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Vitamin Contents of Archaebacteria

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The levels of six water-soluble vitamins of seven archaebacterial species were determined and compared with the levels found in a eubacterium, *Escherichia coli*. Biotin, riboflavin, pantothenic acid, nicotinic acid, pyridoxine, and lipoic acid contents of *Halobacterium volcanii*, *Methanobacterium thermoautotrophicum* ΔH, "*Archaeoglobus fulgidus*" VC-16, *Thermococcus celer*, *Pyrodictium occultum*, *Thermoproteus tenax*, and *Sulfolobus solfataricus* were measured by using bioassays. The archaebacteria examined were found to contain these vitamins at levels similar to or significantly below the levels found in *E. coli*. Riboflavin was found at levels comparable to those in *E. coli*. Pyridoxine was as abundant among the archaebacteria of the methanogen-halophile branch as in *E. coli*. It was only one-half as abundant in the sulfur-metabolizing branch. "*A. fulgidus*," however, contained only 4% as much pyridoxine as *E. coli*. Nicotinic and pantothenic acids were approximately 10-fold less abundant (except for a 200-fold-lower nicotinic acid level in "*A. fulgidus*"). Nicotinic acid may be replaced by an 8-hydroxy-5-deazaflavin coenzyme (factor F_{420}) in some archaebacteria (such as "*A. fulgidus*"). Compared with the level in *E. coli*, biotin was equally as abundant in *Thermococcus celer* and *Methanobacterium thermoautotrophicum*, about one-fourth less abundant in *P. occultum* and "*A. fulgidus*," and 25 to over 100 times less abundant in the others. The level of lipoic acid was up to 20 times lower in *H. volcanii*, *Methanobacterium thermoautotrophicum*, and *Thermococcus celer*. It was over two orders of magnitude lower among the remaining organisms. With the exception of "*A. fulgidus*," lipoic acid, pantothenic acid, and pyridoxine were more abundant in the members of the methanogen-halophile branch of the archaebacteria than in the sulfur-metabolizing branch.

The archaebacteria constitute a group of organisms that are phylogenetically distinct from other bacteria (the eubacteria) but exhibit a wide diversity of physiological traits (10). Although much is known about specific biochemical pathways in some archaebacteria (i.e., methanogenesis or the photosystem of the halophiles), little is known about the basic physiology of most members of the group. Those metabolic processes that have been examined are often unusual and frequently unique. Novel coenzymes are often utilized in these processes.

An initial approach to understanding archaebacterial physiology can be made by determining the coenzymes the organisms use. Leigh approached this problem in a survey of two methanogens and four representative eubacteria for their contents of seven vitamins (18). In that study, he found levels of folic acid and pantothenic acid approximately two orders of magnitude lower in the methanogens than in the eubacteria. The levels of other vitamins were also somewhat lower in the methanogens. Another study, by Nagle et al., found that *Sulfolobus solfataricus* and three methanogens contained little or no folic acid. *Halobacterium volcanii*, however, contained folic acid at a level only slightly lower than that found in eubacteria (D. P. Nagle, Jr., V. E. Worrell, C. W. Jones, and R. Teal, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, I130, p. 194).

The present study was undertaken to expand these observations by surveying organisms spanning the archaebacterial phylogenetic spectrum for their contents of six vitamins. Although the archaebacteria make up a phylogenetic group distinct from the eubacteria, the group is divided into two main branches that are distantly related (27). One branch is composed of the extremely halophilic bacteria, the methanogens, *Thermoplasma acidophilum*, the sulfate reducer "*Archaeoglobus fulgidus*," and the sulfur reducer *Thermococcus celer*. This branch is referred to here as the methanogen-halophile branch. The other branch is composed of the sulfur-metabolizing organisms, including the genera *Thermoproteus*, *Pyrodictium*, *Pyrococcus*, *Thermodiscus*, *Thermofilum*, and the thermoacidophiles of the genus *Sulfolobus*. Selected organisms from each of these two branches were chosen for study to see whether the contents of any vitamins differ between these two major divisions. Such differences may indicate whether these two branches have distinctive physiological traits.

The organisms chosen for this study included representatives of the extreme halophiles (*H. volcanii*) (20), the methanogens (*Methanobacterium thermoautotrophicum* ΔH), the extreme thermophiles (*Thermococcus celer*, "*A. fulgidus*," and *Pyrodictium occultum*) (1, 22, 29), and the thermoacidophiles (*S. solfataricus*) (5, 31).

The evidence presented here demonstrates that, as with the methanogens, the level of pantothenic acid was low in all the archaebacteria compared with that in eubacteria. Nicotinic acid, biotin, and pyridoxine levels varied among archaebacteria but were generally lower than those found in eubacteria. Lipoic acid content was markedly lower in the archaebacteria. Only riboflavin was found in concentrations equivalent to those in eubacteria.

(This work was completed in part as an undergraduate senior thesis project by T.S.B.)

MATERIALS AND METHODS

Organisms. Cultures or cells of the following organisms were kindly provided by the individuals indicated: *H. volcanii*, R. Gupta (University of Southern Illinois, Carbondale); *P. occultum* PL-19 and "*A. fulgidus*" VC-16, K. O. Stetter (University of Regensburg, Regensburg, Federal Republic of Germany); *Thermoproteus tenax* Kra 1 and *Thermococcus celer* Vu 13, W. Zillig (Max-Planck Institut für Biochemie,

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Martinsreid, Federal Republic of Germany); *S. solfataricus* ATCC 35091, J. Risatti (Illinois Natural History Survey, Urbana); and *Escherichia coli* UB1005, J. Cronan (University of Illinois, Urbana).

Lactobacillus casei subsp. *rhamnosus* ATCC 7469 and *E. coli* W1485 *lip-2* ATCC 25645 were used as indicator strains for bioassays and were obtained from the American Type Culture Collection, Rockville, Md.

Media and culture conditions. All organisms tested for vitamin contents were grown on a defined, vitamin-free medium except *Thermococcus celer*. *Thermococcus celer* and *Thermoproteus tenax* were provided as dried cell pastes. *Thermococcus celer* had been grown on a mineral medium containing 0.2% tryptone (Difco Laboratories, Detroit, Mich.) with sulfur as the electron acceptor (3, 29). *Thermoproteus tenax* had been grown autotrophically on a mineral medium (3, 30). Hydrogen and sulfur served as the energy source. *P. occultum* and "*A. fulgidus*" were provided as frozen cell pastes. Each had been grown on a vitamin-free medium (9, 22, 23). *S. solfataricus*, *H. volcanii*, *Methanobacterium thermoautotrophicum* ΔH, and *E. coli* UB1005 were grown on the defined media described below. Growth was monitored by turbidity measurements, and cells were harvested at early stationary phase by centrifugation. Cell pastes were stored at −20°C until analyzed.

S. solfataricus was grown at 78°C on a medium consisting of 1.3 g of $(NH_4)_2SO_4$, 0.2 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 75 mg of $CaCl_2 \cdot 2H_2O$, and 2 g of sucrose per liter. The medium was adjusted to pH 3 with H_2SO_4.

H. volcanii was grown at 37°C with vigorous shaking on a medium consisting of 125 g of NaCl, 50 g of $MgCl_2 \cdot 6H_2O$, 5 g of K_2SO_4, 0.2 g of $CaCl_2 \cdot 6H_2O$, and 20 g of vitamin-free Casamino Acids (Difco) per liter.

E. coli UB1005 was grown at 37°C with vigorous shaking on a medium consisting of 10 g of glucose, 1 g of NH_4Cl, 0.13 g of $MgSO_4$, 3 g of KH_2PO_4, 10 g of vitamin-free Casamino Acids, 0.2 g of tryptophan, 0.1 g of cystine, 0.2 g of methionine, and 20 ml of trace mineral solution (0.05% NaCl, 0.05% $FeSO_4$, 0.05% $MnSO_4$, 0.005% $MgSO_4$) per liter.

Methanobacterium thermoautotrophicum was grown on H_2 and CO_2 using defined medium 2 of Balch et al., except that all vitamins were omitted (2).

Preparation of cell extracts. For *E. coli* UB1005, three types of cell extracts were prepared. (i) Boiled-cell extracts were prepared by suspending 1 g of wet cells in distilled water to a final volume of 2 ml in a crimp-sealed serum bottle followed by incubation at 100°C for 20 min. Insoluble material was removed by centrifugation. (ii) Acid-hydrolyzed extracts were prepared by suspending cells in 6 N HCl (1 g of wet cells in 2-ml final volume) and by heating them under vacuum for 2 h at 100°C in a flame-sealed vial. The hydrolysate was neutralized with NaOH, and insoluble material was removed by centrifugation. (iii) Alkaline-hydrolyzed extracts were prepared by suspending cells in 1 N NaOH (1 g of wet cells in 2-ml final volume) and by heating them under vacuum for 20 min at 100°C in a flame-sealed vial. The hydrolysate was neutralized with an equal volume of 1 N HCl, and insoluble material was removed by centrifugation. The clarified extracts were stored at −20°C.

Lyophilized cell pastes of "*A. fulgidus*," *Thermoproteus tenax*, and *Thermococcus celer* were used for the preparation of cell extracts. These extracts were prepared by adding 0.1 g of dry cells to 0.9 ml of sterile, distilled water. They were then treated as described above for *E. coli*. Extracts of *H. volcanii*, *S. solfataricus*, and *P. occultum* were prepared from wet cell pastes.

For lipoic acid determinations, cells were base hydrolyzed. Each dried cell paste was suspended in 1 N NaOH (100 mg in 0.45 ml of base) and sealed in a glass vial under vacuum. Each vial was heated to 110°C for 30 min and then cooled on ice. The solution was neutralized by the addition of 0.45 ml of 1 N HCl, and debris was removed by centrifugation. The extracts were stored at −20°C until analyzed. Any insoluble material present after the extract was thawed was removed by centrifugation.

Vitamin assays. All media, extracts, and stock solutions were prepared in glassware that had been either acid cleaned or filled with distilled water and autoclaved for 20 min or boiled for at least 2 h. The glassware was then rinsed thoroughly with distilled water before use.

Lactobacillus casei was maintained on a medium consisting of 10 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 5 g of yeast extract, 3 g of glucose, and 2 g of K_2HPO_4 per liter.

Lactobacillus basal assay medium consisted of 5 g of KH_2PO_4, 5 g of K_2HPO_4, 0.2 g of tryptophan, 0.1 g of cystine, 1 g of sodium acetate, 2 g of ammonium citrate, 10 g of vitamin-free Casamino Acids, 1 ml of Tween 80, 10 ml of trace mineral solution (see *E. coli* UB1005 medium), and 20 ml of a stock base solution per liter. The stock base solution consisted of 125 mg each of uracil, adenine, and guanine per liter.

For biotin assays, biotin assay medium (Difco) was prepared and used according to the instructions of the manufacturer.

The vitamin ranges for *Lactobacillus casei* were determined by dispensing a 9-ml portion of *Lactobacillus* basal medium and a 0.4-ml portion of 25% glucose into each screw-cap test tube (12.5 by 1.5 cm). All vitamins other than the one under assay were added in excess. The volume of medium in each tube was increased to 10 ml by the addition of water. The vitamin under assay was added at known, limiting concentrations, and after incubation at 37°C for 48 h, the A_{660} was read. The linear assay range for each vitamin was determined from a standard curve. Each point on a curve represented the average of at least three tubes. To control for any variation in assay conditions, a standard curve was constructed for each determination.

To prepare vitamin assays, a 9-ml portion of *Lactobacillus* basal medium and a 0.4-ml portion of 25% glucose were dispensed into each test tube. To assay for a vitamin, a measured sample of extract was added to a tube of medium lacking that vitamin; all other vitamins were added in at least 10-fold excess of predetermined saturating levels. To test for possible growth inhibition by the extract, a tube of medium containing an excess of all vitamins and the largest volume of extract used in a bioassay was also prepared. The volume of medium in each tube was increased to 10 ml by the addition of water. The medium was sterilized by autoclaving for 10 min at 121°C (101 kPa).

The inoculum was prepared by aseptically centrifuging 1 ml from a 10-ml overnight culture of *Lactobacillus casei* grown in maintenance medium. The pellet was rinsed three times with 10-ml portions of sterile 0.9% saline, resuspended to 10 ml, and diluted 1:100. One drop of inoculum was added to each tube of sterile assay medium. Tubes were incubated at 37°C for 48 h, and the A_{660} of a sample from each tube was measured in a Beckman model DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). Cuvettes with a 1-cm light path were used.

TABLE 1. Growth responses of indicator strains to vitamins[a]

Biotin		Riboflavin		Pantothenic acid		Nicotinic acid		Pyridoxine		Lipoic acid	
μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}
0	0.042	0	0.000	0	0.000	0	0.018	0	0.112	0	0.100
0.005	0.116	3	0.064	0.5	0.264	3	0.210	2	0.207	0.6	0.948
0.02	0.272	5	0.155	0.75	0.428	5	0.355	5	0.291	1.2	1.114
0.03	0.353	10	0.255	2.5	0.645	10	0.530	7	0.389	1.8	1.478
0.04	0.494	15	0.437	3.5	0.724	25	0.785	10	0.475	2.4	1.888
0.06	0.678	25	0.497	5.0	0.804					3.0	2.134
0.07	0.775	50	0.645	7.5	0.859					4.0	2.280

[a] All vitamins other than the one under assay were added in excess amounts. Each value shown represents the average of at least three determinations. *Lactobacillus casei* ATCC 7469 was used for all vitamins except lipoic acid, in which case *E. coli* ATCC 25645 was used.

Lipoic acid was determined by using lipoic acid-requiring *E. coli* W1485 *lip-2* ATCC 25645 (8). Cultures were maintained on a medium consisting of 6 g of K_2HPO_4, 2.5 g of $NaH_2PO_4 \cdot 2H_2O$, 2 g of NH_4Cl, 0.15 g of Na_2SO_4, 5 mg of thiamine, 0.1 g of $MgCl_2 \cdot 6H_2O$, 7 g of sodium succinate, and 5 μg of DL-lipoic acid per liter. The medium was adjusted to pH 7. For assay medium, DL-lipoic acid was omitted.

For lipoic acid determinations, a 5-ml culture of *E. coli* W1485 *lip-2* was grown on maintenance medium at 37°C with vigorous shaking. The cells were harvested by centrifugation and washed three times with 1 ml of sterile 0.9% saline. The cell pellet was suspended in 5 ml of maintenance medium without lipoic acid and incubated overnight at 37°C. One drop of this culture was used to inoculate each tube of the assay medium. An assay tube was prepared by adding a sample of base-hydrolyzed extract to a tube containing 5 ml of assay medium. A standard growth-response curve was established for each assay with DL-lipoic acid. All determinations were performed in duplicate or triplicate. The growth response to lipoamide was determined with DL-lipoamide that had been base hydrolyzed in the same manner as cell extracts. The inoculated assay cultures were incubated for 48 h at 37°C with vigorous shaking. The A_{660} of each culture was determined as described above for *Lactobacillus casei*.

Materials. Vitamins and coenzymes were obtained from Sigma Chemical Co., St. Louis, Mo., and stored at 4°C in the dark.

RESULTS

Vitamin bioassay. The growth responses of *Lactobacillus casei* to various vitamins are shown in Table 1. The quantities of vitamins (micrograms per liter) necessary to elicit half-maximal growth were found to be as follows: biotin, 0.2; riboflavin, 33; pantothenic acid, 1.3; nicotinic acid, 13; and pyridoxine, 6.1. *Lactobacillus casei* also requires folic acid, so this was added to each assay in a saturating amount. The amount of lipoic acid necessary to elicit a half-maximal growth response of *E. coli* W1485 *lip-2* was 2.1 μg/liter. The linear ranges of the growth curves were used to determine vitamin levels in the extracts. All calculations were based on the assumption that 1 g (dry weight) of cells is equivalent to 10 g (wet weight).

The activities of the coenzyme forms of the vitamins likely to be found in cell extracts are listed in Table 2. The concentrations of coenzymes (micrograms per liter) that gave one-half maximal growth responses were as follows: flavin mononucleotide, 11; flavin adenine dinucleotide, 46; pyridoxal phosphate, 1.2; coenzyme A, 641; NAD, 24; NADP, 46; and base-hydrolyzed lipoamide, 2.8. With the exception of coenzyme A, each cofactor was within one order to magnitude as potent in its growth-promoting activity as an equimolar amount of its corresponding vitamin form. Flavin mononucleotide and flavin adenine dinucleotide were, respectively, 3.8 and 1.6 times more potent than an equimolar amount of riboflavin. NAD and NADP were, respectively, 2.9 and 1.8 times more potent than nicotinic acid. DL-Lipoamide gave approximately 10% of the activity of a comparable amount of DL-lipoic acid. After base hydrolysis, however, lipoamide gave 67% of the activity of lipoic acid. Pyridoxal phosphate was 6.1 times more potent than pyridoxine. Approximately 150 times as much coenzyme A as pantothenic acid was required to elicit the same growth response.

To facilitate the comparison of the vitamin contents of archaebacteria determined in our study with those measured

TABLE 2. Vitamin activities of coenzymes in the bioassays

Flavin mononucleotide		Flavin adenine dinucleotide		Pyridoxal phosphate		Coenzyme A		NAD		NADP		Lipoamide[a]	
μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}	μg/liter	A_{660}
0	0.004	0	0.016	0	0.100	0	0.260	0	0.027	0	0.023	0	0.100
25	0.562	25	0.316	2.5	0.543	5	0.165	25	0.498	25	0.367	1	0.932
50	0.791	50	0.540	5.0	0.609	50	0.181	62.5	0.620	125	0.630	3	2.144
250	0.856	250	0.813	12.5	0.677	625	0.273	125	0.748	250	0.731	6	2.160
2,500	0.842	500	0.812	50	0.750	1,250	0.313	250	0.822	625	0.834		
				125	0.785	2,500	0.438						
				1,000	0.832	5,000	0.559						
				5,000	0.810	7,000	0.625						

[a] Values are for base-hydrolyzed DL-lipoamide.

TABLE 3. Comparison of vitamin levels in *E. coli* UB1005

Cell extract	Vitamin level (μg/g [dry wt] of cells)[a]					
	Biotin	Riboflavin	Pantothenic acid	Nicotinic acid	Pyridoxine	Lipoic acid
Boiled cells[b]	0.13	310	170	450[c]	6,300	ND[d]
Boiled cells	0.01	231	23.1	460	103	ND
Acid treated	2.6	46	7.6	160	ND	ND
Base treated	ND	130	4.0	270	ND	3.1

[a] Values were calculated from growth response curves of *Lactobacillus casei* (or *E. coli* ATCC 25645 for lipoic acid) and on the assumption that 1 g (dry weight) of cells is equivalent to 10 g (wet weight). All values are averages of three or more assays.
[b] Values reported by Leigh with a vitamin-requiring *Leuconostoc* strain (18).
[c] Determined with acid-treated extract.
[d] ND, Not determined.

by Leigh (18), the vitamin levels of the same strain of *E. coli* as that used in the study of Leigh were measured in our bioassays. Most of the vitamin levels found in *E. coli* UB1005 determined in our study compare favorably with those found by Leigh when adjustments are made for the use of different test organisms and extraction conditions (Table 3). His determinations of riboflavin and nicotinic acid levels varied by no more than 34% from those measured here. The *Leuconostoc* strain used by Leigh was unable to use NAD or NADP in place of nicotinic acid, so acid-hydrolyzed extracts were used in his study. *Lactobacillus casei* can utilize all three, so boiled extracts were sufficient for nicotinic acid measurements. The nicotinic acid contents of *E. coli* UB1005 were nearly identical in both studies. Our measurement of pantothenic acid was 14% of what Leigh found. However, for *Methanobacterium thermoautotrophicum*, we found 40% of the amount he found (Table 4). We found the biotin and pyridoxine levels to be less than 10% of what Leigh found. These discrepancies can be reconciled (see Discussion).

Table 3 also shows the results of the vitamin level determinations from three types of cell extracts of *E. coli* UB1005. The acid-hydrolyzed extract contained 260-fold more biotin than did the boiled-cell extract. Acid hydrolysis has been shown to enhance biotin recovery from tissues (25). Acid hydrolysis may more efficiently cleave the amide linkage between biotin and a lysine residue of a protein. Therefore, for biotin determinations, acid-hydrolyzed extracts were used. Neither acid nor base hydrolysis of extracts improved the recoveries of the other vitamins tested.

The effects of extracts on the growth of *Lactobacillus casei* and *E. coli* W1485 *lip-2* in the presence of excess amounts of vitamins were determined. The maximum amount of extract used in any vitamin determination was tested for its effect on growth of the test organism. None of the boiled extracts significantly inhibited the growth of *Lactobacillus casei*, but stimulation of 18 to 62% was observed. Although known growth factors were added in excess, some of these factors may have been present in the extracts in a form more accessible to *Lactobacillus casei*. Despite this stimulation, the results obtained for *E. coli* UB1005 with these bioassays were comparable to those found by Leigh (18) and those for other eubacteria found by Thompson (24). Although our data may overestimate the vitamin contents of these organisms, comparisons of relative levels of vitamins among them can be made. Acid-hydrolyzed extracts were used for the biotin assay. These extracts showed either some stimulation (up to 42%) or inhibition (to 20%) of the growth of *Lactobacillus casei*. The base-hydrolyzed extracts gave up to 36% growth stimulation of *E. coli* W1485 *lip-2* when lipoic acid was present in excess.

Vitamin contents of archaebacteria. Table 4 shows the vitamin levels found in selected archaebacteria. The biotin contents of the archaebacteria studied here varied considerably. The amount found in *Thermococcus celer* was over 1,000 times greater than that found in *Thermoproteus tenax*, which had only a trace of biotin. *Methanobacterium thermoautotrophicum* contained levels similar to those found in *Thermococcus celer*. Levels of riboflavin in the archaebacteria appeared to be very similar to those determined for *E. coli* UB1005. The values varied by threefold among the archaebacteria. Levels of pantothenic acid in the archaebacteria were all within about fivefold concentrations of each other. In comparison with the amount found in *E. coli* UB1005 in our study, the archaebacteria contained 4- to 23-fold less pantothenic acid. Except for "*A. fulgidus*," levels of nicotinic acid were lower in the archaebacteria than in *E. coli* UB1005 by factors of 2.4 to 8.5. Levels of nicotinic acid among the archaebacteria varied almost 100-fold. "*A. fulgidus*" appeared to have a significantly lower amount of nicotinic acid than either the other archaebacteria or eubac-

TABLE 4. Vitamin levels in archaebacterial cells

Organism	Vitamin level (μg/g [dry wt] of cells)[a]					
	Biotin	Riboflavin	Pantothenic acid	Nicotinic acid	Pyridoxine	Lipoic acid
H. volcanii	0.07	322	4.1	45	107	0.82
M. thermoautotrophicum	3.6	310	3.3	54	192	0.18
"*A. fulgidus*"	0.79	105	3.5	2	4	0.02
T. celer	4.2	137	5.5	195	85	0.18
P. occultum	0.70	227	2.0	54	39	0.01
T. tenax	<0.01	244	1.0	93	51	0.02
S. solfataricus	0.02	202	1.4	66	49	0.02
M. thermoautotrophicum[b]	0.036	37	8.4	30	900	ND[c]
M. voltae[b]	ND	40	1.4	220	6,100	ND

[a] Values were calculated from growth response curves of *Lactobacillus casei* (or *E. coli* ATCC 25645 for lipoic acid) and on the assumption that 1 g (dry weight) of cells is equivalent to 10 g (wet weight). Most values are averages of three or more assays. *Thermococcus celer* was grown on a medium containing 0.2% tryptone. All other organisms were grown on a vitamin-free medium.
[b] Data from Leigh (18). This biotin measurement was performed on boiled-cell extracts.
[c] ND, Not determined.

teria. Most of the archaebacteria were found to contain pyridoxine at levels comparable to those found in *E. coli* UB1005. "*A. fulgidus*," however, had only 4% of that found in *E. coli* UB1005.

The lipoic acid contents of the sulfur-metabolizing archaebacteria were about 100-fold lower than those found in *E. coli* UB1005. The level in *H. volcanii* was only 3.8-fold lower. *Thermococcus celer* and *Methanobacterium thermoautotrophicum* contained 17 times less lipoic acid. The stereochemistry of the lipoic acid in the archaebacteria is unclear since the growth response of the test organism to the different isomers is unknown. Since a racemic mixture was used to establish the standard growth curves, the values in Tables 3 and 4 may be twice the actual values if the test organism uses only one isomer.

DISCUSSION

Little is known about the intermediary metabolism of archaebacteria. As a beginning step toward understanding these organisms better, this survey has established the presence of six vitamins in the archaebacteria. These results generally confirm and extend the observations Leigh made about the methanogens (18).

Biotin levels in many of the archaebacteria appear to be lower than in the eubacteria. *Methanobacterium thermoautotrophicum* and *Thermococcus celer* contained amounts similar to those of *E. coli* UB1005. *Thermococcus celer* was grown on a medium containing 0.2% tryptone, but *Methanobacterium thermoautotrophicum* was grown on a vitamin-free medium. "*A. fulgidus*" and *P. occultum* had biotin levels similar to those of one another and less than 30% of that in *E. coli* UB1005. The others contained up to two orders of magnitude less biotin than *E. coli* UB1005. Autotrophs (*Thermoproteus tenax* and *Methanobacterium thermoautotrophicum*) showed the same diversity as heterotrophs (*H. volcanii* and *Thermococcus celer*). There appears to be no correlation between biotin content and either phylogenetic position or mode of carbon assimilation.

Leigh (18) reported biotin levels in the methanogens that were much lower than those we have found for other archaebacteria. Since acid treatment enhanced recovery of biotin by almost 200-fold in *E. coli* UB1005, the values of Leigh may be proportionately low. Correcting for this, his determination of biotin in *E. coli* UB1005 would be 10 times that reported here. Using a similar comparison, his value for *Methanobacterium thermoautotrophicum* would be 7.2 μg/g (dry weight), twice the value we found. Thompson reported biotin contents of different eubacteria to be from 1.7 to 7.1 μg/g (dry weight) (24). The levels in *Thermococcus celer* and *Methanobacterium thermoautotrophicum* fell within this range. The other archaebacteria contained one-tenth or less than this.

Riboflavin was present in archaebacteria in amounts similar to those found in eubacteria. Levels varied among archaebacteria by 40% but were only at most twofold lower than in *E. coli* UB1005. Leigh reported riboflavin at one-tenth the level we found in *Methanobacterium thermoautotrophicum* (18). The reason for this discrepancy is not known.

Nicotinic acid was also less abundant in the archaebacteria. In particular, "*A. fulgidus*" had less than 1% the amount of nicotinic acid than *E. coli* UB1005. Interestingly, "*A. fulgidus*" contains amounts of a blue-green fluorescent compound sufficient to cause the cells to fluoresce when viewed under UV illumination (23). This compound appears to be similar to the 8-hydroxy-5-deazaflavin (factor F_{420}) found in methanogens (23). *Methanobacterium thermoautotrophicum* contains a small amount of nicotinic acid but contains one of the highest F_{420} levels (6). *H. volcanii* contains F_{420} in a level slightly lower than that of some methanogens (19) and contains an amount of nicotinic acid similar to that of *Methanobacterium thermoautotrophicum*. *S. solfataricus* has more nicotinic acid than *H. volcanii* but contains little deazaflavin. Leigh found that *Methanococcus voltae* contains seven times more nicotinic acid than *Methanobacterium thermoautotrophicum* (18). The F_{420} content of *Methanococcus voltae* has not been measured. Some methanogens contain as little as 2% of the amount found in *Methanobacterium thermoautotrophicum*. The deazaflavin contents of the other archaebacteria are unknown; but since these cells are neither pigmented nor fluorescent, there is no indication that they contain significant amounts of deazaflavin. They do contain more nicotinic acid than *Methanobacterium thermoautotrophicum*, *H. volcanii*, and "*A. fulgidus*." With their low nicotinic acid contents and apparently high deazaflavin contents, the archaebacteria of the methanogen-halophile branch may use the deazaflavins in place of nicotinic acid cofactors in some physiological processes (17). In methanogens, factor F_{420} serves as a substrate for a variety of redox reactions and can transfer electrons to NADP through an NADP reductase (10, 28). "*A. fulgidus*" may provide many examples of the utility of deazaflavin cofactors in metabolic processes.

Leigh observed higher pyridoxine levels in the methanogens than what we observed in the other archaebacteria (18; Table 4). This apparent difference is due to the differing sensitivities of the test organisms used. Based on the data of Leigh, his *Leuconostoc* strain was 100-fold less sensitive to pyridoxine than pyridoxal phosphate. Since his standard curves were based on pyridoxine, his determinations may be up to 100-fold overestimates. Our *Lactobacillus casei* was only sixfold less sensitive. Leigh reported pyridoxine levels in *E. coli* UB1005 of 6300 μg/g (dry weight), whereas 103 μg/g was found in this survey. Thompson reported values ranging from 6.2 to 18 μg/g for other eubacteria (24).

Pyridoxine levels were lower in some archaebacteria than in the eubacteria. The methanogen-halophile branch had levels similar to those found in *E. coli* UB1005. The sulfur-metabolizing branch had about one-half as much. Again, "*A. fulgidus*" had a much lower level than the others. Boiled-cell extract of "*A. fulgidus*" gave a slight stimulation of growth of the test organism in the presence of excess pyridoxine, so this low value was not due to the presence of inhibitors. *Lactobacillus casei* ATCC 7469 grows better in the presence of pyridoxal, pyridoxamine, and the phosphorylated coenzyme forms (14). If these were present in extracts, enhanced growth should have been observed. Leigh found that the eubacteria and methanogens have comparable levels, although *Methanobacterium thermoautotrophicum* contained about sevenfold less than *Methanococcus voltae* (18). We found *Methanobacterium thermoautotrophicum* to have almost twice as much pyridoxine as *E. coli* UB1005.

Pantothenic acid levels in *E. coli* UB1005 were nearly sevenfold lower in this study than those reported by Leigh (18). Thompson (24) found values in other eubacteria similar to those of Leigh. Rühlemann et al. reported pantothenic acid levels of approximately 500 μg/g (dry weight) in boiled-cell extract of *E. coli* (21), although this organism had been grown in a medium containing yeast extract. Our *Lactobacillus casei* was equally sensitive to coenzyme A and 10 times more sensitive to pantothenic acid than the *Leuco-*

nostoc strain of Leigh (18). Our determinations of pantothenic acid in *Methanobacterium thermoautotrophicum* were only 2.5-fold different. Rühlemann et al. found detectable pantothenic acid in boiled-cell extract of *Methanobacterium thermoautotrophicum* to be 16 μg/g (dry weight) (21). Although comparisons between these studies for *E. coli* may have been affected by culture conditions or strain differences, the *Methanobacterium thermoautotrophicum* values are very similar.

Rühlemann et al. also found that only 10 to 20% is present as free pantothenic acid (including pantothenic acid, pantetheine, and pantethine) (21). The remainder is present as coenzyme A. The pantothenic acid from coenzyme A can be detected only after enzymatic treatment of cell extracts. The measurements reported by Leigh (18) and us measure only this free pantothenic acid pool. Coenzyme A was virtually undetectable in our bioassay. We were unable to assess the contribution coenzyme A makes to the pantothenic acid contents of these organisms.

Leigh observed pantothenate levels in methanogens 20 to 121 times lower than that in *E. coli* UB1005 (18). We found that the other archaebacteria contain 4 to 23 times less. The sulfur-metabolizing archaebacteria had the lowest values. Rühlemann et al. speculated that the low pantothenate content in *Methanobacterium thermoautotrophicum* may in part be due to a lack of phosphopantetheine-containing enzymes, such as acyl carrier protein (21). Since we were unable to detect pantothenic acid from coenzyme A, our values may reflect this enzyme-bound pool of pantothenic acid. Archaebacteria generally lack fatty acids since their cell membranes are composed of isoprenoid acyl chains ether linked to glycerol (15). The halobacteria and methanogens do, however, contain traces of free fatty acids, accounting for up to 10% of their polar lipids (11, 26). *Thermoplasma acidophilum* and *Sulfolobus* species have virtually no fatty acids (16, 17). The fatty acid contents of the other sulfur-metabolizing archaebacteria have not been reported. The slightly elevated levels of pantothenate among the methanogen-halophile organisms suggests that acyl carrier protein may be present in archaebacteria but in very low amounts. Studies of the incorporation of radiolabeled acetate into halobacterial fatty acids suggest that the biosynthesis of fatty acids in the halobacteria may occur by the malonyl-coenzyme A pathway, as in eubacteria (12).

Lipoic acid levels were also generally higher among the organisms of the methanogen-halophile branch than the sulfur metabolizers. Compared with *E. coli*, the organisms of the methanogen branch had 4 to 16 times less lipoic acid, whereas the sulfur metabolizers contained 100-fold less. "*A. fulgidus*" was the lone exception to this observation. Lipoate dehydrogenase has been purified from *Halobacterium halobium*, so its presence might be expected in *H. volcanii* (4). The α-ketoacid dehydrogenases are a major reservoir of lipoic acid in cells. The activities of these dehydrogenases have been demonstrated in several archaebacteria (for a review of this topic, see reference 10). The α-ketoacid dehydrogenases of *H. halobium* are unusual in that the prosthetic groups lipoic acid and flavin adenine dinucleotide have been replaced by two 4Fe:4S clusters (13). Thus, a potential major source of lipoic acid is missing. Whatever remaining role lipoic acid plays in the archaebacteria must be of more importance to the organisms of the methanogen-halophile branch, since most of them have higher contents of this vitamin.

This survey indicates that the archaebacteria utilize the same variety of water-soluble vitamins as the eubacteria and eucaryotes. The fact that many of them are present in smaller amounts suggests possible differences from the eubacteria in the relative importance of certain biochemical pathways. The archaebacteria are known to use altered versions of known metabolic pathways and to use novel cofactors for biochemical reactions (10). This survey has also revealed that pantothenic acid, lipoic acid, and pyridoxine contents differ between the two major phylogenetic branches of the archaebacteria. These differences may be one indication of the physiological nature of the schism.

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