CURRENT MICROBIOLOGY, Vol. 10 (1984), pp. 195–198

Current Microbiology

Lysine Biosynthesis in *Methanobacterium thermoautotrophicum* Is by the Diaminopimelic Acid Pathway

Nouna Bakhiet,† Fred W. Forney,‡** Donald P. Stahly,† and Lacy Daniels†*

†Department of Microbiology, University of Iowa, Iowa City, Iowa 52242; and ‡Division of Natural Sciences and Mathematics, College of St. Teresa, Winona, Minnesota 55987, USA

Abstract. *Methanobacterium thermoautotrophicum,* an archaebacterium, possesses the first and last enzymes of the diaminopimelic acid pathway for lysine biosynthesis, dihydrodipicolinate synthase, and diaminopimelate decarboxylase. It does not have saccharopine dehydrogenase, the last enzyme of the aminoadipate pathway for lysine biosynthesis. The dihydrodipicolinate synthase is inhibited but not repressed by lysine. We conclude that this microbe uses the diaminopimelate pathway for synthesis of lysine.

Methanobacterium thermoautotrophicum is a thermophilic archaebacterium capable of making methane and cell material from H_2 and CO_2 [5, 22]. Cells of archaebacteria are considered to be sufficiently different from typical procaryotic bacterial cells and eucaryotic cells to justify classifying them in a separate kingdom [5]. Unusual characteristics of the methanogenic archaebacteria include the lack of D-alanine and peptidoglycan and the presence of distinctly different ribosomal RNA species and unusual cofactors. In some ways, e.g., RNA polymerases and elongation factor 2, archaebacteria more closely resemble eukaryotes. Because of these differences it is conceivable that some of these organisms synthesize their amino acids differently from eubacteria. In *M. thermoautotrophicum* (and probably most methanogens) all amino acids ultimately arise from CO_2. The organic precursors from which the amino acids are derived result from operation of a non-Calvin cycle autotrophic pathway resembling a partial reversed tricarboxylic acid cycle [4, 6, 7]. The amino acids alanine, aspartate, and glutamate are formed in a normal fashion by transamination of the complementary keto acids [12]. Early work on "*Methanobacterium omelianskii*" [3, 13] indicated that aspartate, alanine, glycine, serine, threonine, lysine, proline, methionine, and valine were made by normal bacterial pathways. However, the culture is now known to have been composed of at least two species, one of which was a eubacterium [3].

The mechanism of lysine biosynthesis is of particular interest to those studying phylogenetic relationships, because two different pathways exist in nature; the diaminopimelic acid pathway and aminoadipic acid pathway [15, 18, 19, 20]. The diaminopimelic acid pathway is used by bacteria, some fungi, some algae, and higher plants. The aminoadipic acid pathway is used by most of the fungi and some algae. No organism is known to possess both pathways [14]. In this study we found that one archaebacterium, *M. thermoautotrophicum,* has the first and last enzymes of the diaminopimelate pathway, dihydrodipicolinate synthase and diaminopimelate decarboxylase. Therefore, the microbe derives its lysine from the diaminopimelate pathway [1]. Our conclusions are supported by recent ^{13}C-NMR data of Ekiel et al. (I. Ekiel, I. Smith, and G. D. Sprott, unpublished observations) on *Methanospirillum hungatei.*

Materials and Methods

Growth of organisms. *Methanobacterium thermoautotrophicum* strain ΔH (ATCC #29096) [22] was grown anaerobically at 62°C in a medium containing the following components (g/liter): KH_2PO_4, 0.6; NH_4Cl, 2.0; $MgCl_2 \cdot 6H_2O$, 0.04; mineral elixir, 10; Na_2CO_3, 0.28. Na_2S was added after the medium was made anaerobic to make the culture 1 m*M* in sulfide. The mineral elixir contained the following components (mg/liter): nitrilotriacetic acid, 5000; $FeCl_3 \cdot 4H_2O$, 500; $CoCl_2 \cdot 6H_2O$, 5; $Na_3Mo_4 \cdot 2H_2O$, 5; $NiCl_2 \cdot 6H_2O$, 24. Cells used for most of the experiments were grown in a 14-liter fermentor (New Brunswick Scientific Co., Inc.), continually gassed with a mixture of H_2 and CO_2 (80:20, vol/vol). Cells used for the repression study were grown in the

* To whom reprint requests should be addressed.
** Deceased.

Table 1. Specific activities of selected lysine biosynthetic enzymes in initial cell extracts of *Methanobacterium thermoautotrophicum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae*

Enzyme	Specific activity		
	M. thermo-autotrophicum	*B. subtilis*	*S. cere-visiae*
Dihydrodipicolinate synthase[a]	0.1	2.0 [10][d]	NDA[e]
Diaminopimelate decarboxylase[b]	0.0002	0.049 [9]	NDA
Saccharopine dehydrogenase[c]	0	NDA	0.1 [8]

[a] Specific activity equals ΔA_{520}/min/mg protein.
[b] Specific activity equals μmoles of CO_2 evolved/min/mg protein.
[c] Specific activity equals μmoles of NADH oxidized/min/mg protein.
[d] [ ] denotes reference.
[e] NDA denotes no data available, assumed to be zero.

presence and absence of D, L-lysine in the same medium (70 ml) in 250-ml tightly stoppered serum bottles (Wheaton Scientific Co.) pressurized to 20 psi with a mixture of H_2 and CO_2 (80:20, vol/vol), using an adaptation of the method described by Balch and Wolfe [2]. The bottles were shaken in a waterbath at 62°C and repressurized three times per day for three days. Fermentor cultures were harvested in a Sharples centrifuge, frozen in pellets with liquid N_2, and kept at −70°C until used. Bottle-grown cells were harvested by centrifugation and washed once with 50 m*M* tris (hydroxymethyl) aminomethanehydrochloride (Tris-HCl) buffer, pH 7.5. Both types of cells were suspended in the same buffer and broken in a French pressure cell at approximately 20,000 psi. Cell debris was removed by centrifugation at 23,600 *g* for 10 min. The supernatant dialyzed against the same buffer was used for assay of dihydrodipicolinate synthase and saccharopine dehydrogenase. Cells grown for assay of diaminopimelate decarboxylase were broken in buffer A of Rosner [17], and the supernatant resulting from centrifugation was dialyzed against the same buffer.

Enzyme assays. The enzymatic assay procedure for dihydrodipicolinate synthase was previously described [11]. Unless otherwise specified, the pyruvate and L-aspartic β-semialdehyde concentrations were 50 m*M* and 10 m*M*, respectively, the pH was 8.6, and the temperature was 37°C. Diaminopimelate decarboxylase was measured at 37°C and 57°C by the procedure of Rosner [17] using 10 m*M* [1, 7-^{14}C] diaminopimelate as substrate (5550 disintegrations per minute [dpm]/μmol of DL and meso isomers; Amersham Searle Corp., Arlington Heights, IL). The assay procedure used for saccharopine dehydrogenase was described by Nakatani et al. [16]. The concentrations of L-lysine, α-ketoglutarate, and NADH were 2.67 m*M*, 2.67 m*M*, and 0.0867 m*M*, respectively, and the assay temperature was 22°C.

Lysine assimilation. Uptake of lysine was studied by growing cells in serum bottles (as described above) in medium containing 5 m*M* [^{14}C] D, L-lysine (95,000 dpm/μmol). The ml samples were taken at intervals, examined for turbidity at 660 nm, centrifuged at 2000 *g* for 30 min, and the cell pellet was washed twice with 15-ml portions of 5% trichloroacetic acid. The final pellet was resuspended with 0.5 ml of 0.5 *M* tris-HCl buffer, pH 7.5, and the radioactivity was determined by scintillation counting.

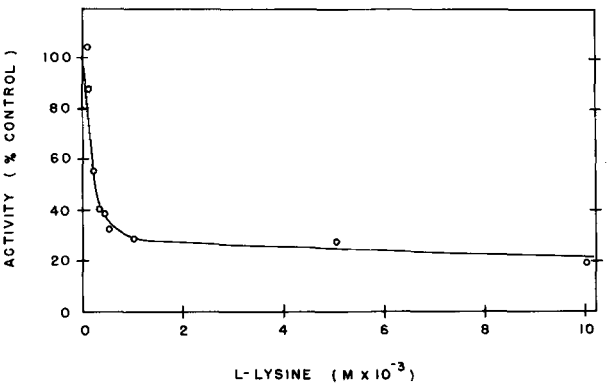

Fig. 1. Inhibition of dihydrodipicolinate synthase from *Methanobacterium thermoautotrophicum* by L-lysine.

Results and Discussion

Presence of diaminopimelic acid pathway. Table 1 shows the specific activities of the three lysine biosynthetic enzymes in initial cell extracts of *M. thermoautotrophicum*. These values are compared with published values for the same enzymes in a "typical" eubacterium, *Bacillus subtilis*, and in the yeast, *Saccharomyces cerevisiae*. *Bacillus subtilis* uses the diaminopimelate pathway for synthesis of lysine, whereas *S. cerevisiae* uses the aminoadipate pathway. The activities of the enzymes of the diaminopimelic acid pathway were detected in cell extracts of *M. thermoautotrophicum*, whereas the activity of the one enzyme of the aminoadipic acid pathway was not detected. These results strongly suggest that *M. thermoautotrophicum* derives its lysine from the diaminopimelate pathway. As indicated in Table 1, the specific activities of dihydropidicolinate synthase and diaminopimelate decarboxylase in *M. thermoautotrophicum* were 20 times and 245 times, respectively, less active than the same enzymes in *B. subtilis*. The generation times of *B. subtilis* and *M. thermoautotrophicum* (fermentor grown) were approximately 50 min and 15 h, respectively, an 18-fold difference. This may partially explain the difference in levels of lysine biosynthetic enzymes observed in the two microbes. Another factor to note is that the assays for these two enzymes were performed at 37°C with cell extracts from both microbes. It is probable that the activities of *M. thermoautotrophicum* enzymes would have been much higher if the assays were performed at 62°C, the optimum growth temperature. (Support for this was obtained from one

experiment in which diaminopimelate decarboxylase of *M. thermoautotrophicum* was assayed at 57°C. Specific activity of the enzyme was 7.1 times higher than that obtained when the assay was performed at 37°C.) Lysine auxotrophs of *B. subtilis* lacking diaminopimelate decarboxylase and lysine independent revertants were isolated by Rosner [17]. His results indicated that lysine independent growth of revertants occurred with a diaminopimelate decarboxylase specific activity at least 24 times less than that existing in the wild type strain. Thus, we conclude that the specific activities of these enzymes in *M. thermoautotrophicum* are sufficiently high to allow lysine biosynthesis.

After the conclusion of our work we became aware (D. Sprott, personal communication) of ^{13}C-NMR experiments (I. Ekiel, I. Smith, and G. D. Sprott, unpublished observations) demonstrating a ^{13}C-labeling pattern in *Methanospirillim hungatei* that was consistent with the operation of the diaminopimelic acid pathway normally found in bacteria. Thus, it seems certain that the methanogens, and perhaps other archaebacteria, use this pathway for lysine biosynthesis.

Properties of dihydrodipicolinate synthesis. We have partially characterized the dihydrodipicolinate synthase of *M. thermoautotrophicum*. The dihydrodipicolinate synthase activity as a function of time was linear. The pH optimum for activity was 8.6, with no activity below pH 6.5. The Km (pyruvate) and Km (L-aspartic β-semialdehyde) values were 0.83 m*M* and 22.2 m*M*, respectively. The enzyme activity was inhibited by lysine, but not diaminopimelate. As shown in Fig. 1, 20 m*M* lysine caused 80% inhibition. The concentration of lysine necessary to cause 50% inhibition was 0.25 m*M*. Cell extracts were prepared from cells grown in the absence of lysine and the presence of 8 and 15 m*M* D, L-lysine to determine whether lysine represses synthesis of dihydrodipicolinate synthase. No repression was observed.

The inhibition of dihydrodipicolinate synthase by lysine is similar to that observed in *Escherichia coli* [21]. No generalization can be made about inhibition and repression of this enzyme in different bacteria, since it is subject to negative control in some bacteria and not in others. It could be argued that lack of repression by lysine is due to the absence of uptake and intracellular accumulation of lysine. Although it is not known what cellular concentration of lysine results from growth in excess lysine, *M. thermoautotrophicum* does assimilate this amino acid into its protein from the medium during growth at a rate of about 0.8 nmol/mg cell dry weight/h. Assuming a cellular volume of approximately 1.5–2.5 μl/mg dry weight [14], the data of Kenealy et al. [12] suggest an internal soluble lysine concentration of about 1–2 m*M* in autotrophically grown cells. It is interesting to note that the approximate lysine level in cells grown on H_2/CO_2 alone is in a range that would allow lysine biosynthesis to be controlled by dihydrodipicolinate synthase activity variations resulting from small changes in cellular lysine concentrations (see Fig. 1).

Conclusions

Methanobacterium thermoautotrophicum (and probably all methanogens) biosynthesizes lysine via the diaminopimelic acid pathway. Dihydrodipicolinate synthase is inhibited by lysine, but extracellular lysine does not repress its synthesis.

Literature Cited

1. **Bakhiet, N., Forney, F. W., Stahly, D. P., Daniels, L.** 1983. Lysine biosynthesis in *Methanobacterium thermoautotrophicum* is by the diaminopimelic acid pathway. Abstracts of the Annual Meeting of the American Society for Microbiology **1983**:271.
2. **Balch, W. E., Wolfe, R. S.** 1976. New approach to cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Applied and Environmental Microbiology **32**:781–791.
3. **Bryant, M. P., Wolin, E. A., Wolin, M. J., Wolfe, R. S.** 1967. *Methanobacillus omelianskii,* a symbiotic association of two species of bacteria. Archives for Microbiology **59**:20–31.
4. **Daniels, L., Zeikus, J. G.** 1978. One-carbon metabolism in methanogenic bacteria: analysis of short term fixation products of $^{14}CO_2$ and $^{14}CH_3OH$ incorporated into whole cells. Journal of Bacteriology **136**:75–84.
5. **Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrsen, K. R., Chen, K. N., Woese, C. R.** 1980. The phylogeny of prokaryotes. Science **209**:457–463.
6. **Fuchs, G., Stupperich, E.** 1978. Evidence for an incomplete reductive carboxylic acid cycle in *Methanobacterium thermoautotrophicum*. Archives for Microbiology **118**:121–125.
7. **Fuchs, G., Stupperich, E., Thauer, R. K.** 1978. Acetate assimilation and the synthesis of alanine, aspartate and glutamate in *Methanobacterium thermoautotrophicum*. Archives for Microbiology **117**:61–66.
8. **Fujioka, M., Nakatani, Y.** 1970. A kinetic study of saccharopine dehydrogenase reaction. European Journal of Biochemistry **16**:180–186.
9. **Grandgenett, D. P., Stahly, D. P.** 1971. Repression of diaminopimelate decarboxylase by L-lysine in different Bacil-

lus species. Journal of Bacteriology **105**:1211–1212.

10. **Hoganson, D. A., Irgens, R. L., Doi, D. H., Stahly, D. P.** 1975. Bacterial sporulation and regulation of dihydrodipicolinate synthase in ribonucleic acid polymerase mutants of *Bacillus subtilis*. Journal of Bacteriology **124**:1628–1629.
11. **Hoganson, D. A., Stahly, D. P.** 1975. Regulation of dihydrodipicolinate synthase during growth and sporulation of *Bacillus cereus*. Journal of Bacteriology **124**:1344–1350.
12. **Kenealy, W., Zeikus, J. G.** 1982. Ammonia assimilation and synthesis of alanine, aspartate, and glutamate in *Methanosarcina barkeri* and *Methanobacterium thermoautotrophicum*. Journal of Bacteriology **150**:1357–1365.
13. **Knight, M., Wolfe, R. S., Elsden, S. R.** 1966. The synthesis of amino acids by *Methanobacterium omelianskii*. Biochemical Journal **99**:76–86.
14. **Maloney, P., Kashnet, E., Wilson, T.** 1975. Methods for studying transport in bacteria. Methods in Membrane Biology **5**:1–49.
15. **Meister, A.** 1965. Biochemistry of the amino acids, vol. 2, pp. 928–940, New York: Academic.
16. **Nakatani, Y., Fujioka, M., Higashino, K.** 1972. Enzymatic determination of L-lysine in biological materials. Analytical Biochemistry **49**:225–231.
17. **Rosner, A.** 1975. Control of lysine biosynthesis in *Bacillus subtilis:* inhibition of diaminopimelate decarboxylase by lysine. Journal of Bacteriology **121**:20–28.
18. **Vogel, H. J.** 1959. Lysine biosynthesis in *Chlorella* and *Euglena:* phylogenetic significance. Biochimica et Biophysica Acta **34**:282–283.
19. **Vogel, H. J.** 1959. On biochemical evolution: lysine formation in higher plants. Proceedings of the National Academy of Sciences **45**:1717–1721.
20. **Vogel, H. J.** 1960. Two modes of lysine synthesis among lower fungi: evolutionary significance. Biochimica et Biophysica Acta **41**:172–173.
21. **Yugari, Y., Gilvarg, C.** 1965. The condensation step in diaminopimelate synthesis. Journal of Biological Chemistry **240**:4710–4716.
22. **Zeikus, J. G., Wolfe, R. S.** 1972. *Methanobacterium thermoautotrophicum* sp. n., an anaerobic, autotrophic, extreme thermophile. Journal of Bacteriology **109**:707–712.