NOTICE

CERTAIN DATA CONTAINED IN THIS DOCUMENT MAY BE DIFFICULT TO READ IN MICROFICHE PRODUCTS.

DOE/ER/13995--2

DE91 005722

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

Progress Report

March 1, 1990 to February 28, 1991

Edward DeMoll
Department of Microbiology and Immunology
University of Kentucky
Chandler Medical Center
Lexington, Kentucky 40536-0084

October 22, 1990

PREPARED FOR THE U.S.DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG05-89ER13995

Personnel. The technician I had for a year decided to go to the Seminary. She quit March 1, 1990. I was able to hire another technician at the beginning of June, 1990. I hired her with the assurance that she would work for me at least two years. She quit around the October 1st, because of boyfriend problems. I have now hired another technician, who already has some lab experience. He is excellent and is married.

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. If I ever get to this item in the proposal, it will be toward the end of the project.

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 now know that tetrahydromethanopterin (MPT), is required in this reaction. We can get a dose response with added MPT for the conversion of formiminoglycine to glycine catalyzed by a relatively cofactor-free extract of M. vannielii. MPT is time-consuming and expensive to make, so we are trying to recover it from assays and reuse it in others. One other problem that we have been having is that around 25 to 50% of the MPT we purify appears to be irreversibly oxidized, so will not function biologically. I have spoken to others who are studying reactions that involve MPT, and they also see a certain fraction of their purified MPT in the oxidized form. We can purify the oxidized form away, but do not really need to for most of our qualitative tests. We are probably ready to start purifying an enzyme activity now.

I believe I have now characterized to a reasonable degree, the entire purine-degrading pathway used by M. vannielii. I have prepared a manuscript and will send it off when the figures are finished. After I do, I will send in the appropriate forms and a copy of the paper.

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 formerly reported that we had seen no glycine degradation by by M. vannielii. We have now, by employing a more sensitive radioactive assay, and also by our finding that glycine can supply the nitrogen requirement for M. vannielii. One problem that we were evidently having before was that the reaction requires MPT, and without the methane biosynthetic to rejuvinate the free form of MPT by sending C_1 units to CoM then methane, the assay quickly runs out of free MPT and slows to a stop. By adding more MPT to the assay, we can extend the length of the linear response (release of CO_2 from glycine). Glycine is cleaved by a glycine decarboxylase-like activity. This is an enzyme activity found in several bacteria, including E. coli. We can easily see $^{14}CO_2$ formed from [1-^{14}C]glycine.

4) Elucidation of the route by which the formyl moiety of formiminoglycine is incorporated into the methane biosynthetic pathway. I plan to do as much of this work as I can with the mass spectrometry experiments described in my proposal and in last year's report. Toward this end I have gotten the mass spectrometric analysis of amino acids working. I am ready to do labeling experiments as soon as I get my technician trained to do a few more things. These studies eventually will likely depend upon successful resolution of goal We have started trying to synthesize various forms of MPT and identify them by their spectra. This is using up a lot of MPT, so we will do as much enzymology as we can with MPT first.

Determination of the percent methane and amino acid synthesis originating from the purine degrading pathway of M. vannielii. The main problem with getting a maximum value for methane synthesis originating from purines is that purines are not very soluble in aqueous solution. Our results so far show that as far as they are soluble, xanthine and uric acid are capable of replacing a certain amount of formate as carbon and energy source. But we don't see a big difference, and we are right at the limit where we can see a difference between purine and no purine.

Our finding that glycine is metabolized by M. vannielii does allow us to determine to what extent glycine can replace formate or CO_2 as carbon and energy source. In doing these experiments we won't have to lower the formate or CO_2, as glycine is very soluble, we will be able to simply measure by how much unlabeled glycine is able to reduce the incorporation of labeled formate or CO_2 into methane. We should be able to raise glycine levels at will. We will have to check for growth inhibition by excessive glycine levels.

That glycine is metabolized by M. vannielii will also allow us to determine the extent to which carbon atoms are free to exchange in and out of the methane biosynthetic pathway. This allows us to introduce a double label into the methanogen. Carbon of natural abundance (99% C-12, 1% C-13) is added as formate and carbon enriched (99%) in C-13 is added as either [1-^{13}C]glycine or [2-^{13}C]glycine. Since MPT evidently replaces tetrahydrofolic acid in methanogen biochemistry, an obvious question posed is whether carbon atoms that enter the methane biosynthetic pathway remain more or less channeled into that pathway or whether they are free to be used in other metabolic reactions. For instance, N^5-methyl-MPT is a component of the methane biosynthetic pathway, but it is also the source of the 6-C atom of methionine in its synthesis from homoserine. We are now doing mass spectrometry experiments to measure the amount of carbon in the methane biosynthetic pathway that is diverted for use in other metabolic steps.

RELATED STUDIES.

Studies on xanthine dehydrogenase from M. vannielii. I had previously purified this enzyme and did some partial characterizations. I have begun some additional characterization of the enzyme. We are trying to determine the selenium content of the enzyme. Also we are trying to determine the chemical linkage of the Mo-cofactor to the enzyme. It has been shown that mammals have the cofactor linked through GMP and that E. coli has its cofactor linked through CMP. We would like to see how Archaebacteria do it, so we are using xanthine dehydrogenase from M. vannielii as a model.

Pyrimidine degradation by M. vannielii. We have done kinetic experiments with crude extracts of M. vannielii. These studies show that uracil can competitively inhibit the degradation of purines in this bacterium. Preliminary evidence indicates that this takes place in the first one or two steps of the degradation. This likely indicates that M. vannielii is using the same enzymes, at least initially, for the degradation of both purines and pyrimidines. This is not surprising in light of their structural similarities, and because they may both be used as nitrogen sources.

END

DATE FILMED

01 / 30 / 91