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THE ROLE OF PURINE DEGRADATION IN METHANE BIOSYNTHESIS AND ENERGY PRODUCTION IN METHANOCOCCUS VANNIELII

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Progress Report

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Personnel. My grant began March 1, 1989. I was able to hire a technician in late May, 1989. In August, 1989 I hired an undergraduate primarily for the purpose of growing cells for enzyme and cofactor preparations. The technician has now been trained in various aspects of anaerobic microbiology and biochemistry. The undergraduate can successfully grow and harvest large cultures of M. vannielii.

Below are listed the specific goals of the proposal. Included with each of the goals is a summary of the progress toward that specific goal.

1) Survey of other methanogens to assess how widespread the pathway of purine degradation is. This is not the high priority item in the proposal, so these studies will likely be among the last done.

2) Isolation and characterization of the enzyme and the products of the formiminoglycine cleavage reaction in M. vannielii. We plan to reconstitute a defined enzymatic reaction in which formiminoglycine is cleaved. The enzyme involved will be purified from large quantities of M. vannielii. We suspect that the methanogen cofactor, tetrahydromethanopterin (MPT), is required in this reaction. Unfortunately, this cofactor is not, to my knowledge, commercially available. For both the enzyme and the cofactor, we must grow large quantities of cells. We have, are currently, and will in the future continue to grow M. vannielii for these purposes. We have purified MPT from approximately 120 g of M. vannielii to be used in the assay of formiminoglycine cleavage. We have not started on the isolation of the enzyme that catalyzes this reaction.

I had not completely characterized all of the steps previous to formiminoglycine cleavage in the purine degrading pathway of M. vannielii. We have been attempting to identify 4-imidazolone as an intermediate in the pathway, but have not been successful to date.

3) Ascertaining of the metabolic fate of glycine formed from the cleavage of formiminoglycine. Certain Clostridia can metabolize glycine by either of two pathways, thereby driving the synthesis of one mole of ATP from ADP. These two reactions are catalyzed by either glycine reductase or glycine decarboxylase. We have not detected any degradation of glycine by M. vannielii. We have specifically assayed for glycine reductase, but have seen no enzymatic activity. The proposal is not concerned with anabolic biosynthetic fates of glycine, so these pathways have not been investigated. As far as we can tell, there is no catabolism of glycine by M. vannielii.

4) Elucidation of the route by which the formyl moiety of formiminoglycine is incorporated into the methane biosynthetic pathway. These studies eventually will likely depend upon successful resolution of goal 2) above. However the results of our stable isotope-mass spectrometry studies should shed some light on this goal. Originally I described experiments in which [8-^{13}C]xanthine would be provided to cells of M. vannielii growing with formate as the main carbon source. The atom percent ^{13}C in various metabolites of cellular compounds would then be examined by mass spectrometry. If xanthine is metabolized by M. vannielii in a manner similar to the pathways used by the Clostridia, then it is possible, by examining the atom percent ^{13}C in certain carbon atoms of specific compounds isolated from cells grown under these conditions, to conclude whether these carbon atoms passed through

any of the various forms of MPT. For example, if as the result of such an experiment, it were found that the atom percent ^{13}C in methane, was the same as the atom percent ^{13}C in the methyl group of methionine or the methyl group of thymine, then it may be concluded that the 8-carbon of xanthine is metabolized through various forms of MPT in a manner analogous to the routes used by the Clostridia with tetrahydrofolic acid.

After some additional thought, I decided that we should be able to obtain similar answers by doing the experiments in an easier and cheaper fashion. In these experiments we would grow the cells on $^{13}CO_2$ and H_2 and add unlabeled uric acid. The 2- and 6-carbons are lost as CO_2 so should only slightly dilute the ^{13}C content of that pool, whereas the 8-carbon should, if it is metabolized via derivatives of MPT, significantly dilute the atom percent ^{13}C in those pools. If we could grow M. vannielii with CO_2 as the main carbon source instead of formate, yet still have purines provide the sole nitrogen requirement of the bacterium, then we could take advantage of several things. 1) We would not have to synthesize and purify [8-^{13}C]xanthine, 2) we could use any purine, uric acid being the number one choice because of its solubility in aqueous solutions relative to the other purines, and 3) we would save money on labeled chemicals. The latter is possible because labeled sodium bicarbonate is cheaper than the labeled formate and formic acid required for synthesis of [8-^{13}C]xanthine. Also, by switching to metabolism on CO_2/H_2, less label is wasted. With formate as an energy source, only one-fourth of the substrate actually gets converted to methane. With the carbon source CO_2/H_2, all (except that required for anabolic reactions) of the substrate gets converted to methane.

Toward the end described above we have accomplished several things. We have successfully grown M. vannielii without formate, but with CO_2/H_2 as the main carbon and energy source and with uric acid as the sole nitrogen source. We have determined the minimum culture size (approximately 100 ml) from which we may obtain significant results. We have successfully carried out, with unlabeled cultures, the cellular amino acid isolation, derivatization, and analysis by gas chromatography-mass spectrometry. We have successfully isolated and analyzed methane and CO_2 from these cultures. As soon as we come up with the best way of introducing labeled CO_2 to the cultures, we should have some answers.

Determination of the percent methane and amino acid synthesis originating from the purine degrading pathway of M. vannielii. In order to do these experiments in the most efficient manner, we need to discover the minimum amount of formate or CO_2 required by M. vannielii to grow. We will then try to lower this amount by adding uric acid with the anticipation that the carbon atoms released by degradation of this purine will be able to replace the more usual single-carbon substrates.

Our results thus far have indicated that we should do these experiments with cells growing with CO_2/H_2 as the main carbon and energy source. The main problem with growth on formate is that it requires continuous pH adjustment through addition of formic acid, so there are wide fluctuations of substrate present in the growth medium. Also, the constant additions do not make for accurate determinations of substrate. It seems likely, from our results so far that the absolute contribution of purines toward carbon and energy metabolism in methanogens is constrained by the somewhat low solubilities of these compounds in aqueous media. However, we are trying to raise the rela-

tive contribution of purines by lowering the concentration of CO_2 in the cultures. Thus far we have managed to get growth of M. vannielii in cultures with 11 mM uric acid and 70 mM CO_2. We are also trying to determine the best way of presenting these data. It is likely that as we lower the CO_2 levels, the final amount of cell mass will be proportionally lower. Consequently, we are currently presenting our data as percent carbon supplied by some purine per mg protein synthesized per day.