogenic species. The analogs differ with respect to substitutions at the pterin and aniline moieties and/or amino acid composition. A methyl group is present at the 7-pterin position of methanopterin and sarcinapterin. The aniline group is replaced by 2-aminoresorcinol in thermopterin. Sarcinapterin contains a glutamate whereas aspartate is present in tatiapterin and thermopterin.

Notwithstanding the structural diversity the function is unique; within a single species MPT derivatives obtained from other methanogens are catalytic active. Like its ubiquitous counterpart, H₄folate, MPT acts in the 5,6,7,8-tetrahydropterin state of reduction (H₄MPT) as the carrier of C₁ units in the formyl through methyl state of reduction. The structural modifications compared to H₄folate make H₄MPT pre-eminently designed to function as a catalyst in the specific methanogenic reactions.

Concluding Remarks

Ralph S. Wolfe

University of Illinois

The theme of this symposium has been microbial diversity, metabolism and physiology, and the Department of Microbiology at the University of Illinois has been a fertile place for me to have pursued these research topics. Diverse microbes and their physiology have included *Gallionella*, *Beggiatoa*, *Streptococcus allantoicus*, *Sphaerotilus natans*, *Arthrobacter crystallopoietes*, *Mycobacter* AL-1, *Clostridium tetanomorphum*, *Geodermatophilus*, *Aquaspirillum magnetotacticum*, *Rhodospseudomonas chromatium*, *Chlorobium*, *Rhodomicrobium*, *Methanogenium cariaci*, *Methanogenium marisnigri*, *Methanococcus voltae*, *Methanococcus jannaschii*, *Methanogenium thermophilum*, *Acetobacterium woodii*, *Methanospirillum hungatei*, *Acetogenium kivii*, and *Sarcina ventriculi*. My research program has involved 7 new coenzymes, the first being ferredoxin. Study of the biochemistry of methanogenesis led to the discovery and structural determination of coenzyme M (the coenzyme of methyl transfer), coenzyme F₄₂₀ (a deazaflavin involved in hydride transfer), 7-mercaptoheptanoylthreonine phosphate (the electron donor for the methylreductase), and methanofuran (the coenzyme of formylation). The formaldehyde activation factor was found to be tetrahydromethanopterin and its role as a C₁ carrier at the formyl, methenyl, methylene and methyl levels was elucidated. The structure of methenyltetrahydromethanopterin was determined in the laboratory of Vogels. Factor (F₄₃₀) was found to contain nickel and to be the chromophore of the methylreductase. Its structure was determined by the laboratories of Thauer and Eschenmoser as a tetrahydrocophin, the first nickel tetrapyrrole to be found. It is a pleasure to thank all of the graduate students and postdoctoral students who have made contributions to my research program in microbial diversity, metabolism, and physiology.

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