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Carbon assimilation pathways in sulfate reducing bacteria. Formate, carbon dioxide, carbon monoxide, and acetate assimilation by *Desulfovibrio baarsii*

Kathrin Jansen [1], Rudolf K. Thauer [1], Fritz Widdel [2], and Georg Fuchs [3]

[1] Fachbereich Biologie, Universität Marburg, Lahnberge, D-3550 Marburg, Federal Republic of Germany
[2] Fakultät für Biologie, Universität Konstanz, Universitätsstraße 10, D-7750 Konstanz, Federal Republic of Germany
[3] Abteilung Angewandte Mikrobiologie, Universität Ulm, Oberer Eselsberg, D-7900 Ulm, Federal Republic of Germany

Abstract. *Desulfovibrio baarsii* is a sulfate reducing bacterium, which can grow on formate plus sulfate as sole energy source and formate and CO_2 as sole carbon sources. It is shown by ^{14}C labelling studies that more than 60% of the cell carbon is derived from CO_2 and the rest from formate. The cells thus grow autotrophically. Labelling studies with [^{14}C]acetate, ^{14}CO and [^{14}C]formate indicate that CO_2 fixation does not proceed via the Calvin cycle. The labelling patterns of alanine, aspartate, glutamate, and glucosamine indicate that acetate (or activated acetic acid) is an early intermediate in formate and CO_2 assimilation; the methyl group of acetate is derived from formate, and the carboxyl group from CO_2 via CO; pyruvate is formed from acetyl-CoA by reductive carboxylation. The capacity to synthesize an acetate unit from two C_1-compounds obviously distinguishes *D. baarsii* from those *Desulfovibrio* species, which require acetate as a carbon source in addition to CO_2.

Key words: *Desulfovibrio baarsii* – Autotroph – Sulfate reducing bacteria – Activated acetic acid pathway – Formate – Carbon monoxide dehydrogenase – Pyruvate synthesis – Ribulose-bisphosphate carboxylase

It has been a point of controversy for many years whether sulfate reducing bacteria exist which are capable of autotrophic growth. It has also been controversial whether such organisms – supposed they exist – use the Calvin cycle for CO_2 assimilation or not.

As to their status as autotrophs, Postgate (1979) stated positively that "… these bacteria are recognized not to be true autotrophs …". The early work of Butlin and Adams (1947) and Sisler and Zobell (1950, 1951) and failures to confirm autotrophy have been critically reviewed (Rittenberg 1969; Postgate 1979). In fact, all sulfate reducing bacteria studied since then required acetate for growth, in addition to CO_2, if grown on H_2 or formate as sole electron donors. CO_2 accounted for only ~30% of cell carbon (Sorokin 1966a–c; Rittenberg 1969; Postgate 1970, 1979; Badziong et al. 1978, 1979; Brandis and Thauer 1981). However, Widdel and Pfennig recently isolated many different strains of sulfate reducing bacteria which definitely were capable of utilizing CO_2 as sole carbon source (Widdel 1980; Pfennig et al. 1981; Pfennig and Widdel 1981, 1982; Widdel et al. 1983; K. Brysch and F. Widdel personal communication). Among the sulfate reducers, *Desulfovibrio baarsii* takes a mid-position. It uses formate but not H_2 as electron donor. In addition to formate, it can also oxidize fatty acids as large as stearate completely to CO_2. As will be shown here, both formate and CO_2 are used as carbon sources. This study was undertaken to learn how carbon from CO_2 and formate, respectively, is assimilated into cell compounds. Three findings directed our research: (1) The organism produced small amounts of acetate during growth on formate; (2) it contained a very active carbon monoxide dehydrogenase, measured with methyl viologen as the electron acceptor; (3) ribulose-1,5-bisphosphate carboxylase could not be detected.

These findings pointed to a pathway of CO_2 assimilation via acetyl CoA as intermediate as has recently been described for autotrophic methanogenic and acetogenic bacteria (Fuchs and Stupperich 1983; Stupperich et al. 1983; Stupperich and Fuchs 1984a, b; Eden and Fuchs 1982; Ljungdahl and Wood 1982).

Materials and methods

The strain of *Desulfovibrio baarsii* 2st14 was enriched from anaerobic fresh water mud with stearate plus sulfate and isolated in anaerobic agar dilution series with formate plus sulfate (Widdel 1980, 1981). Purity of the strain was controlled by inoculating the medium used for *Acetobacterium woodii* (Bache and Pfennig 1981) containing yeast extract plus fructose, yeast extract plus trimethoxybenzoic acid, and yeast extract plus $H_2 + CO_2$ gas mixture, respectively. Neither growth nor cell forms other than vibrioid bacteria were observed, indicating that homoacetogenic bacteria were not present. Radioisotopes were purchased from Amersham Buchler (Braunschweig, FRG) and from NEN (Dreieich, FRG). ^{14}CO was prepared from [^{14}C]formate as described elsewhere (Stupperich and Fuchs 1984b). Enzymes and coenzymes, if not otherwise stated, were from Boehringer Mannheim (Mannheim, FRG).

Growth

D. baarsii was routinely grown at 37° C in a stoppered 250 ml flask. It contained 120 ml anaerobic mineral medium

Offprint requests to: G. Fuchs

routinely used for freshwater species (Pfennig et al. 1981) under 1.5 bar N_2/CO_2 [80%/20% (v/v)] gasphase. Formate plus sulfate was the sole energy source, carbon sources were formate and CO_2. The medium contained per l: Sodium formate, 3.4 g; Na_2SO_4, 3 g; KH_2PO_4, 0.2 g; NH_4Cl, 0.25 g; NaCl, 1 g; $MgCl_2 \cdot 6\,H_2O$, 0.4 g; KCl, 0.5 g; $CaCl_2 \cdot 2\,H_2O$, 0.15 g; $NaHCO_3$, 2.5 g; $Na_2S \cdot 9\,H_2O$, 0.36 g. Trace element solution SL 10 (Widdel et al. 1983), 1 ml; $Na_2SeO_3 \cdot 5\,H_2O$, 3 µg; $Na_2WO_4 \cdot 2\,H_2O$, 4 µg; resazurin as redox indicator, 0.8 mg. Bicarbonate and sulfide were added from sterile stock solutions after autoclaving the mineral medium. The initial pH was about 7. The medium was inoculated with 10% of the culture volume. Growth was followed by measuring the optical density at 578 nm (d = 1 cm) against the medium blank. The medium became alkaline due to sulfate and formate consumption. The pH was therefore readjusted and formate supplied by adding 10 ml/l of a solution of 0.25M formic acid and 0.75M sodium formate, when the $\Delta A_{576\,nm}$ of the culture was approximately 0.17. The cultures were harvested at an optical density of 0.25 – 0.3.

Long term tracer studies

Tracer studies were conducted using the same medium, but under slightly modified conditions. [U-^{14}C]Acetate assimilation was studied in a 250 ml flask under 1.5 bar N_2/CO_2 [80%/20% (v/v)] containing 120 ml medium supplemented with 0.19 mM (initial concentration) [U-^{14}C]acetate (initial specific radioactivity, 10.5 kBq/µmol = 6.3×10^5 dpm/µmol). ^{14}CO assimilation was studied in a 1 l flask containing 580 ml medium and 535 ml N_2/CO_2 gas mixture [80%/20% (v/v)] at a pressure of 1.5 bar. Eight microliter ^{14}CO (specific radioactivity, 6 kBq/µmol = 360,000 dpm/µmol) were added. [^{14}C]Formate assimilation was studied in a 1 l fermentor with 580 ml medium supplemented with [^{14}C]formate (initial specific radioactivity, 723 Bq/µmol = 43,000 dpm/µmol) which was gassed with N_2/CO_2 gas mixture [80%/20% (v/v)] at a rate of 60 ml/min. The [^{14}C]acetate-, [^{14}C]formate-, and ^{14}CO-cultures were grown for approximately 3, 0.5, and 1 generations, respectively, in the presence of the tracer molecule.

Incorporation of ^{14}C into growing cultures

At different times during growth 1 – 2 ml samples were withdrawn for determination of pH, cell density, and radioactivity in the medium including cells. A 1 ml aliquot of the sample was centrifuged, and acetate and formate were determined in the supernatant. The label in the supernatant was determined after acidifying the sample with $HClO_4$ and flushing with 80% N_2/20% CO_2 gas mixture in an ice bath. Then the acid was neutralized with $KHCO_3$. The cell pellets were washed several times, solubilized with Lumasolve (Baker, Groß-Gerau, FRG), and radioactivity in the dissolved cell material was determined. Cells were fractionated as described by Fuchs et al. (1978). The specific radioactivity of cell carbon was determined after wet combustion of washed and dried [^{14}C]-labelled cells (Van Slyke and Folch method, modified by Watson and Williams 1970). The $^{14}CO_2$ was trapped in 1 M NaOH, the amount of carbon in an aliquot was quantitated as barium carbonate (Simon and Floss 1967), and ^{14}C in an aliquot was determined by liquid scintillation counting.

Isolation of alanine, aspartate, glutamate and glucosamine

The amino acids were isolated from the hydrolysate of the protein fraction of labelled cells (Fuchs et al. 1978; Fuchs and Stupperich 1980). Glucosamine was isolated from the hydrolyzed cell wall fraction (Jansen et al. 1982).

Degradation of isolated labelled compounds and determination of radioactivity

Alanine and aspartate were sequentially degraded to CO_2, glutamate was decarboxylated at C-1 (Simon and Floss 1967; Fuchs et al. 1980). Radioactivity in aqueous solution was determined by liquid scintillation counting in Aqualuma cocktail, dissolved cell material was counted in Lipoluma (Baker, Groß-Gerau, FRG).

Determination of compounds

Aspartate, glutamate and glucosamine were quantitated and their purity was proven in an amino acid analyzer by Dr. Linder, Universität Gießen. Alanine, which was enzymatically converted into lactate, was isolated as L-lactate and determined enzymatically (Gutmann and Wahlefeld 1974). Acetate (using acetyl CoA synthetase) and formate (Höpner and Knappe 1974) were determined enzymatically. CO was quantitated by gas chromatography (Daniels et al. 1977).

Results

1. Incorporation of [^{14}C]formate

Desulfovibrio baarsii was grown at 37°C and pH 7 in a mineral medium containing 50 mM formate and 20 mM sulfate. The gas phase contained 20% CO_2 and 80% N_2. Formate was oxidized to CO_2 with the concomitant reduction of sulfate to H_2S. Fermentor cultures gassed with 20% CO_2/80% N_2 gas mixture grew mostly exponentially with a generation time of ~20 h. The cells were harvested at the end of growth ($\Delta A_{578\,nm}$ = 0.3). At this time, all of the formate had been consumed by the culture.

When [^{14}C]formate was added (Fig. 1), radioactivity in the culture decreased faster than formate; most of the ^{14}C lost was recovered as $^{14}CO_2$. This was due to a rapid isotope exchange between [^{14}C]formate and $^{12}CO_2$. As a result, the specific radioactivity of [^{14}C]formate continuously decreased.

[^{14}C]Formate was incorporated into the cells. The rate of uptake continuously decreased, most likely because the specific radioactivity of [^{14}C]formate decreased from 383 Bq/µmol (23,000 dpm/µmol) at the beginning to 37 Bq/µmol (2,200 dpm/µmol) after 76 h, when the culture was harvested. After harvest the specific radioactivity of cell carbon was 73 Bq/µmol (4,380 dpm/µmol). In order to follow the incorporation of [^{14}C]formate into specific cell compounds, an exponentially growing culture (20 h generation time) was incubated for only half a generation in the presence of [^{14}C]formate (Table 1). The culture was vigorously gassed with N_2/CO_2 gas mixture in order to continuously dilute and blow out the $^{14}CO_2$ formed from [^{14}C]formate. During the incubation period, the formate concentration decreased from 22 mM to 11 mM, the specific radioactivity of [^{14}C]formate decreased by approximately 50%.

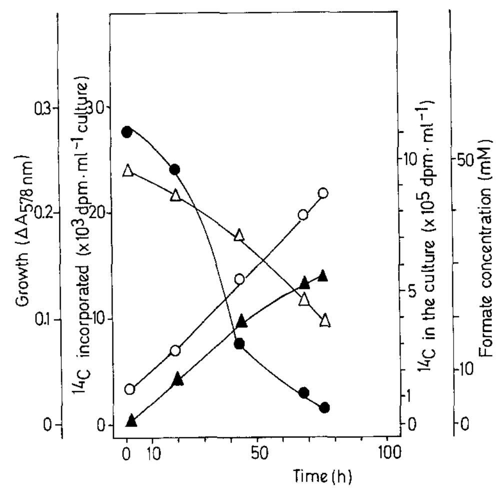

Fig. 1. Incorporation of ^{14}C from [^{14}C]formate into *Desulfovibrio baarsii* growing on formate, CO_2 and sulfate. (○) Growth; (●) total ^{14}C in culture (liquid phase); (△) formate concentration; (▲) ^{14}C incorporated into cells. For experimental details see Materials and methods

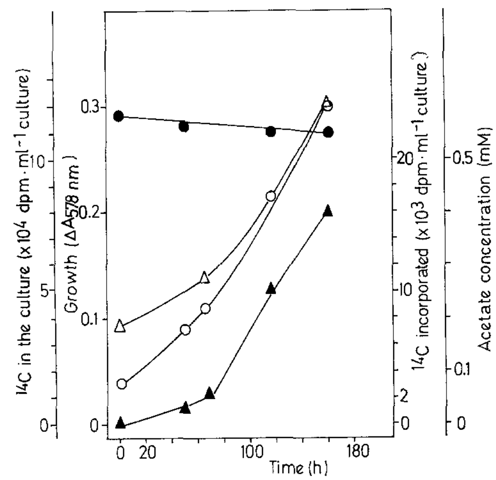

Fig. 2. Incorporation of ^{14}C from [U-^{14}C]acetate into *Desulfovibrio baarsii* growing on formate, CO_2, and sulfate in presence of 0.19 mM [U-^{14}C]acetate. (○) Growth; (●) total ^{14}C in culture (liquid phase); (△) acetate concentration; (▲) ^{14}C incorporated into cells. For experimental details see Materials and methods

Table 1. Incorporation of ^{14}C from [^{14}C]formate into alanine by *Desulfovibrio baarsii.* The organism was grown on formate and sulfate in a fermentor with 580 ml mineral medium, which was gassed with N_2/CO_2 [80%/20% (v/v)] at a rate of 60 ml/min. [^{14}C]Formate was added to the growing culture at an $\Delta A_{578\,nm} = 0.155$. The culture was harvested at an $\Delta A_{578\,nm} = 0.237$; alanine was isolated from the ^{14}C-labelled protein fraction. Cell material synthesized in presence of [^{14}C]formate was 34.6% of total cell material. The specific radioactivities of alanine and its individual carbon atoms were corrected correspondingly

Labelled compound or carbon atom	Specific radioactivity (Bq/µmol)
Formate at $\Delta A = 0.155$	723
Formate at $\Delta A = 0.237$	342
Alanine	145
Alanine corrected[a]	420
C-1 of alanine	20
C-2 of alanine	119
C-3 of alanine	281

[a] By subtraction of the amount of unlabelled alanine present in cells before addition of the tracer

From the protein of labelled cells alanine was isolated; its specific radioactivity was 145 Bq/µmol (8,720 dpm/µmol). The actual specific radioactivity of the alanine synthesized from [^{14}C]formate was three times higher, because 2/3rd of the cells were present before label was added. The amino acid was chemically degraded to evaluate the ^{14}C-content of its individual carbon atoms. C-3 of alanine carried 67% of the total label in the molecule, C-2 and C-1 contained only 28% and 5%, respectively.

2. *Incorporation of [U-^{14}C]acetate*

During growth the organism consistently produced small amounts (0.6 mmole/l) of acetate. It was tested whether acetate was assimilated. [U-^{14}C]Acetate (0.19 mM) was initially added to a 120 ml culture growing on formate, CO_2, and sulfate; [^{14}C]incorporation was followed during 3 generations (Fig. 2).

Table 2. Incorporation of ^{14}C from [U-^{14}C]acetate into cell compounds of *Desulfovibrio baarsii.* The organism was grown for 3 generations on formate, CO_2 and sulfate in presence of [U-^{14}C]acetate (0.19 mM initially added). Alanine, aspartate and glutamate were isolated from the protein fraction, glucosamine from the cell wall fraction of ^{14}C-labelled cells and the specific radioactivity was determined. Cell material synthesized in presence of [^{14}C]acetate was 87% of total cell material. The specific radioactivities were corrected correspondingly

Labelled compound	Specific radioactivity (Bq/µmol)
[U-^{14}C]Acetate added	
initially present	10,500
at the end of growth	2,500
Alanine	441
Aspartate	424
Glutamate	836
Glucosamine	914

The specific radioactivity of acetate at the beginning was 10.5 kBq/µmol (630,000 dpm/µmol). The culture grew exponentially with a doubling time of 40 h up to a cell density of $\Delta A_{578\,nm} = 0.25$ (≙ 0.25 g wet cells/l) and was harvested at $\Delta A_{578\,nm} = 0.3$. Due to acetate production (Fig. 2), the specific radioactivity of acetate decreased approximately 4-fold (Table 2). 14% of the radioactivity initially added was taken up into 30 mg wet cells. Almost the same amount of [^{14}C]disappeared from the medium. The data indicate, that even in the presence of exogeneous acetate the cells grew autotrophically, because there was always a net production of acetate.

When the ^{14}C-labelled cells were fractionated, label was found in the cell wall, nucleic acids, protein, lipid, and low molecular weight fractions. The proportions of ^{14}C in the individual fractions were similar to those found in *Methanobacterium* grown in presence of [^{14}C]acetate (Taylor et al. 1976; Fuchs et al. 1978). Alanine, aspartate and glutamate were isolated from the protein fraction, and glucosamine was isolated from the cell wall fraction. Their specific radioactivites were determined (Table 2) and the label distribution was analyzed by degradation. The specific radioactivity of alanine and of aspartate was nearly the same. It amounted to 4% of the specific radioactivity of the [^{14}C]acetate present at the beginning and to 18% of that present at the end of growth. The specific radioactivities of glutamate and glucosamine were similar to each other, but twice as high as that of alanine. The label distribution in alanine was: 4% in C-1, 41% in C-2, and 48% in C-3 (93% recovery). Aspartate contained 9% of its label in C-1 plus C-4, and 91% in C-2 plus C-3. Glutamate contained 19% of its total label in the molecule in C-5. The amount of [^{14}C]glucosamine isolated from the cells was not sufficient for degradation.

Table 3. Incorporation of ^{14}C from [^{14}C]CO into cell compounds of *Desulfovibrio baarsii*. The organism was grown for 1 generation on formate, CO_2, and sulfate in the presence of ^{14}CO (1.5 vol% CO initially added). Alanine, aspartate and glutamate were isolated from the protein fraction of ^{14}C-labelled cells and their specific radioactivity was determined. Cell material synthesized in presence of ^{14}CO was 45% of total cell material. The specific radioactivities were corrected correspondingly

Labelled compound	Specific radioactivity (Bq/μmol)
[^{14}C]CO added	6,000
Alanine	252
Aspartate	239
Glutamate	424

3. Incorporation of ^{14}CO

A 580 ml culture was grown in a sealed 1 l flask on formate, CO_2, and sulfate. At an optical density of $\Delta A_{578\,nm} = 0.08$, ^{14}CO was added (1.5% v/v; 6 kBq/μmol = 360,000 dpm/μmol). After approximately one generation time (41 h; $\Delta A_{578\,nm} = 0.145$), when the ^{14}CO had just been completely metabolized, the cells were harvested. ^{14}C incorporation from ^{14}CO into alanine, aspartate, and glutamate was studied (Table 3). The specific radioactivities of alanine and aspartate were virtually identical and each 4% of that of the added ^{14}CO. The specific radioactivity of glutamate was twice as high as that of alanine. Table 4 shows the label distribution within the molecules. Alanine was predominantly labelled in C-2 (72%); C-1 and C-3 each contained only 14% of the total label. Aspartate was predominantly labelled in C-2 and C-3. Glutamate C-5 contained 3.3 times more ^{14}C as compared to C-1.

Discussion

Desulfovibrio baarsii can grow on formate and CO_2 as sole carbon sources. By [^{14}C]formate labelling, we have tried to ascertain the distribution of cell carbon derived from formate and from CO_2. It is shown (Fig. 1) that one cannot differentiate exactly between the two species, because of the rapid isotope exchange between [^{14}C]formate and $^{12}CO_2$. From the data of the [^{14}C]formate labelling experiment (Table 1), however, it can be concluded: If all carbon atoms of alanine were derived from [^{14}C]formate, the specific radioactivity of the amino acid would be at least three times that of [^{14}C]formate at the end of growth (3×342 Bq/μmol = 1,026 Bq/μmol). We only found 420 Bq/μmole alanine (~40%). This means that at least 60% of cell carbon

Table 4. Degradation of [^{14}C]-labelled alanine, aspartate and glutamate from cells of *Desulfovibrio barrsii* grown with CO_2 and formate as carbon sources in presence of ^{14}CO. The label distribution in % refers to the total label in the individual compound ≙ 100%

Compound degraded	Degradation method	CO_2 recovered from	Radioactivity added in individual degradation step (Bq)	Radioactivity recovered in CO_2 (Bq)	Percentage of total radioactivity in individual carbon atoms (%)
Alanine	alanine → lactate → acetate + CO_2	C-1	175.8	25.7	14.6
	acetate → $CH_3NH_2 + CO_2$	C-2	94.5	72.4	71.7
	CH_3NH_2 → $NH_3 + CO_2$	C-3	n.d.	13.8	13.7
Aspartate	aspartate → $C_2 + 2\,CO_2$	C-1 + C-4	84.2	20.5	24.3
	aspartate → lactate + CO_2	C-4	140.5	14.3	10.2
	lactate → acetate + CO_2	C-1	86.5	8.7	9.0
	acetate → $CH_3NH_2 + CO_2$	C-2	53.3	24.7	46.3
	CH_3NH_2 → $CO_2 + NH_3$	C-3	n.d.	18.4	34.5
Glutamate	glutamate → $C_4 + CO_2$	C-1	110.9	12.2	11.0
	glutamate → $C_4 + CO_2$	C-5	195.8	71.5	36.5

was derived from CO_2, since no other carbon source besides formate and CO_2 was present. *D. baarsii* thus was shown to grow autotrophically.

The labelling data presented in this paper further indicate, that in *D. baarsii* formate, carbon dioxide, carbon monoxide, and acetate are assimilated, with acetate or activated acetic acid as an intermediate, as depicted in Fig. 3. Synthesis of cell compounds from acetyl CoA requires further carboxylations. From acetyl CoA on, biosynthesis resembles acetate assimilation by *Desulfovibrio vulgaris* (Marburg) except that glutamate labelling is consistent with a si-stereospecificity of citrate synthase, but not with a re-stereospecificity (Gottschalk and Barker 1967; Gottschalk 1968; Badziong et al. 1979). Yet, si-stereospecificity has been reported for the *Desulfovibrio gigas* enzyme (Gottschalk 1968) and for the enzyme of *Desulfobacter postgatei* (Brandis-Heep et al. 1983; Gebhardt et al. 1983). The labelling data are not at all consistent with the operation of the Calvin cycle. This is supported by the failure to demonstrate ribulose-1,5-bisphosphate carboxylase in cell extracts (B. Bowien, personal communication; unpublished results). It should be noted however, that *Desulfovibrio vulgaris* (Hildenborough) growing on lactate and sulfate has been reported to contain the carboxylase (Alvarez and Barton 1977; Barton 1981). This is a surprising finding, since this strain cannot grow autotrophically.

Acetyl CoA in *D. baarsii* appears to be synthesized in a process analogous to the clostridial total synthesis of acetate from two CO_2 (Ljungdahl and Wood 1982). This is supported by the following findings: 1. The labelling pattern indicated that the methyl position of pyruvate, which corresponds to the methyl position of activated acetic acid, was preferentially synthesized from [^{14}C]formate (Table 1). This has also been established in acetogenic bacteria (Ljungdahl and Wood 1982). A minor part of C-2 of alanine, which corresponds to the carboxyl group of activated acetic acid, was also synthesized from [^{14}C]formate. However, most of carbon position 2 came from unlabelled $^{12}CO_2$. This can be explained if $^{14}CO_2$ formed from [^{14}C]formate was not in free equilibrium with $^{12}CO_2$ and was preferentially incorporated into the carboxyl group of acetate. A similar effect has been observed in *Methanosarcina barkeri* (Kenealy and Zeikus 1982). 2. *D. baarsii* produced small amounts of acetate, when grown on formate plus sulfate. This acetate was assimilated into all cell fractions. 3. *D. baarsii* contained high activities of carbon monoxide dehydrogenase (see also Yagi 1958, 1959; Postgate 1970). The specific activity with methylviologen as electron acceptor was 2.5 μmol CO oxidized min^{-1} mg^{-1} cell protein; the apparent K_M-values were 18 μM for CO and 2 mM for methylviologen (unpublished). Carbon monoxide dehydrogenase has been shown to be involved in the total synthesis of acetate from CO_2 in acetogenic bacteria (Diekert and Thauer 1978; Hu et al. 1982; Diekert and Ritter 1983) and in methanogenic bacteria (Stupperich et al. 1983; Stupperich and Fuchs 1984a, b). This enzyme was postulated to reduce CO_2 in a reversible reaction to a bound intermediate in the oxidation state of carbon monoxide, which can exchange with gaseous ^{14}CO (Fig. 4). This was shown to occur also in *D. baarsii*. The C-2 of alanine was preferentially labelled from ^{14}CO (Table 4), which indicates that the carboxyl of acetate was synthesized from CO. The specific radioactivities (Table 3) and labelling patterns (Table 4) of alanine, aspartate, and glutamate correspond to the hypothetical pattern, if acetyl CoA was de-novo synthesized and then assimilated. Since ^{14}CO was oxidized to $^{14}CO_2$, the slight label incorporation into other carbon positions can easily be explained.

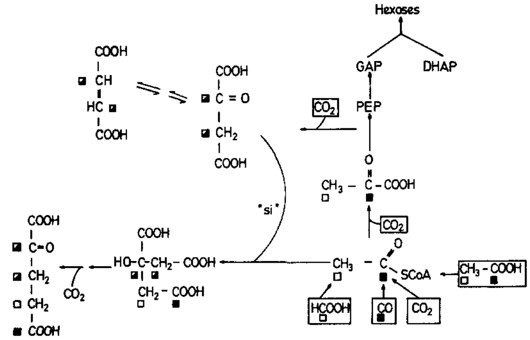

Fig. 3. Proposed synthesis in *Desulfovibrio baarsii* of pyruvate (≙ alanine), oxaloacetate (≙ aspartate), α-ketoglutarate (≙ glutamate) and hexoses (≙ glucosamine) from acetate, formate, CO, and CO_2. The scheme visualizes the observed labelling pattern. *PEP* Phosphoenolpyruvate; *GAP* glyceraldehyde-3-phosphate; *DHAP* dihydroxyacetonephosphate; *"si"* si-stereospecific citrate synthase

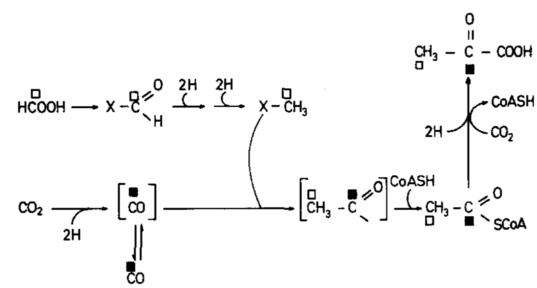

Fig. 4. Proposed pathway of formate, CO_2, acetate, and CO assimilation in *Desulfovibrio baarsii*. The scheme is consistent with the observed labelling pattern. This hypothetical pathway is similar to that proposed for acetogenic (Ljungdahl and Wood 1982; Eden and Fuchs 1982) and methanogenic bacteria (Stupperich et al. 1983). [CO] bound carbon monoxide. *X* One-carbon-carrying coenzyme, possibly a pteridine

Our results show that certain species of sulfate reducing bacteria represent a third physiological group of chemolithotrophic anaerobes, besides autotrophic homoacetogenic and autotrophic methanogenic bacteria. These three groups are capable of synthesizing all their cell carbon from C_1-compounds and appear to use a similar "activated acetic acid pathway".

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