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2,3-Cyclopyrophosphoglycerate in Methanogens: Evidence by ^{13}C NMR Spectroscopy for a Role in Carbohydrate Metabolism†

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ABSTRACT: The novel compound 2,3-cyclopyrophosphoglycerate (CPP) is the major small molecule carbon pool in *Methanobacterium thermoautotrophicum*. High-field ^{13}C NMR $^{13}CO_2$ pulse/unenriched CO_2 chase experiments have shown that the labeled CPP rapidly loses its ^{13}C to an insoluble pool, while the CPP steady-state concentration is maintained (as monitored by ^{31}P NMR spectroscopy). The biosynthesis of CPP from CO_2, acetyl coenzyme A, and pyruvate as precursors has been established by a ^{13}C NMR study of ethanol extracts of *Mb. thermoautotrophicum* fed with $^{13}CO_2$, [1-^{13}C]- and [2-^{13}C]acetate, and [1-^{13}C]pyruvate. That CPP is a post-phosphoenolpyruvate metabolite has been confirmed by in vitro experiments with cell extracts. A role for CPP in carbohydrate metabolism was established when [1-^{13}C]glucose fed to cells resulted in the formation of [3-^{13}C]CPP *exclusively*. Possible functions of CPP within the cell are discussed.

Methanogens are anaerobic organisms capable of reducing CO_2 to CH_4 in an atmosphere of H_2. As such, they are interesting from an evolutionary standpoint and have been classified as archaebacteria (Fox et al., 1980), which is a class distinct from eukaryotes and eubacteria. For their unusual chemistry, methanogens have evolved an interesting set of coenzymes, examples of which include coenzyme F_{420} (Eirich et al., 1978), coenzyme F_{430} (Ellefson et al., 1982; Pfaltz et al., 1982), coenzyme M (Taylor & Wolfe, 1974), methanofuran (Leigh et al., 1984), and methanopterin (van Beelen et al., 1984). They also possess unusual phospholipids and cell wall structures. In short, nearly every aspect of the biochemistry of these organisms shows unique and fascinating features.

While methanogenesis from CO_2 and H_2 is the source of energy for methanogens, CO_2 also acts as the sole carbon source. The first known cell carbon fixation product in *Methanobacterium thermoautotrophicum* is acetyl-CoA (Fuchs et al., 1978; Fuchs & Stupperich, 1980; Stupperich & Fuchs, 1981). Neither the Calvin cycle nor the reductive tricarboxylic acid cycle operates in CO_2 assimilation in this organism. Work by Jansen et al. (1982) has established that gluconeogenesis starts from acetyl-CoA, and activities for many of the enzymes in the pathway have been demonstrated (Zeikus et al., 1977; Eyzaguirre et al., 1982; Fuchs et al., 1983).

In preliminary studies (Kanodia & Roberts, 1983; Seeley & Fahrney, 1983), ^{31}P NMR spectroscopy was used in vivo to examine *Mb. thermoautotrophicum*, and a unique cyclized pyrophosphate of 2,3-diphosphoglycerate [2,3-cyclopyrophosphoglycerate (CPP)[1]] was identified. The intracellular concentration of this novel compound in cells grown under conditions of phosphate abundance was 10–12 mM. This cyclic pyrophosphate (CPP) appears to be unique to methanobacteria (C. J. Tolman, S. Kanodia, L. Daniels, and M. F. Roberts, unpublished results). In this paper we report studies using high-field ^{13}C NMR spectroscopy, directed toward

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[1] Abbreviations: CPP, 2,3-cyclopyrophosphoglycerate; FID, free induction decay; PIPES, 1,4-piperazinediethanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; P-enolpyruvate, phosphoenolpyruvate; CoA, coenzyme A.

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elucidating the biosynthesis of CPP and its role in carbohydrate metabolism in *Mb. thermoautotrophicum*.

Materials and Methods

Chemicals. $^{13}CO_2/H_2$ (1:4 v/v) was obtained either from Cambridge Isotopes (Cambridge, MA) or from Mound Isotopes (St. Louis, MO). [1-^{13}C]- and [2-^{13}C]acetate and [1-^{13}C]glucose were obtained from Cambridge Isotopes, and [1-^{13}C]- and [2,3-^{13}C]pyruvate and [1-^{13}C]propionate were obtained from Merck Isotopes (Montreal, Quebec, Canada). Unenriched CO_2/H_2 (1:4 v/v) was obtained unanalyzed from Matheson Gas (Gloucester, MA). All other chemicals were of reagent grade.

Cell Growth. Cells of *Mb. thermoautotrophicum* strain ΔH were initially grown in bottles, pressurized with CO_2/H_2 as previously described (Kanodia & Roberts, 1983), except that PIPES was omitted from the medium in later experiments (see figures). Bottles containing cells with $OD_{660} \simeq 1$ (measured in a Turner Model 330 spectrophotometer) were used to inoculate (10% inoculum) anaerobically 1 L of medium in a 2-L fermenter, and the cells were grown at 62 °C, pH 7.1, under continuous purging of CO_2/H_2 (flow rate = 210 cm^3 min^{-1} at 12 psi) and H_2S (flow rate = 0.8 cm^3 min^{-1} at 12 psi) with stirring (750 rpm). Under these conditions the culture exhibited a doubling time of ca. 5 h. When the OD_{660} reached $\simeq 1$ (mid-log growth), the pH was adjusted (7.1 for CO_2 and glucose feeding experiments and 6.5 for others), and the cells were transferred anaerobically to five 500-cm^3 bottles (200 cm^3 each) for use in feeding experiments.

Feeding Experiments. The bottles containing cells were pressurized with CO_2/H_2 (1:4 v/v) or $^{13}CO_2/H_2$ (1:4 v/v), and sodium sulfide (0.2 cm^3, 500 mM in water) was added to each anaerobically. Anaerobic solutions of [^{13}C]acetate (20 mM in water), [1-^{13}C]pyruvate (20 mM in water), [2,3-$^{13}C_2$]pyruvate (10 mM in water), [1-^{13}C]propionate (20 mM in water), or [1-^{13}C]glucose (10 mM in water) were added to bottles containing unenriched CO_2. All bottles were then shaken at 62 °C in a shaker bath for times referred to in the figure legends. For each experiment one of the five bottles was an unenriched control. In pulse–chase experiments, the cells were grown in the fermenter to $OD_{660} = 1$, and the $^{13}CO_2/H_2$ (1:4 v/v) was purged for 0.5 h before flushing with unenriched CO_2/H_2 (1:4 v/v). Aliquots of cells (200 cm^3) were removed from the fermenter at the chase times referred to in the figure legends. Early pulse–chase experiments were done under conditions where the cells had a doubling time of 22–24 h; later series had shorter doubling times, as indicated.

Cell Extracts. Cells were harvested aerobically by centrifugation (8000*g*, 10 min), washed with potassium phosphate (10 mM, pH 7.2), and centrifuged again (8000*g*, 10 min). The cells were extracted with ethanol (70% in water, 20 cm^3 per bottle), stirred at room temperature for 30 min, and centrifuged (12000*g*, 10 min). The supernatant was decanted and the residue reextracted with ethanol (10 cm^3). After centrifugation (12000*g*, 10 min), the supernatants from both centrifugations were combined and concentrated to dryness in vacuo.

In Vitro CPP Biosynthesis Experiments. Cells were harvested anaerobically by centrifugation as for cell extracts, except that all transfers were conducted under strictly anaerobic conditions. The cell pellet (3 g wet weight) was suspended in anaerobic buffer (20 cm^3, pH 7.0) containing PIPES (50 mM), $MgCl_2$ (4 mM), β-mercaptoethanol (5 mM), PMSF (1 mM, a protease inhibitor), and NaF (1 mM, a phosphatase inhibitor). The resuspended cells were sonicated with a Branson Sonifier 350 probe sonicator for 3 min under a constant stream of nitrogen. Sonicated cells (1-cm^3 final volume) were combined anaerobically with 10 mM carbon source (acetate, pyruvate, or P-enolpyruvate) and 10 mM phosphate source (P_i, P-enolpyruvate, ATP, or ADP). Samples were incubated under CO_2/H_2 (1:4 v/v) at 60 °C for 6 h, and the reaction was stopped by freezing. Thawed samples were diluted to 1.5 cm^3 with D_2O, adjusted to 10 mM in EDTA, and assayed by ^{31}P NMR.

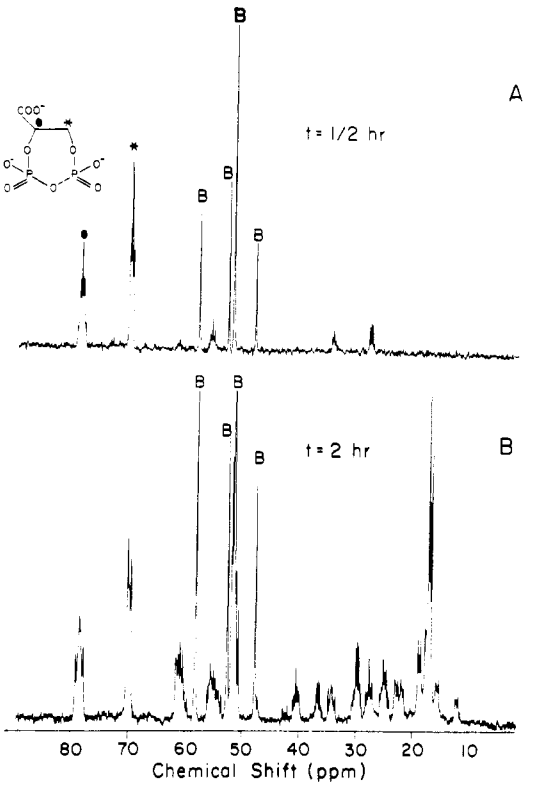

FIGURE 1: ^{1}H-decoupled ^{13}C NMR spectra at 67.9 MHz of ethanol extracts of *Mb. thermoautotrophicum* exposed to $^{13}CO_2/H_2$ (1:4 v/v) for (A) 0.5 h and (B) 2 h. Data were obtained by using a flip angle of 45°, a recycle time of 1.0 s, a sweep width of 16 000 Hz, and a data table size of 8K points; number of scans = 10 000. The FID's were exponentially multiplied with a line broadening of 2 Hz prior to Fourier transformation. Peaks marked B arise from PIPES and EDTA in the buffer.

NMR Spectroscopy. Spectra of extracts were obtained with a 6.34-T Bruker 270 instrument operating at 67.9 MHz for ^{13}C and 109.3 MHz for ^{31}P (Francis Bitter National Magnet Laboratory, MIT) and a 9.39-T Bruker AM-400 WB instrument operating at 100.6 MHz for ^{13}C (Tufts University Medical School). Dry samples obtained as described above were dissolved in buffer (10 mM potassium phosphate, 0.1 mM EDTA, pH 7.2, 50% D_2O). The ^{13}C chemical shifts in each sample were indirectly referenced to *p*-dioxane (δ_C = 67.4 ppm).

Results

When *Mb. thermoautotrophicum* was fed with $^{13}CO_2/H_2$ over 0.5 h, the major labeled compound that accumulated was CPP (see Figure 1A). For comparison, the β-carbons of glutamate and alanine resonate at 28.9 and 17.7 ppm, respectively. With 0.5-h $^{13}CO_2$ incubation, neither compound is appreciably labeled. After 2 h of exposure to $^{13}CO_2/H_2$ many other signals appeared upfield of CPP in the spectrum (Figure 1B). The signals arising from $^{13}CO_2$-labeled CPP occur at 70.09 (C-3), 78.55 (C-2), and 174.45 ppm (C-1, not shown). These carbons have been identified previously from ^{1}H-coupled spectra of purified CPP (Kanodia & Roberts,

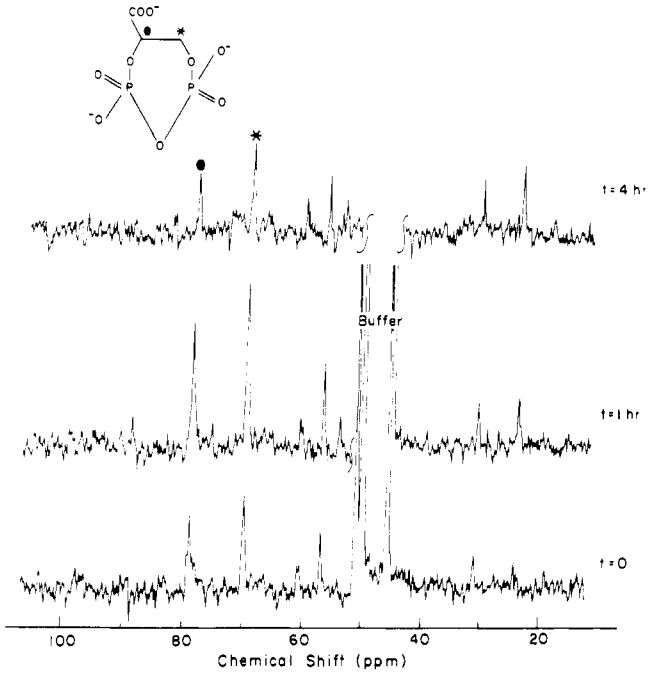

FIGURE 2: ^{1}H-decoupled ^{13}C NMR spectra at 67.9 MHz of *Mb. thermoautotrophicum* cells exposed to a $^{13}CO_2/H_2$ (1:4 v/v) pulse for 0.5 h followed with an unenriched CO_2/H_2 (1:4 v/v) chase, harvested as a function of time, and examined at 4 °C. Data were obtained and processed as in Figure 1, except that the number of scans = 800.

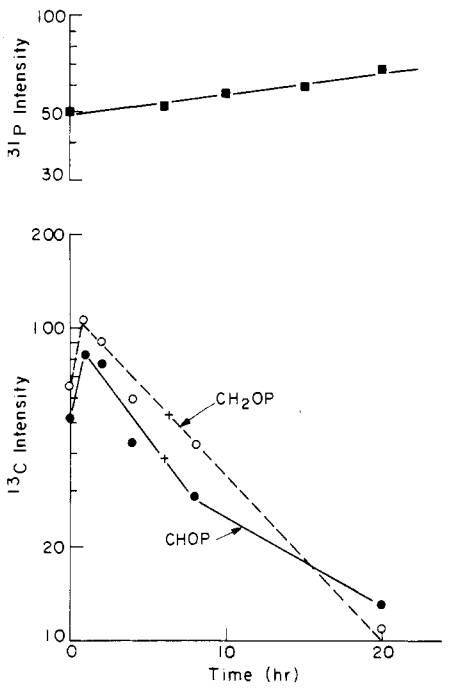

FIGURE 3: ^{13}C intensity of C-2 and C-3 of CPP and ^{31}P intensity of the pyrophosphate of CPP plotted against time after $^{13}CO_2/H_2$ (1:4 v/v) pulse/unenriched CO_2/H_2 (1:4 v/v) chase as shown in Figure 2. The (+) symbols indicate the half-times (from each individual carbon) for CPP in these cells.

1983). The multiple splittings for each carbon reflect ^{13}C–^{13}C and ^{13}C–^{31}P spin–spin couplings, with a superimposed doublet arising from ^{13}C–^{12}C species split by ^{31}P coupling. These patterns provide information on the C_1 to C_2 condensation and have been considered in detail elsewhere (Evans et al., 1985).

In addition to CPP being the major labeled carbon pool, it also turns over rapidly. In a series of $^{13}CO_2$ pulse/$^{12}CO_2$ chase experiments, we found rapid turnover of CPP (see Figure 2) compared to the cell doubling time. Figure 3 shows the in vivo intensity of [^{13}C]CPP (C-2, C-3) resonances after a $^{13}CO_2$ pulse (0.5 h) followed by an unenriched CO_2 chase as a function of time after the start of the chase. The CPP carboxyl carbon has no directly bonded proton and hence has only a small nuclear Overhauser enhancement and a long T_1. Under our spectral conditions this carbon may be partially saturated; therefore, we have not used it to follow loss of label from the CPP molecule in the pulse–chase experiment. ^{31}P NMR spectra were also obtained for each aliquot of cells. The level of ^{13}C incorporation into CPP initially increases as the remaining $^{13}CO_2$ is used up and then decreases to natural abundance levels. In this in vivo experiment, the CPP half-life is ca. 5 h [indicated by (+) on the ^{13}C decay curves in Figure 3] when the cell doubling time is about 22–24 h (measured by monitoring the asborbance of the cell suspension at 660 nm). Other pulse–chase experiments in which the cells are grown with faster doubling times (e.g., 10–12 h) yield a proportionately shorter CPP half-life (e.g., 2–3 h). Significantly, no new resonances appear with the concomitant loss of intensity of CPP resonances, either in the in vivo spectra or in spectra of ethanol extracts, which can be accumulated longer to achieve greater signal-to-noise ratios. Such new resonances would have shed light on the direct fate of CPP. The disappearance of ^{13}C intensity from the soluble fraction suggests that the CPP carbons are incorporated into a macromolecular structure in the cells, and this has been confirmed by ^{13}C CP-MASS of cell wall material (J. N. S. Evans and M. F. Roberts, unpublished results). The ^{31}P NMR spectra confirm that the steady-state concentration of CPP does not decrease over the same time period. Therefore, the enzymes that synthesize and degrade CPP are well regulated.

Since all cell carbons in *M. thermoautotrophicum* are derived from acetyl-CoA, it seems likely that acetate would act as a precursor to CPP. The results of feeding experiments of both [1-^{13}C]- and [2-^{13}C]acetate are shown in Figure 4. Clearly acetate is specifically incorporated into CPP, with C-1 of acetate labeling C-2 of CPP and C-2 of acetate labeling C-3 of CPP. The carboxylate of CPP (C-1) is almost certainly derived from CO_2. As pyruvate is biosynthesized from acetyl-CoA and CO_2 (Fuchs et al., 1978; Fuchs & Stupperich, 1980), it could presumably act as a precursor for CPP. Figure 5 shows the results of a feeding experiment with [1-^{13}C]pyruvate, in which the C-1 of CPP is the most significantly labeled species in the ethanol extract. Purification of CPP from this extract confirmed the specific enrichment of the carboxyl group and not C-2 or C-3. In order to show that pyruvate was a specific C_3 precursor, [1-^{13}C]propionate was employed in a feeding experiment, and no incorporation into CPP was observed (data not shown). Propionate is known to label isoleucine (Eikmans et al., 1983a), although propionyl-CoA is not an obligatory intermediate (Eikmans et al., 1983b).

In vitro experiments were also undertaken to see if cell extracts could synthesize CPP. A variety of combinations of pyruvate, P-enolpyruvate, acetate, ATP, or ADP were used as carbon and phosphate donors with anaerobic cell-free extracts (Table I). Levels of CPP in extracts were estimated from the integrated intensities of ^{31}P NMR spectra, and limited but significant synthesis of CPP occurred only when P-enolpyruvate was added, together with ATP or ADP. This implies that CPP biosynthesis from CO_2 occurs *after* P-enolpyruvate biosynthesis. *Mb. thermoautotrophicum* cell extracts have enzymatic activity that scrambles ATP and ADP. This activity must be inhibited by K^+, since cell extract combined with P-enolpyruvate and ADP shows CPP biosynthetic

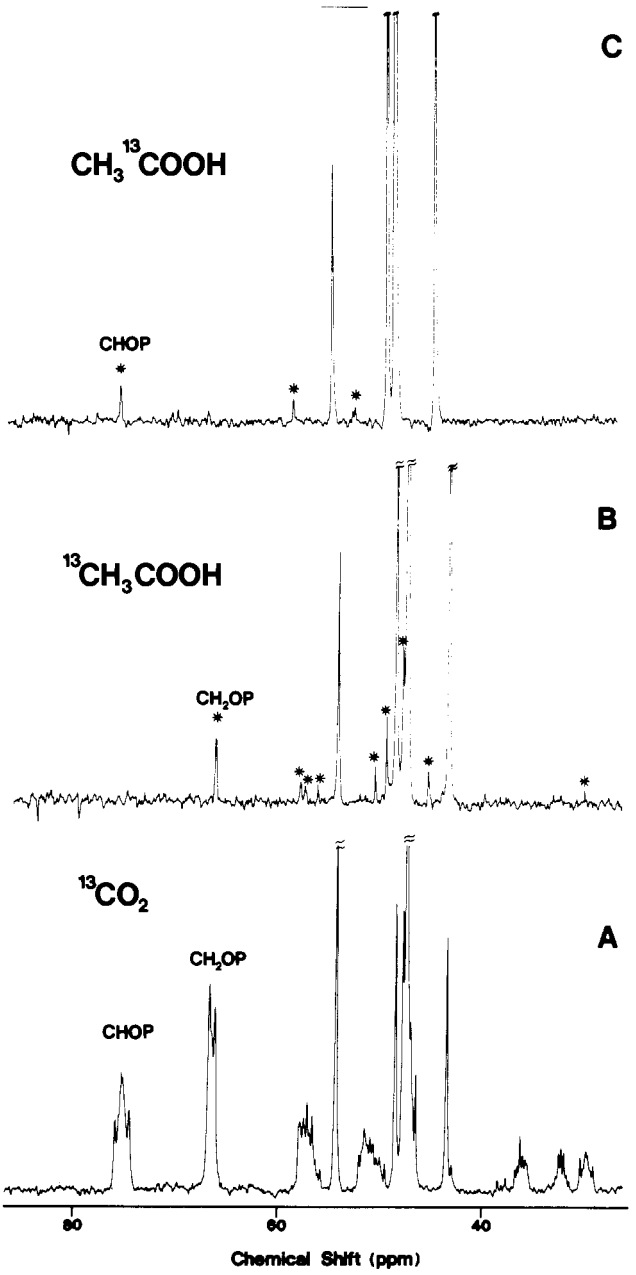

FIGURE 4: ^{1}H-decoupled ^{13}C NMR spectra at 67.9 MHz of ethanol extracts of *Mb. thermoautotrophicum* exposed to (A) $^{13}CO_2$ (2 h), (B) [2-^{13}C]acetate (20 mM), and (C) [1-^{13}C]acetate (20 mM) all for 6 h prior to extraction. Data were obtained and processed as in Figure 1. In (B) and (C) peaks marked (*) arise from labeled species derived from ^{13}C-labeled acetate; those remaining are buffer peaks.

Table I: Relative Increase in CPP Concentration in in Vitro Experiments with a Cell-Free Extract and Various Additives

additives	[CPP]/[CPP]$_0$[a]	additives	[CPP]/[CPP]$_0$[a]
control	1.0	PEP[b] + P_i/KCl	0.8
acetate + P_i	1.2	PEP + ATP	4.9*[c]
acetate + PEP	1.1	PEP + ATP/KCl	6.4*
acetate + ATP	1.5	PEP + ADP	3.8*
acetate + ADP	1.5	PEP + ADP/KCl	0.8
pyruvate + PEP	1.0	2,3-DPG[b] + PEP	1.0
pyruvate + ADP	1.1	2,3-DPG + ATP	1.0
PEP + P_i	0.8	2,3-DPG + ADP	0.9

[a] Ratio of CPP produced relative to the original concentration. [b] Abbreviations: PEP, phosphoenolpyruvate; 2,3-DPG, 2,3-diphosphoglycerate. [c] Asterisks indicate a significant increase in [CPP].

activity, which is abolished with added KCl. Likewise, the addition of KCl to P-enolpyruvate/ATP/cell extract samples

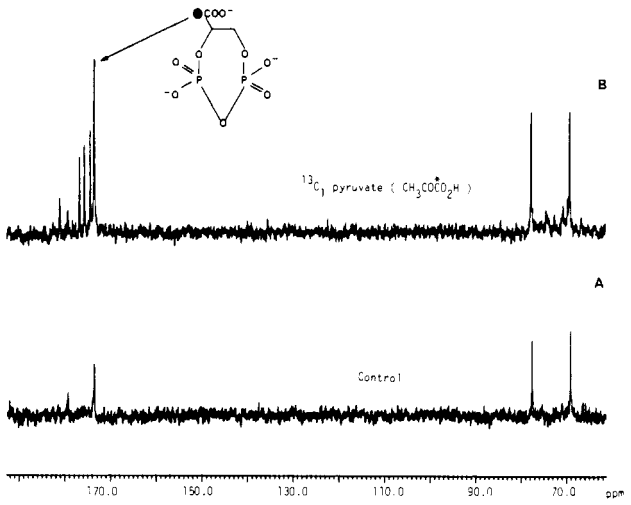

FIGURE 5: ^{1}H-decoupled ^{13}C NMR spectra at 100.6 MHz of ethanol extracts of *Mb. thermoautotrophicum* incubated for 6 h with (A) unenriched CO_2/H_2 (1:4 v/v) and (B) unenriched CO_2/H_2 and [1-^{13}C]pyruvate (20 mM). Data were obtained by using a flip angle of 30°, a recycle time of 1.0 s, a sweep width of 25 000 Hz, and a data table size of 16K points; number of scans = 400. The FID's were exponentially multiplied with a line broadening of 5 Hz prior to Fourier transformation. Spectral intensities in (A) are normalized to those in (B).

increases the biosynthesis of CPP, since the ADP-forming reaction is prevented.

The biosynthesis of CPP therefore takes place by CO_2 fixation into acetyl-CoA, which is further carboxylated to pyruvate and then P-enolpyruvate. The structure of CPP, bearing a close resemblance to 2,3-diphosphoglycerate, together with its metabolism into an insoluble pool is highly suggestive of a role in gluconeogenesis. However, like higher plants, no free glucose or similar hexoses accumulate in *Mb. thermoautotrophicum*, as indicated by the absence of appropriate resonances in the spectra in Figures 1, 2, and 4. Thus, the only method available for testing the role of CPP in gluconeogenesis would be to make use of the intracellular glycolytic enzymes to label CPP from added [^{13}C]glucose. Figure 6 shows that, by starting with [1-^{13}C]glucose, the predicted labeling of CPP would be *exclusively* in C-3. Figure 7 shows the result of a feeding with [1-^{13}C]glucose, showing that C-3 is significantly labeled. No incorporation of label into acetate, alanine, glutamate, or pyruvate was detected, implying that the back-reaction from CPP to P-enolpyruvate or pyruvate is not favored.

Discussion

Since CPP is the major soluble carbon- and phosphorus-containing species in many methanogenic bacteria, its role is of major importance. The work described here establishes unequivocally its biosynthesis from CO_2 via acetyl-CoA, pyruvate, and P-enolpyruvate and its position in the primary metabolic pathways of these organisms. The questions that remain concern the function of CPP: Is it (i) a storage pool for energy or carbon, (ii) an obligatory primary metabolite, or (iii) a metabolic regulator? The high cellular concentration of CPP suggests that it could function in carbon storage. Since the formation of the pyrophosphate linkage requires energy, it also may have a function in energy storage, although CPP is not directly linked to maintenance of ATP levels (Tolman et al., 1985). The rapid turnover of CPP implies that it is also an intermediate in a major pathway.

There is no doubt, therefore, that CPP is the principal product of CO_2 fixation. It has been suggested (Daniels &

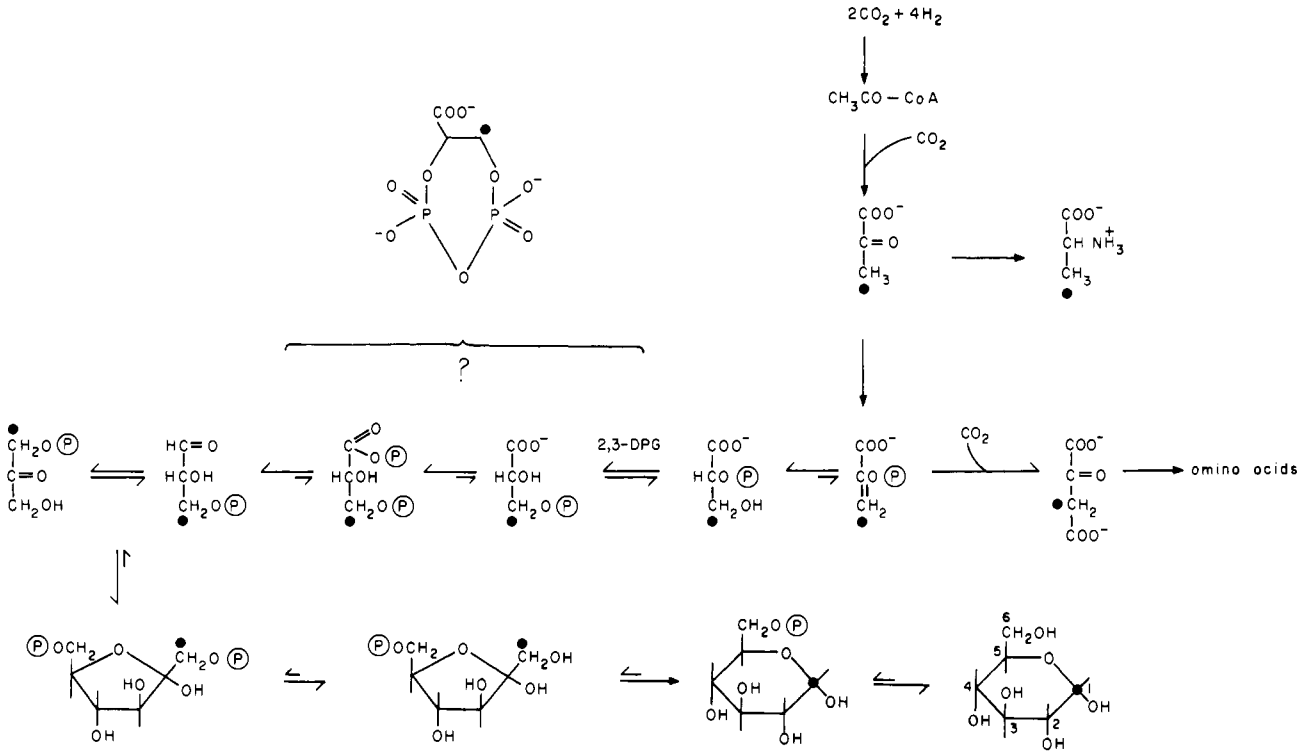

FIGURE 6: Gluconeogenesis pathway showing predicted labeling of CPP from [1-^{13}C]glucose.

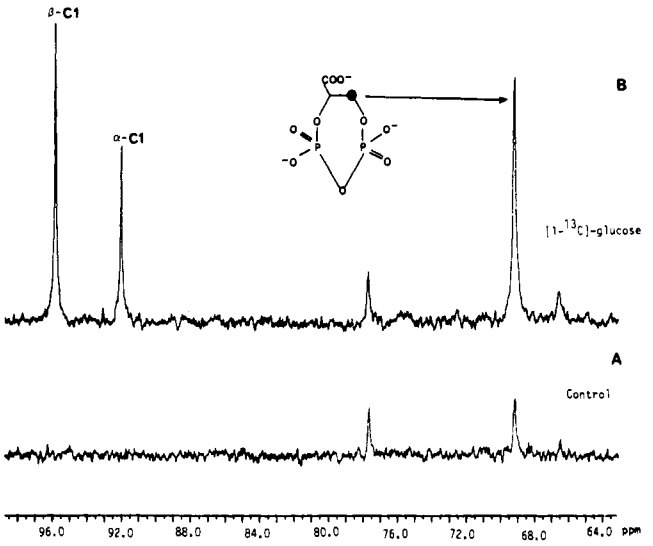

FIGURE 7: ^{1}H-decoupled ^{13}C NMR spectra at 100.6 MHz of ethanol extracts of *Mb. thermoautotrophicum* fed with (A) unenriched CO_2/H_2 (1:4 v/v) and (B) unenriched CO_2/H_2 and [1-^{13}C]glucose (10 mM). Data were obtained by using a flip angle of 90°, a recycle time of 1.0 s, a sweep width of 25 000 Hz, and a data table size of 16 K points; number of scans = 400. Data were processed as in Figure 5. Spectral intensities in (A) are normalized to those in (B). Peaks marked α-C1 and β-C1 refer to the anomeric carbons of glucose.

Zeikus, 1978; Stupperich & Fuchs, 1981) that during short CO_2 fixation periods alanine and other amino acids become labeled rather than phosphorylated compounds. The ^{13}C NMR studies presented here contradict this (amino acids are detected at lower levels of enrichment at the same time as the appearance of CPP), and ^{15}N NMR studies in this laboratory (B. S. Choi, J. N. S. Evans, and M. F. Roberts, unpublished results) also corroborate this. Fuchs et al. (1983) have asserted that carbohydrate metabolism is unidirectional and glycolysis (i.e., metabolism of glucose as a source of energy) does not take place in *Mb. thermoautotrophicum*. Furthermore, Sprott & Jarrell (1981) have asserted that glucose penetrates to the cytoplasmic membrane but does not penetrate into the cytoplasm because intact cells do not metabolize glucose. Clearly, our work indicates that glucose is metabolized by *Mb. thermoautotrophicum*. Many of the gluconeogenesis enzymes can work in the opposite direction in the presence of added glucose, as indicated by the specific incorporation of ^{13}C into CPP.

In addition to its apparent function in carbon assimilation, presumably as an obligatory gluconeogenic intermediate, CPP or a derivative could be involved in metabolic regulation. For example, Fuchs et al. (1983) have shown that phosphorylated C_3 intermediates are inhibitors of fructose-1,6-bisphosphate phosphatase, the pacemaker enzyme of gluconeogenesis. Work in this laboratory is currently under way in an attempt to establish possible sites for metabolic regulation by CPP or a direct metabolite formed from it in the gluconeogenic pathway.

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Right- and Left-Handed (Z) Helical Conformations of the Hairpin $d(C\text{-}G)_5T_4(C\text{-}G)_5$ Monomer and Dimer†

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ABSTRACT: The partial self-complementary 24-mer oligodeoxynucleotide $d(C\text{-}G)_5T_4(C\text{-}G)_5$ forms a hairpin which can be enzymatically dimerized to a dumbbell structure. The blunt-ended nature of the hairpin is indicated by its ability to inhibit the T_4 DNA ligase catalyzed joining of ϕX174 *Hae*III fragments. The hairpin monomer and dimer (dumbbell) undergo a reversible B to Z transition as shown by ultraviolet, circular dichroism, and ^{31}P NMR spectroscopy. The Z form of the hairpin monomer and dimer is supported by monovalent ions (Na^+), divalent ions (Mg^{2+} but *not* Mn^{2+}), and dehydrating (ethanol) conditions. The conformational transition of $d(C\text{-}G)_5T_4(C\text{-}G)_5$ monomer requires higher ionic or dehydrating conditions than those necessary for the corresponding linear oligomer $d(C\text{-}G)_5$. The contribution of the loop ($-T_4-$) of the hairpin to the apparent free energy change for the B to Z conformational transition at the midpoint was calculated to be 3.8 kJ mol^{-1}.

Alternating purine/pyrimidine sequences in linear DNA (Pohl & Jovin, 1972; Wang et al., 1979; Jovin et al., 1983) and RNA (Hall et al., 1984) or plasmids (Singleton et al., 1982; Nordheim et al., 1982; Haniford & Pulleyblank, 1983) can, under appropriate conditions, adopt a left-handed conformation. Supercoiled plasmids containing inverted repeats can also extrude into a cruciform structure (Panayotatos & Wells, 1981; Mizuuchi et al., 1982; Courey & Wang, 1983; Lilley, 1981). Self-complementary alternating purine/pyrimidine sequences, such as $d(G\text{-}C)_n$ present in supercoiled DNA, have the inherent ability to either adopt a Z conformation or extrude into a cruciform. A minimal insert length is required for the latter mechanism (Frank-Kamenetskij & Vologodski, 1984). The conformational freedom of DNA hairpin structures, parts of cruciforms, and the possible structural restrictions in hairpin loops have not been studied in detail. In particular, can hairpin structures, which contain potential Z-DNA sequences, undergo a B to Z transition, or does the loop provide a barrier to this transition? Superhelical free energy is not expected to promote a conformational change in cruciforms, since the hairpin arms are topological independent domains from the rest of the superhelical DNA molecule. However, Z-binding proteins or Z-specific ligands may provide the necessary free energy for a conformational transition in the constituent hairpin arms of a cruciform.

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To answer these questions, the conformational flexibility of DNA hairpins has been studied by using $d(C\text{-}G)_5T_4(C\text{-}G)_5$ as a model substrate. Here we report on a B to Z conformational transition of the monomer hairpin and dimer dumbbell of $d(C\text{-}G)_5T_4(C\text{-}G)_5$.

Experimental Procedures

Synthesis, Purification, and Characterization of $d(C\text{-}G)_5T_4(C\text{-}G)_5$. Deoxyoligonucleotide $d(C\text{-}G)_5T_4(C\text{-}G)_5$ was synthesized on an Applied BioSystems Model 380A DNA synthesizer using phosphoramidate chemistry (Beaucage & Caruthers, 1981). Step yields greater than 99% were obtained. After deblocking and detritylation, the synthesis product was purified by anion-exchange chromatography at pH 13.0 on NACS-20 (Bethesda Research Laboratories).

The purified oligonucleotide was 5′-end labeled with [γ-^{32}P]ATP and T_4 polynucleotide kinase (Chaconas & van de Sande, 1980) and then analyzed by digestion to 5′-mononucleotides (Kleppe et al., 1970). Thermal denaturation profiles were determined in 0.5 mM sodium phosphate and 10^{-5} M ethylenediaminetetraacetic acid (EDTA), pH 7.0.

T_4 DNA Ligase Reactions. Ligation of ϕX174 *Hae*III restriction fragments (3.6 μg) in 10 μL of ligase buffer [50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), and 1 mM ATP] and T_4 DNA ligase (6 units) was carried out at ambient temperatures for 18 h in the presence or absence of a 70-fold excess of $d(C\text{-}G)_5T_4(C\text{-}G)_5$. Dimerization

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