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Distribution of 10-Formyltetrahydrofolate Synthetase in Eubacteria

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The distribution of 10-formyltetrahydrofolate synthetase, which activates formate for use as a one-carbon donor in a variety of biosynthetic reactions, was determined for a variety of eubacteria. Organisms from several genera were found to lack detectable synthetase activity; however, all organisms tested were found to contain 5,10-methylenetetrahydrofolate dehydrogenase activity.

One-carbon metabolism in procaryotes and eucaryotes involves the one-carbon derivatives of the coenzyme tetrahydrofolate (THF) at various states of oxidation. The principal sources of one-carbon units are serine and glycine (10). Serine is derived from carbohydrate precursors in most organisms and is then cleaved to form glycine and 5,10-CH_2-THF (5,10-methylene-THF). This product can also be formed in a nonenzymatic reaction between THF and formaldehyde generated in cellular metabolism. The active methylene carbon is oxidized by 5,10-CH_2-THF dehydrogenase (EC 1.5.1.5) to yield 5,10-CH^+-THF (5,10-methenyl-THF), which may then undergo enzymatic hydrolysis by 5,10-CH^+-THF cyclohydrolase (EC 3.5.4.9) to yield 10-CHO-THF (10-formyl-THF).

10-CHO-THF functions as a one-carbon donor in purine biosynthesis and in the formation of the initiator of protein synthesis, fMet-$tRNA^{fMet}$, in procaryotes and mitochondria. In addition to its biosynthesis from serine described above, 10-CHO-THF can also be formed by the enzymatic activation of formate by the enzyme 10-CHO-THF synthetase (EC 6.3.4.3). It has been proposed that this enzyme is ubiquitous (8, 24), based on the recognition of the importance of one-carbon metabolism and on the fact that 10-CHO-THF synthetase activity was detected in all bacteria tested (26). However, it has more recently been reported that the enzyme does not occur in *Escherichia coli* (5).

In eucaryotes, the synthetase, cyclohydrolase, and dehydrogenase are associated with a single trifunctional protein (2, 15, 17, 23), C_1-THF synthase (3). In all procaryotes studied, these activities are associated with monofunctional proteins, except for a bifunctional cyclohydrolase/dehydrogenase in *E. coli* (4) and *Clostridium thermoaceticum* (14). Our laboratory has been studying the potential structural, functional, and evolutionary relationships between the different forms of 10-CHO-THF synthetase (19–21, 25). We have found extensive amino acid homology (>50%) between the synthetase from *Clostridium acidiurici* ("*Clostridium acidi-urici*") and the synthetase domain of C_1-THF synthase from *Saccharomyces cerevisiae* (T. R. Whitehead and J. C. Rabinowitz, manuscript in preparation). The possibility therefore exists that a procaryotic synthetase may be associated with a multifunctional protein. Since relatively few procaryotic synthetases have been studied, we decided to investigate the distribution of the synthetase among different eubacteria. The only other similar study was reported almost 30 years ago (26). In addition, we determined the level of 5,10-CH_2-THF dehydrogenase in these bacteria. The bacteria were chosen for this study for their morphological and physiological differences so as to encompass a wide spectrum of organisms and are listed in Table 1. Bacteria were grown to mid-log phase in L broth (11), except for the anaerobic bacteria, which were grown on peptone-yeast extract basal medium (9). *C. acidiurici* was grown on uric acid medium (16), and *Mycobacterium smegmatis* was grown on glycerol medium containing 0.2% Tween 80 (7). Cells were harvested by centrifugation at 5,000 × *g* at 4°C and were suspended in 50 mM Tris chloride (pH 7.5)–10 mM KCl–10 mM 2-mercaptoethanol (buffer A). Cells were broken with a French pressure cell at 12,000 lb/in^2, and crude extract was prepared by centrifugation at 30,000 × *g* at 4°C for 30 min. The supernatant fluid was recovered and used for enzyme assays. 10-CHO-THF synthetase was assayed essentially as previously described (13), except ammonium formate (pH 8.0) was used in place of sodium formate and the reaction was terminated with 2% perchloric acid to precipitate protein. The precipitate was pelleted by centrifugation, and the supernatant fluid was used to determine the A_{350}. 5,10-CH_2-THF dehydrogenase was assayed as previously described (18), except 2% perchloric acid was used to terminate the reaction.

The synthetase and dehydrogenase activities determined in these studies are given in Table 1. The variation in detectable synthetase activity ranged from 13,000 mU/mg in *C. acidiurici* to 1 mU/mg in *Branhamella catarrhalis*. We were unable to detect synthetase activity in a number of organisms. These included *E. coli*, as has been reported previously (5), as well as other representatives of the family *Enterobacteriaceae*, such as *Salmonella typhimurium* and *Klebsiella pneumoniae*. However, one member of this group, *Proteus mirabilis*, contained detectable activity. All of the gram-positive organisms tested contained detectable activity, with the exception of *Micrococcus luteus*.

We have not been able to discern a pattern related to the presence or absence of the enzyme based on morphological or physiological characteristics of the organisms tested. Examples of the presence and the absence of activity were found in aerobic, facultative, and anaerobic bacteria. The distribution of the enzyme among the organisms tested, arranged by phylogenetic and evolutionary relationships on the basis of their 16S rRNA sequences, was also examined (6). The enzyme was found in all members of the clostridial group tested but did not show uniform distribution among the members of the purple bacterial group, or actinomycetes, tested.

One pattern that does emerge from this data is that organisms which have a DNA G+C content of greater than

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TABLE 1. Synthetase and dehydrogenase activities in representative eubacteria

Group	Organism	% G+C	Activity (mU/mg)[a] of:	
			Synthetase	Dehydrogenase
Gram negative				
Aerobic rod	*Pseudomonas aeruginosa* PA01	67	0	10
Facultative rod	*Escherichia coli* JM83	50	0	25
	Salmonella typhimurium LT2	51	0	32
	Klebsiella pneumoniae 13833	57	0	19
	Shigella flexneri 12022	51	0	35
	Proteus mirabilis 25433	40	13	35
Anaerobic rod	*Bacteroides fragilis* 25285	43	100	90
Aerobic coccus	*Branhamella catarrhalis* 25238	42	1	300
Gram positive				
Aerobic rod	*Bacillus subtilis* W168	43	2	28
Anaerobic rod	*Clostridium acidiurici* 9a	28	13,000	550
	Clostridium absonum 27555	29	20	88
	Clostridium bifermentans	26	570	260
	Clostridium pasteurianum	27	59	89
	Eubacterium sp. strain VPI 12708	35	772	490
Aerobic coccus	*Micrococcus luteus* 4698	73	0	1
Facultative coccus	*Staphylococcus epidermidis* 14990	33	5	1
	Streptococcus faecalis 19433	35	1	11
Anaerobic coccus	*Peptostreptococcus anaerobius* 27337	34	65	48
Acid fast				
Aerobic rod	*Mycobacterium smegmatis* 356	66	1	177

[a] One unit is defined as the amount of enzyme required to produce 1 μmol of 10-formyl-THF or 5,10-CH$^+$-THF at 37°C in 1 min.

50% lack detectable synthetase activity, except for *M. smegmatis*, which had a very low level of activity. This pattern correlated to some degree with the bacterial classification based on 16S RNA sequence, since the clostridial group is characterized by a low G+C content, whereas the actinomyces group has a high G+C content. The purple bacterial group contains organisms that have a wide range of G+C contents. However, the presence or absence of the synthetase was more likely to reflect the physiology of the particular organism.

The extremely low values for the synthetase activity in crude extracts of some bacteria, such as 2 mU/mg of protein in *Bacillus subtilis*, relative to values of over 10,000 mU/mg of protein found in others, led us to question whether the low values obtained resulted from the presence of the enzyme or whether they were an artifact of the assay method. To demonstrate that a value of 2 mU/mg did represent enzyme, we purified the activity from such a low-activity source by chromatography on heparin-agarose (22). A crude extract of *B. subtilis* was passed over a heparin-agarose column, which was then treated with a linear salt gradient from 10 to 500 mM KCl in Buffer A. The column purification resulted in a 93-fold purification of the synthetase from the crude extract (data not shown). These results indicate that the assay used here can detect activity of as little as 2 mU/mg of protein in crude bacterial extracts. In addition, immunoblot analysis of the purified *B. subtilis* enzyme with antibody raised against purified *C. acidiurici* synthetase demonstrated a cross-reacting protein which comigrated with the purified clostridial synthetase (data not shown), indicating that the *B. subtilis* enzyme is structurally similar to the *C. acidiurici* synthetase. Similar immunoblot results have also been found with the synthetase from *Bacteroides fragilis*, *Clostridium bifermentans*, and *Clostridium absonum* (T. R. Whitehead and J. C. Rabinowitz, Fed. Proc. **44**:1076, 1985).

The wide range in concentrations of the enzyme and its total absence from some eubacteria leads to a reconsideration of the physiological role of the synthetase in different organisms. One prevailing concept is that the synthetase is used to scavenge formate into the folate one-carbon pathway for biosynthetic purposes. The results of our study suggest that a variety of organisms do not have this ability. It has been reported that purine C-8 of *E. coli* can be derived from formate by an unknown mechanism that does not appear to involve folate derivatives (4). While some bacteria lack synthetase activity, all bacteria tested expressed 5,10-CH_2-THF dehydrogenase activity. This observation suggests that 10-formyl-THF is obtained independently of formate in these cases. Such a pathway has been shown for *E. coli*, which derives its one-carbon units primarily from the degradation of serine to glycine by serine hydroxymethyltransferase with the formation of 5,10-methylene-THF, which can then be converted to 10-CHO-THF by the dehydrogenase and cyclohydrolase (5). This conclusion is consistent with the observation that the formyl donor required for the transformylation to generate formyl-Met-tRNA^{fMet} exists in extracts of *E. coli*, but that formate cannot be converted to the active formyl donor by these *E. coli* extracts (12). This reaction requires the addition of a pigeon liver extract for activation to yield the formyl donor, 10-CHO-THF (1). The results of our study suggest that other bacteria are also unable to incorporate formate into the one-carbon pool and that although 10-formyl-THF synthetase is not ubiquitous, 5,10-CH_2-THF dehydrogenase is present in all eubacteria examined and functions in the formation of the primary one-carbon metabolite, 10-CHO-THF.

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