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Energetics and Kinetics of Anaerobic Aromatic and Fatty Acid Degradation

Progress Report

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I. Summary of work accomplished.

The kinetics of benzoate degradation by the anaerobic syntrophic bacterium, *Syntrophus buswellii*, was studied in coculture with *Desulfovibrio* strain G11. The threshold value for benzoate degradation was dependent on the acetate concentration with benzoate threshold values ranging from 2.4 μM at 20 mM acetate to 30.0 μM at 65 mM acetate. Increasing acetate concentrations also inhibited the rate of benzoate degradation with a apparent K_i for acetate inhibition of 7.0 mM. Interestingly, hydrogen concentrations after the benzoate threshold value was reached were lower (4×10^{-7} atm) compared to hydrogen concentrations before the threshold was reached (1×10^{-5} atm). Also, lower threshold values were obtained when nitrate rather than sulfate was the terminal electron acceptor. The Gibbs free energy for benzoate degradation when the benzoate threshold was reached was -43 to -46 kJ mol^{-1}. These data are consistent with a thermodynamic explanation for the threshold, and suggest that there is a minimum Gibbs free energy value required for the degradation of benzoate.

An acetoacetyl-CoA thiolase has been isolated from *Syntrophomonas wolfei* ; it is apparently a key enzyme controlling the synthesis of poly-ß-hydroxyalkanoate from acetyl-CoA in this organism. Kinetic characterization of the acetoacetyl-CoA thiolase from *S. wolfei* showed that it is similar in its structural, kinetic, and apparent regulatory properties to other biosynthetic acetoacetyl-CoA thiolases from phylogenetically distinct bacteria that synthesize PHA. Intracellular concentrations of CoA and acetyl-CoA are believed to be critical factors regulating the activity of the acetoacetyl-CoA thiolase in *S. wolfei*.

We have also isolated and characterized several new halophilic anaerobic fermentative anaerobes. Phylogenetic analysis indicates that one of these bacteria is a new species in the genus, *Haloanaerobium*. Two other species appear to be members of the genus, *Halobacteroides*. Several halophilic acetoclastic methanogenic bacteria have also been isolated and their physiological properties are currently under investigation. We have also isolated an acetate-using dissimilatory iron-reducing bacterium. The physiological properties are similar to strain GS-15.

II. Work Accomplished.

A. Energetics of benzoate degradation.

The kinetics of benzoate degradation by the anaerobic syntrophic bacterium, *Syntrophus buswellii*, in coculture with *Desulfovibrio* strain G-11 was studied. Benzoate consumption reached a threshold value below which no further degradation of benzoate was observed even after extended incubations. The benzoate threshold value depended on the amount of substrate and/or acetate added. A benzoate threshold value of 2.4 μM was observed when 20 mM sodium acetate was added. The benzoate threshold value was higher (30 μM) when 65 mM sodium acetate was added. The rate of benzoate degradation was inhibited by acetate with a k_i of 7 mM for acetate. Equal concentrations of sodium chloride did not result in a benzoate threshold

value, and had no effect on benzoate degradation rates compared to unamended controls. When nitrate rather than sulfate was the electron acceptor, the benzoate threshold value was not observed even in the presence of 65 mM sodium acetate. This showed that the benzoate threshold value was not caused by high concentrations of the undissociated form of acetate. Interestingly, the hydrogen concentration was lower after the benzoate threshold value was reached (4×10^{-7} atm) compared to hydrogen concentrations before the threshold value was reached (1×10^{-5} atm). This shows that the benzoate threshold was not caused by hydrogen inhibition. Similar values for the Gibbs free energy for benzoate degradation were obtained when the threshold value was reached, ranging from -43 to -46 kJ mol^{-1}. These observations are consistent with a thermodynamic explanation for the benzoate threshold value, and suggest that a minimum Gibbs free energy value is required for the degradation of benzoate by the syntrophic coculture.

An acetoacetyl-CoA thiolase has been isolated form *Syntrophomonas wolfei*. Studies on the substrate specificity of the enzyme support its role in the synthesis of poly-ß-hydroxyalkanoate from acetyl-CoA in *S. wolfei*. The acetoacetyl-CoA thiolase was separated from the majority of the enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities and purified to homogeneity. The purified acetoacetyl-CoA thiolase fractions contained low levels of crotonase activity, but a separate crotonase enzyme was not discernible by native gel electrophoresis. This suggests that the thiolase may form an easily dissociated complex with the other ß-oxidation enzymes. The acetoacetyl-CoA thiolase had a native molecular weight of about 160,000, and was composed of a single polypeptide of 41,000 molecular weight. This suggest a homoterameric arrangement of the native enzyme. Kinetic parameters for the thiolytic cleavage reaction of the acetoacetyl-CoA thiolase were a k_m^{app} of 23 µM and a V_{max}^{app} of 161 µmol $min^{-1}mg^{-1}$; those for the condensation reaction were a k_m^{app} of 294 µM and a V_{max}^{app} 31 µmol $min^{-1}mg^{-1}$. The reaction mechanism is Ping Pong Bi Bi. The enzyme is product inhibited in both directions with a k_i^{app} of 3.2 µM for CoA and of 0.5 mM for acetyl-CoA. The regulation of PHA synthesis is thought to be achieved by regulating the activity of the acetoacetyl-CoA thiolase. The concentrations of CoA and acetyl-CoA may be critical factors governing the activity of this enzyme.

Our benzoate-degrading syntrophic bacterium grows in coculture with *Desulfovibrio baarsii* which uses formate, but not H_2. This indicates that *S. buswellii* can produce formate, and suggests that formate may be an intermediate during syntrophic metabolism. In addition to benzoate, our strain uses ferulic acid and *p*-hydroxybenzoate in cocuiture with *Desulfovibrio* strain G11. This suggests that this organisms may be a different species than *Syntrophus buswellii*.

We have isolated and characterized several halophilic anaerobic fermentative bacteria from oil field brines. All strains required a minimum of 6 to 9% NaCl for growth in complex medium. All strains were gram-negative, catalase negative, non-spore-forming rods that were inhibited by chloramphenicol, tetracycline, and penicillin, but

not cyclohexamide or azide, indicating that the isolates are eubacteria and not archaebacteria. All strains utilized carbohydrates, but did not use amino acids or several of the tested aromatic compounds as energy sources, except VS-752 and TTL-30 which used pyrogallic acid. Glucose was fermented to H_2, CO_2, ethanol, and acetate by all strains. Formate was an additional product from glucose in the case of strains VS-732 and VS-751. The change in gas phase from N_2/CO_2 to H_2/CO_2 stimulated the growth of strains VS-732 and VS-511, but completely inhibited the growth of strain TTL-30. The presence of a carbon monoxide gas phase at any of the tested concentrations was inhibitory to all strains. Methane was not produced by any of the strains. These data provide further evidence for the importance of eubacteria in extremely saline environments.

A dissimilatory Fe(III)-reducing microorganism was isolated from sediments of a hydrocarbon contaminated ditch in Norman, Okla. The isolate was an obligately anaerobic, non-motile, gram-negative rod and was designated strain PCA. PCA grew in a defined medium with acetate as an electron acceptor, and ferric phosphate, ferric oxyhydroxide, or ferric citrate as the sole electron acceptor. PCA did not reduce Fe(III) with glucose, lactate, formate, butyrate, propionate, ethanol, succinate, isobutyrate, isovalerate, phenol, benzoate, yeast extract, or hydrogen as an electron donor. Acetate oxidation was coupled to Co(III)-EDTA reduction, but not to Mn(IV), nitrate, sulfate, sulfite, or fumarate reduction. PCA contains *c*-type cytochromes and has a membrane fatty acid profile similar to *Geobacter metallireducens*. Atypical of previously described Fe(III)-reducing bacteria, PCA exhibits a very narrow substrate range, and is thus a unique addition to the relatively small group of respiratory metal-reducing microorganisms in pure culture.

III. Publications and Presentations

A. Manuscripts in press (preprints attached)

1. M. J. McInerney and N. Q. Wofford. 1992. Enzymes involved in crotonate metabolism in *Syntrophomonas wolfei*. Arch. Microbiol. 158: in press.

2. D. A. Amos and M. J. McInerney. 1992. Formation of D-3-hydroxybutyryl-coenzyme A by an acetoacetyl-coenzyme A reductase in *Syntrophomonas wolfei* subsp. *wolfei*. Arch. Microbiol. 158: in press.

3. M. J. McInerney, D. A. Amos, K. S. Kealy, and J. A. Palmer. 1992. Synthesis and function of polyhydroxyalkanoates in anaerobic syntrophic bacteria. FEMS Microbiol. Rev. in press.

B. Manuscripts in preparation.

1. B. S. Hopkins and M. J. McInerney. Benzoate degradation by a syntrophic coculture: evidence for a threshold for benzoate degradation, and growth of the

benzoate degrader in pure culture. in preparation for Appl. Environ. Microbiol.

2. V. Warikoo and M. J. McInerney. Energetic and kinetics of benzoate degradation by a syntrophic coculture. in preparation for Appl. Environ. Microbiol.

3. K. Kealy and M. J. McInerney. Purification and properties of an acetoacetyl-coenzyme A thiolase involved in polyhydroxyalkanoate synthesis in *Syntrophomonas wolfei*. in preparation for J. Bacteriol.

4. M. J. McInerney and N. Q. Wofford. H_2 production from NADH by cell-free extracts of *Syntrophomonas wolfei*. in preparation for Appl. Environ. Microbiol.

C. Presentations.

1. V. Warikoo and M. J. McInerney. 1992. Energetics and kinetics of anaerobic benzoate degradation. Abstr. Ann. Meet. Amer. Soc. Microbiol. I7, p. 238. American Society for Microbiology, Washington D. C.

2. M. J. McInerney. 1992. Synthesis and function of polyhydroxyalkanoates in anaerobic syntrophic bacteria. International Symposium on Bacterial Polyhydroxyalkanoates, ISBP'92, L4. Gottingen, Germany.

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