Glycine Fermentation via a Glycine Reductase in *Peptococcus glycinophilus* and *Peptococcus magnus*

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Abstract. *Peptococcus glycinophilus* and *P. magnus* (*P. variabilis*) utilized only glycine-containing compounds for growth and required selenium compounds for the fermentation of glycine in an optimized medium. Under these conditions an active glycine reductase was expressed in vivo as demonstrated for both species by tracer experiments and additionally in vitro for *P. glycinophilus* in cell extracts. It is concluded that the availability of selenium determines the fermentation pathway of glycine. In the presence of selenite glycine is converted via the glycine reductase rather than the hitherto known glycine-serine-pyruvate interconversion. The molar growth yield of *P. magnus* was determined to be 8.9 g of dry weight per mol of glycine. The significance of the low vitamin B₁₂ content and a low carbon monoxide dehydrogenase activity in *P. glycinophilus* for the reduction of CO₂ to acetate is discussed.

Key words: *Peptococcus glycinophilus* — *Peptococcus magnus* — Glycine fermentation — Glycine reductase — Selenium — CO₂ reduction to acetate — B₁₂ content — CO dehydrogenase

phosphate to acetate 2 mol are required for the production of 2 mol methylenetetrahydrofolate from CO₂, only 1 mol ATP is available for anabolic reactions.

Recently, a more economic pathway of glycine degradation has been described to occur in *Clostridium purinolyticum* (Dürre and Andreesen 1982a). This organism oxidizes 1 mol glycine completely to CO₂ and NH₃, and uses the reducing equivalents formed to reduce 3 further mol glycine directly to acetate. The last reaction is coupled to ATP production so that a maximum of 4 mol ATP can be gained from the fermentation of 4 mol glycine with 1 mol formed via the formyltetrahydrofolate synthetase reaction and 3 mol via the glycine reductase reaction. *C. purinolyticum* strictly depends on selenium compounds for growth which suggests that the glycine reductase might be a selenoenzyme as has been demonstrated for the enzyme from *C. sticklandii* (Turner and Stadtman 1973).

This work presents evidence that both *P. glycinophilus* and *P. magnus* reduce glycine directly to acetate via a glycine reductase after supplementation of the growth medium with selenite. Under these conditions, the organisms no longer use the energetically less efficient glycine-serine-pyruvate interconversion for the fermentation of glycine.

Materials and Methods

Peptococcus glycinophilus (ATCC 23195, DSM 20474) and *P. magnus* (ATCC 14955 and ATCC 14956) were used in this study and were obtained from Dr. N. Weiss, DSM, München, FRG. *P. glycinophilus* was grown at 37°C either in a complex medium (Cardon and Barker 1947) or in a medium containing the trace element selenium and only a low amount (0.1%, w/v) of yeast extract (Dürre and Andreesen 1982a). *P. magnus* was routinely grown at 37°C in a basal medium containing (per l): yeast extract, 10 g; Tween 80, 1 ml; salt solution, 40 ml; trace element solution SL 8 (Pfennig et al. 1981), 1 ml; Na₂SeO₃, 10⁻⁷ M; Na₂MoO₄, 10⁻⁷ M; Na₂WO₄, 10⁻⁷ M, cysteine-HCl, 0.5 g. The salt solution consisted of (g per l): CaCl₂, 0.2; MgSO₄ × 7 H₂O, 0.2; K₂HPO₄, 1.0; KH₂PO₄, 1.0; NaHCO₃, 10; NaCl, 2.0. The substrate glycine, 100 mM, was added to the basal medium, the final pH was 7.2. All media were prepared under strictly anaerobic conditions. Substrate utilization tests were performed as described previously (Dürre et al. 1981). Turbidity was measured at 600 nm in a Bausch & Lomb Spectronic 88 or a Zeiss PM 4 spectrophotometer.

The protein content of whole cells was determined according to Stickland (1951), the dry weight of cells on membrane filters (Dürre and Andreesen 1982a). Hydrogen, carbon

Peptococcus (*Diplococcus*) *glycinophilus* and *P. (Micrococcus) variabilis* are unique organisms because of their very restricted substrate spectrum which includes only glycine and some glycine-containing compounds (Cardon and Barker 1946, 1947; Foubert and Douglas 1948; Douglas 1951, 1957). Meanwhile the official name for *P. variabilis* has been changed to *P. magnus* (Holdeman and Moore 1972; Skerman et al. 1980). The pathway of glycine fermentation was only elucidated for *P. glycinophilus* by radiochemical and enzymatic investigations (Barker et al. 1948; Sagers and Gunsalus 1961; Klein and Sagers 1962). The main reactions are the splitting of glycine into methylenetetrahydrofolate, CO₂, and NH₃, and the subsequent condensation of the methylenetetrahydrofolate formed with another glycine yielding serine (Scheme 1). Serine then is converted into pyruvate from which acetate is finally formed. The acetate kinase reaction represents the only ATP-yielding step in the whole reaction sequence. The overall fermentation balance shows that 4 mol glycine are transformed into 3 mol acetate, 2 mol CO₂, and 4 mol NH₃. Since out of the 3 mol ATP formed during the conversion of acetyl

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Scheme 1. Glycine fermentation to acetate via the glycine-serine-pyruvate interconversion. Labelled position of glycine is indicated by * or +, respectively. THF, tetrahydrofolate; CH₂-THF, methylene THF; 2H, reducing equivalents; □, final product. The consequences of labelling studies are that the labelled products of 1-¹⁴C-glycine will be 2-¹⁴C-acetate (75%) and CO₂ (25%), whereas 2-¹⁴C-glycine yields acetate labelled in the carboxyl-group (75%) and in the methyl-group (25%) and no labelled CO₂ in the absence of exchange reactions

dioxide, and carbon monoxide were determined gas chromatographically (Braun 1979; Dürre and Andreessen 1982a; Meyer and Schlegel 1978). Quantitative analyses for acetate, glycine, formate, and ammonium were carried out employing enzymatic and colorimetric procedures, respectively (Dorn et al. 1978; Sardesai and Provideo 1970; Lang and Lang 1972; Kaplan 1969). The concentration of amino acids was calculated by the ninhydrin-method (Singh et al. 1978) using individual calibration curves for each amino acid tested and subtracting the ninhydrin-value for ammonium whose concentration was separately determined by the Berthelot-method. The vitamin B₁₂ content of cells was determined microbiologically using a B₁₂-methionine-auxotrophic mutant of *Escherichia coli* (ATCC 10799) as test organism (Guttman 1963). Tracer experiments were performed as described recently in detail (Dürre and Andreessen 1982a). Electron microscopy was carried out as before (Dürre et al. 1981).

Cells for enzymatic investigations were always harvested at the end of the exponential growth phase and stored at -70°C. Cell extracts were prepared by French press treatment as described (Dürre and Andreessen 1982a). Protein determination of extracts was performed according to Beisenherz et al. (1953). Glycine reductase was measured using the radiochemical assay of Stadtman (1970). Carbon monoxide dehydrogenase activity was determined according to Diekert and Thauer (1978) except that 100 mM KPO₄ buffer (pH: 7.3) and 4 mM methylviologen were used. One unit of enzyme activity was defined as 1 μmol substrate transformed or product formed per min at 37°C.

Results

Influence of Selenium on Growth of Peptococcus glycinophilus in Various Media

P. glycinophilus could be grown in a medium containing 100 mM glycine and only 0.1% (w/v) yeast extract after

supplementation with 0.1 μM selenite. When transferred from the original very complex medium [40 mM glycine, 1% (w/v) peptone and yeast extract; Cardon and Barker 1947] into the new selenite-containing medium, the cells required an adaptation period of 2–3 days to grow to a high turbidity. However, beginning with the second transfer, stationary growth phase was always reached within 35 h. Growth strictly depended on the trace element selenium (Fig. 1). Molybdate or tungstate alone did not show any stimulatory effect, but in combination with selenite, molybdate somewhat increased the final turbidity of the cultures whereas tungstate exhibited the contrary effect. A combination of all three elements was as effective as selenite alone. The optimal concentration of selenite proved to be 1 μM (Fig. 2) if the cultures had been starved for selenium by two transfers (1%, v/v, inoculum) into trace element-free medium. If the organism was grown for several transfers in a medium containing 0.1 μM selenite, a turbidity was reached which corresponded to that shown for 1 μM in Fig. 2. Therefore, a concentration of 0.1 μM selenite was chosen for routine culturing. *P. glycinophilus* did not survive more than 3 transfers into a selenium-deficient medium. Selenocystine and selenomethionine showed clear stimulation of growth but were only 55% and 37% as effective as selenite, respectively. Chemically related elements such as tellurium (in form of tellurite) and sulphur (in form of sulphate, sulphite, sulphide, and cysteine) could not substitute for selenium. A possible toxic effect of these compounds could be excluded in most cases, since even high concentrations (0.1 mM) in combination with selenite (0.1 μM) resulted in normal growth responses. Only in the case of tellurite and sulphite the final turbidities of the cultures were decreased by about 30%.

A stimulatory effect of selenium could also be demonstrated with the complex medium that was used in all earlier studies with *P. glycinophilus* (Fig. 3). Addition of selenite to the medium resulted in an extended exponential growth phase and a final turbidity which was about twice as

Fig. 1. Influence of trace elements on growth of *Peptococcus glycinophilus* in a medium containing 100 mM glycine and 0.1 % (w/v) yeast extract. Data shown give the maximal optical densities reached after inoculation with starved cells

Fig. 2. Growth responses of *P. glycinophilus* to increasing concentrations of selenium and tellurium compounds. Conditions were the same as described in Fig. 1: (Δ), selenite; (●), selenocystine; (■), selenomethionine; (○), tellurite

Fig. 3

Growth of *P. glycinophilus* in various media with and without supplementation of selenite and bicarbonate. Cells were grown for two transfers on the respective medium before inoculation. (●), 100 mM glycine, 0.1 % (w/v) yeast extract, 0.2 % (w/v) KHCO_3 , selenite, molybdate, and tungstate (10^{-7} M each); (○), 40 mM glycine, 1 % (w/v) peptone and yeast extract, no supplements; (■), 40 mM glycine, 1 % (w/v) peptone and yeast extract, 0.2 % (w/v) potassium bicarbonate; (Δ), 40 mM glycine, 1 % (w/v) peptone and yeast extract, 10^{-7} M selenite

high as in an unsupplemented culture. Supplementation of the medium with potassium bicarbonate caused a shorter lag-phase but did not effect the growth yields as judged from final turbidity. No differences could be found between cultures that were supplemented with selenite either at the time of inoculation or at the beginning of the logarithmic growth phase. For comparison, growth in the medium with lower yeast extract but higher glycine content is shown in Fig. 3. The doubling time of the cells was 4.7 h (judged from turbidity) compared to more than 10 h in the complex medium without selenite supplementation. All these experiments were repeated with a separate culture of *P. glycinophilus* to which selenium compounds had never been added before. This strain showed an identical behavior. Using selenite-supplemented media hydrogen evolution was never observed even under conditions reported earlier to be stimulatory for H_2 production (Cardon and Barker 1947). However, under an atmosphere of H_2 and CO_2 (80:20, %) an uptake of 1 mol hydrogen gas could be observed concomitantly with the consumption of 5 mol glycine. The acetate formation was higher than under an atmosphere of nitrogen, indicating that glycine served as electron acceptor. Formate could be used instead of hydrogen to produce more acetate. In addition, some hydrogen was formed from formate.

The substrate spectrum of *P. glycinophilus* was tested in the presence of 0.1 % yeast extract, 0.2 % bicarbonate, 0.03 % ammonium sulfate and 10^{-7} M selenite. Since selenium proved to be an important growth factor, the ability of *P. glycinophilus* to grow on other carbon compounds and to use CO_2 as an electron acceptor might have changed compared to former studies. Heterocyclic compounds were included because selenium has a marked influence on their degradation (Dürre et al. 1981) and *P. glycinophilus* is known to contain enzymes for the degradation of formiminoglycine as do purine fermenting clostridia (Sagers and Gunsalus 1961; Klein and Sagers 1962; Champion and Rabinowitz 1977). No growth was observed in the presence of: adenine, 2-amino-6,8-dihydroxypurine, 4-amino-2,6-dihydroxypyrimidine, 5-amino-2,6-dihydroxypyrimidine, 4-amino-5-imidazolecarboxamide, 2-aminopurine, 6-chloropurine, 4,5-diamino-2,6-dihydroxypyrimidine, 2,6-diaminopurine, 6,8-dihydroxypurine, fructose, glucose, guanine, 2-hydroxypurine, 4-hydroxypteridine, hypoxanthine, isoguanine, lactate, 6-mercaptapurine, 3-methylpurine, orotic acid, purine, 2,4,5-triamino-6-hydroxypyrimidine, 2,3,5-trihydroxypyrimidine, uramil, and xanthine. However, formiminoglycine was fermented by whole cells.

Nutritional Requirements of *Peptococcus magnus*

Both strains studied, ATCC 14955 and 14956, were known before as *P. variabilis* (Foubert and Douglas 1948). So far, these strains have not been subject to a thorough study (Douglas 1957). In order to achieve an optimal growth response on glycine, the investigations were started with a complex medium [1% (w/v) yeast extract] containing the formerly mentioned trace elements. The final turbidity reached by cells of both strains was proportional to the glycine concentration present up to 100 mM glycine. Strain ATCC 14955 was up to 20% less efficient according to the final turbidity. 150 mM glycine was completely degraded but growth was not further enhanced. The starting pH and the bicarbonate concentration had no effect on growth of both strains within the range of pH 6.2 to 8.2 and 0 to 100 mM potassium bicarbonate. A starting pH of 7.2 was routinely chosen since the pH change observed during growth was then very moderate. According to the doubling time of the cells, the temperature optimum was 37 to 40°C (ATCC 14955; $t_d = 2.6$ h) or 40°C (ATCC 14956; $t_d = 2.1$ h). Strain ATCC 14955 failed to grow at 45°C contrary to ATCC 14956 ($t_d = 12.3$ h). Growth at 24°C was slow for both strains ($t_d = 33$ and 44 h, respectively).

Complex nutrients may mask a requirement for trace elements, therefore, the necessity of adding 1% yeast extract as supplement was investigated. 0.5% yeast extract (Difco) gave about the same final turbidity and growth rate as obtained with 1% using strain ATCC 14955. However, strain ATCC 14956 exhibited exponential growth up to a turbidity of $A_{600} = 6$ only in the presence of 1% yeast extract; with 0.5% the growth rate decreased after reaching a turbidity of $A_{600} = 2$, although the same final turbidity was reached. In the presence of 0.1% yeast extract the doubling time of the cells was twice as high as with 1%, and turbidities of only $A_{600} = 1.6$ and 1.4 were obtained for strain ATCC 14955 and ATCC 14956, respectively. In the absence of glycine 1% yeast extract allowed growth to a final turbidity of $A_{600} = 0.4$ and 0.6, respectively. The requirement for yeast extract (1%) could be substituted for best by tryptose, meat extract, and proteose-peptone, followed by trypticase, peptone, and tryptone, which promoted final turbidities between $A_{600} = 6$ and 4 in both strains. Gelatin, casamino acids, and bovine serum albumine were much less efficient and allowed growth on glycine only to a turbidity $A_{600} = 1.6$ to 1.9. Therefore, the required growth factors were present in sufficient amounts only in more complex ingredients. A mixture of branched and long chain fatty acids (Pfennig et al. 1981), sodium acetate (0.3%), tryptophan (0.004%), or a vitamin mixture (Pfennig et al. 1981) did not show any stimulatory effect when growth on glycine was limited by 0.1% yeast extract. However, a requirement for Tween 80 was demonstrated for both strains when they were cultivated in a medium used to grow *Clostridium purinolyticum* on glycine (Dürre and Andreesen 1982a). Addition of 0.1% (v/v) Tween 80 restored growth to about the level otherwise obtained in the presence of 0.1% yeast extract. Contrary to *P. magnus* growth of *P. glycinophilus* was not affected by 0.1% Tween 80.

An influence of selenite on growth of *P. magnus* could be shown despite the necessity to add 1% yeast extract (Fig. 4). The cells had to be starved for selenium and transferred (1% v/v) three to five times into a medium to which selenium was not added and where precautions had been taken to use only material without contact to this trace element. The strains

Fig. 4. Effect of selenite on growth of *P. magnus* with glycine. The cells were starved of selenium by five transfers. The arrow indicates the addition of 10^{-7} M selenite (final concentration). (▲) *P. magnus* ATCC 14955, (●) *P. magnus* ATCC 14956

differed in the extent to which growth was still possible in the selenium-deprived medium. When growth ceased after three transfers, strain ATCC 14955 had consumed 15% of the glycine compared to 23% for strain ATCC 14956. After five transfers the glycine consumption was only marginal at this point. However, exponential growth and glycine consumption started immediately after adding 10^{-7} M selenite indicating a strict interdependence.

The substrate spectrum of the organism was tested in the presence of selenite. However, both strains of *P. magnus* were unable to grow on glucose, fructose, serine, threonine, uric acid, hypoxanthine, or adenine. In addition, the possibility of a Stickland-reaction with 100 mM glycine as oxidant and alanine, aspartate, arginine, glutamate, histidine, lysine, serine, or threonine (each 50 mM) as reductant can be excluded for strain ATCC 14956, since no differences could be observed compared to growth on glycine alone (final turbidities of $A_{600} = 5.9$ to 6.5). Glycine was completely fermented by the cells, however, the second amino acid was recovered in the supernatant to at least 40 mM. Therefore, these amino acids did not serve as a reductant to a significant extent, whereas they might act as precursors for synthesis of cell material.

The variability in size was a main characteristic of both strains according to their original description (Foubert and Douglas 1948). The spheres were reported to vary from 0.5 to 1.5 μ m in diameter, whereas the size of *P. magnus* is 1.5 to 1.8 μ m (West and Holdeman 1973). However, in our hands both strains showed no significant variation in size (Fig. 5). The cells occurred as single cocci, in pairs, or in chains of up to 8 cells, but rarely in groups. The cells were coccoid to ellipsoid and their size was 0.72–0.88 μ m long and 0.42–0.66 μ m wide. The cell wall was that of a typical Gram-positive organism (Fig. 5).

Fig. 5. Ultrastructure of *P. magnus* ATCC 14956. Bar represents 1 μ m

Glycine Fermentation by *Peptococcus magnus*

The growth response of *P. magnus* strain ATCC 14956 on glycine in the presence of 1% yeast extract and 10^{-7} M selenite is given in Fig. 6. Exponential growth occurred in two phases according to turbidity. The fastest doubling time of the cells was 1.8 h (according to turbidity). The protein: dry weight ratio of the cells was 45–56%. The maximum turbidity reached ($A_{600} = 4.2$, Zeiss PM4; equivalent to 5.6 measured with a Spectronic 88) corresponded to a dry weight of 1.5 mg/ml. The molar growth yield, as obtained from the slope of the plot of the increase in dry weight versus glycine consumption, was 8.9 g of dry cell mass per mol glycine. The products formed during growth were 0.74 mol acetate and 1.07 mol NH_3 per mol glycine. CO_2 was formed but not quantitatively determined, formate was not detected. The pH shifted from 7.25 to 6.8 during growth.

Determination of Glycine Reductase Activity by Tracer Experiments and Enzymatic Assays

P. glycinophilus and *P. magnus* ATCC 14956 fermented *in vivo* glycine via a glycine reductase system as shown by tracer experiments using resting cells and ^{14}C -labelled glycine (Table 1). Most of the radioactive glycine was directly converted into the correspondingly labelled acetate. Less than 1% of the radioactivity was found to be incorporated into the cells of both species. The deviations from the theoretical value of 100% for the formation of 1- ^{14}C -acetate and 2- ^{14}C -acetate are in same range as determined for the purine-degrading clostridia *C. purinolyticum* and *C. cylindrosporum* and might be due to exchange reactions (Dürre and Andreesen 1982a; 1983).

Glycine reductase activity could be measured in cell extracts of *P. glycinophilus*. The specific activity was 0.07 U per mg of protein, which is in the same range as that determined for *C. sticklandii* and *C. purinolyticum* (Dürre and Andreesen 1982a). However, no glycine reductase activity could be detected in extracts of freshly harvested cells of *P. magnus* ATCC 14956. No effect was obtained by changing the buffer system to TES-KOH, pH 7.2, by deleting centrifugation or using sonification to disrupt the cells.

Fig. 6A and B. Growth, substrate utilization, and product formation by *P. magnus* ATCC 14956; A (●) turbidity, (■) dry weight, (▲) protein; B (▲) glycine, (●) acetate, (■) ammonium

Physiological Features of *P. glycinophilus* Related to its Ability to Reduce CO_2 to Acetate

Earlier studies had demonstrated that *P. glycinophilus* was able to reduce CO_2 to acetate although the pathway still remained unknown (Barker et al. 1948; Schulman et al. 1972). An active carbon monoxide dehydrogenase has a key function in the so-called "corrinoid pathway" of CO_2 reduction to acetate (Diekert and Thauer 1978; Drake et al. 1981; Hu et al. 1982; Clark et al. 1982). Therefore, cell extracts of *P. glycinophilus* were tested for the activity of such an enzyme. A low activity of 0.017 U per mg of protein could be determined. However, CO was not oxidized by growing cells when present

Table 1. Distribution of radioactivity in the products of ^{14}C -labelled glycine after fermentation by resting cells of *Peptococcus glycinophilus* and *P. magnus*

Organism	Specific radioactivity (dpm/mmol)					¹⁴ C balance (%)
	Labelled glycine ^a	Substrate	Products			
			CH ₃ -group of acetate ^b	COOH-group of acetate ^b	CO ₂	
<i>P. glycinophilus</i>	1- ¹⁴ C-glycine	3.7 × 10 ⁵	22,560 (6.7)	312,380 (93.3)	235,620	99.7
	2- ¹⁴ C-glycine	3.7 × 10 ⁵	444,220 (95.4)	21,190 (4.6)	56,320	101.9
<i>P. magnus</i>	1- ¹⁴ C-glycine	1.85 × 10 ⁶	256,650 (16.2)	1,327,590 (83.8)	1,255,610	97.3
	2- ¹⁴ C-glycine	3.19 × 10 ⁶	2,459,200 (84.8)	441,000 (15.2)	885,210	82.1

^a Glycine was used at a concentration of 1.2 mmol/30 ml in all experiments. The ^{14}C -balance was calculated according to the fermentation equation: 4 glycine + 2 H₂O → 3 acetate + 2 CO₂ + 4 NH₃

^b Numbers in parentheses indicate the percentage of acetate labelled in the group specified

in the gas phase in a concentration of up to 5% although sufficient equilibrium between gas and liquid phase was provided. Neither an inhibitory nor a stimulatory effect was observed under these conditions.

The content of vitamin B₁₂ in *P. glycinophilus* was only 0.002 µg per mg of protein. Using the same assay, B₁₂ amounts up to 1.84 µg per mg of protein in *C. formico-aceticum* and to 0.025–0.055 µg per mg of protein in purine-utilizing clostridia (Dürre and Andreesen 1982b).

Discussion

The species of the genus *Peptococcus* seem to form a quite specialized group because they depend on fermentation of amino acids and purines (Douglas 1957). Both *P. glycinophilus* and *P. magnus* attack only glycine derivatives, however, they are genotypically unrelated (V. Huss, personal communication) and are different in their nutritional requirements. Whereas *P. glycinophilus* grew already optimal in the presence of 0.1% yeast extract, *P. magnus* required 1% yeast extract and Tween 80 in addition. The requirement for selenium compounds is common to both species as observed before for the glycine fermentation performed by *Clostridium purinolyticum* (Dürre and Andreesen 1982a) and the reduction of glycine in the Stickland reaction of *C. sticklandii*, *C. lentoputrescens*, *C. sporogenes*, and *C. histolyticum* (Turner and Stadtman 1973; Costilow 1977; Lebertz and Andreesen, unpublished results). According to the 16S rRNA catalogue, all these organisms and the species of the genus *Peptococcus* in general are phylogenetically unrelated (Tanner et al. 1982). Therefore, a selenium requirement seems to be a characteristic of anaerobic glycine fermentation.

In the presence of selenium both *P. glycinophilus* and *P. magnus* fermented glycine via a glycine reductase system. According to the labelling data the organisms oxidized 1 mol glycine completely to CO₂ and used the reducing equivalents generated thereby to reduce a further 3 mol glycine directly to acetate as has recently been found in *C. purinolyticum* (Dürre and Andreesen 1982a). The enzymes necessary for the complete oxidation of glycine, such as glycine decarboxylase, methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase, and formate dehydrogenase, are present in *P. glycinophilus* (Sagers and Gunsalus 1961). The glycine reductase pathway is energetically more favourable than the glycine-serine-pyruvate route since it provides two sites of

ATP-generation, i.e. the formyltetrahydrofolate synthetase reaction and the glycine reductase reaction. By this pathway 4 mol ATP can be gained from the degradation of 4 mol glycine compared to only 1 mol ATP per 4 mol glycine by the glycine-serine-pyruvate interconversion (Dürre and Andreesen 1982a).

The molar growth yield of *P. magnus* on glycine was higher than the one determined for *C. purinolyticum* (Dürre and Andreesen 1982a). However, due to the higher amount of yeast extract present in the medium the energy expenditure for cell material formation and maintenance (Stouthamer 1979) will be lower for *P. magnus* than for *C. purinolyticum*, thus explaining the higher molar growth yield observed. The inability to detect a glycine reductase activity in cell extracts of *P. magnus* might be due to the generally observed lability or to inappropriate assay conditions. Although the glycine reductase system is energetically more advantageous, *P. glycinophilus* and perhaps also *P. magnus* may switch to some extent between the two systems depending on the degree of selenium availability. Such a shift might allow survival under conditions of selenium limitation, although both species probably do not grow at all on glycine in the absence of selenium. In any case, the glycine-serine-pyruvate system is useful for anabolic reactions since serine and pyruvate are starting metabolites for many biosyntheses.

The importance of the trace element selenium for growth of both species might be explained by the possibility that this element is a component of the glycine reductase system as was shown for the enzyme complex in *C. sticklandii* (Turner and Stadtman 1973). An additional or alternative explanation is that the formate dehydrogenase is a selenoenzyme, as it is the case in several anaerobic bacteria (Andreesen 1980; Yamamoto et al. 1983). However, only the glycine decarboxylase complex of *P. glycinophilus* has been investigated in detail (Klein and Sagers 1966a, b, 1967a, b; Baginsky and Huennkens 1966, 1967; Robinson et al. 1973).

The participation of a glycine reductase in glycine fermentation was already indicated by the first tracer experiments using *P. glycinophilus* (Barker et al. 1948). From the published results it can be calculated that the acetate formed from 1- ^{14}C -glycine was labelled to 77% in the C-1-position whereas acetate derived from 2- ^{14}C -glycine showed 58% of the radioactivity in the C-2-position. However, according to a glycine-serine-pyruvate interconversion those values should be 0 and 25%, respectively (Scheme 1). This is due to a shift of the labelling in the condensation reaction. On the other hand

a glycine reductase system should yield 100% of the correspondingly labelled acetate in both cases. This would represent 75% of the totally added radioactivity, since 25% should be found as CO₂ originating from the complete oxidation of 1 mol glycine per 4 mol glycine fermented. Thus, a combination of both systems seems to have been operating during the earlier experiments as is also indicated by the high amount of labelled CO₂ found using 1-¹⁴C-glycine as a substrate (Barker et al. 1948). Also Sagers et al. (1977) preliminarily reported that 2-¹⁴C-glycine was converted first to 2-¹⁴C-acetate and then also to 1-¹⁴C- and doubly labelled acetate. In this respect the remark of Cardon and Barker (1946) that the use of distilled water often resulted in bad growth of *P. glycinophilus* which could be overcome by the addition of tap water could be interpreted as an early observation of a trace element dependence of this bacterium. A sufficient concentration of selenium in tap water seems to be the probable cause of growth because of the geographical location of both places (Berkeley, California, and Provo, Utah) where the experiments had been performed (National Research Council 1976).

The finding that *P. glycinophilus* was able to perform a CO₂ reduction to acetate (Barker et al. 1948) led to investigations using ¹³CO₂ and ¹⁴CO₂ to elucidate the pathway of this reduction (Schulman et al. 1972). Due to insufficient incorporation of CO₂ into acetate, no clear-cut results were obtained. So far, three different pathways are known which lead to the formation of acetate from CO₂. They all have in common the first reaction sequence which includes the formation of methylenetetrahydrofolate from CO₂, reducing equivalents, tetrahydrofolic acid, and ATP. *C. thermoaceticum*, and probably *C. formicoaceticum*, *C. thermoautotrophicum*, *C. aceticum* and *Acetobacterium woodii* use the so-called "corrinoid pathway", for all these organisms have a high corrinoid content (Ljungdahl and Wood 1982; Drake et al. 1981; Clark et al. 1982). Characteristic reactions are the conversion of carbon monoxide into a formyl derivative and the formation of acetyl-CoA from a formyl derivative and a methyl group derived from methylenetetrahydrofolate (Hu et al. 1982).

A second pathway is used by *C. purinolyticum* and probably *C. cylindrosporum* which form glycine from CO₂, methylenetetrahydrofolate, NH₃, and reducing equivalents, which in turn is converted into acetate and NH₃ by a glycine reductase (Dürre and Andreesen 1982b, 1983). This pathway is at least balanced with respect to energy expenditure because the ATP used for the activation of formate to formyltetrahydrofolate is compensated by the ATP formed during the glycine reductase reaction. *C. acidiurici* was assumed to use the glycine-serine-pyruvate interconversion for the formation of pyruvate which then could be transformed into acetate (Waber and Wood 1979). However, recent results indicate the occurrence of glycine reductase in this organism, too (Dürre and Andreesen, 1983).

According to the data presented in this paper it appears highly probable that *P. glycinophilus* uses the more economical glycine reductase pathway of CO₂ reduction to acetate (Dürre and Andreesen 1982b) as indicated by the following lines of evidence: (I) presence of the key enzyme glycine reductase; (II) presence of all enzymes necessary for the formation of glycine from CO₂ (Sagers and Gunsalus 1961); (III) extremely low B₁₂ content; (IV) low CO dehydrogenase activity [the activity of the CO dehydrogenase of *C. for-*

micoaceticum (Diekert and Thauer 1978), is more than 500 times higher than that of *P. glycinophilus*]; (V) carbon monoxide is totally inert for cells of *P. glycinophilus* as for *C. purinolyticum* (Dürre and Andreesen 1982b) in contrast to organisms such as *C. thermoaceticum* and *C. formicoaceticum* that oxidize this substance during growth (Diekert and Thauer 1978).

So far the physiological role of the CO₂ reduction to acetate remains unclear, since glycine as the most important substrate of *P. glycinophilus* is more oxidized than acetate. Interestingly, Cardon and Barker (1946, 1947) observed under certain conditions formation or uptake of hydrogen. According to our results *P. glycinophilus* was cultivated by these authors under suboptimal conditions. A removal of reducing equivalents derived from the oxidation of glycine to CO₂ was not possible or only to a limited extent by glycine reductase or by formate dehydrogenase (CO₂ reductase) due to selenium starvation. Under these conditions glycine was partly converted to pyruvate which yielded acetate, CO₂, and electrons at low redox potential suited for hydrogen formation. The same might be true for *P. magnus* (*variabilis*) for these strains were originally described as not (hydrogen) gas forming microorganisms (Foubert and Douglas 1948; Douglas 1951). However, Holdeman and Moore (1972) noted the ability to produce traces to moderate amounts of hydrogen gas. In the same report strain ATCC 14956 was transferred from *P. variabilis* to the species *P. magnus* presumably because of an increase in size. The anaerobic cocci of Hare group IV produce gas from pyruvate (Hare 1967) and increase considerable in size from 0.6–0.8 µm to 1.2–1.6 µm in diameter in the absence of oleate (Tween 80) (Wildy and Hare 1953). These cocci and strains labelled *P. magnus*, *P. variabilis*, and *P. anaerobius* have to be regarded as one species according to their peptidoglycan structure (Weiss 1981) and DNA/DNA hybridization studies (V. Huss, personal communication). Both strains, ATCC 14955 and ATCC 14956, required Tween 80 for good growth as the cocci of Hare group IV. The name *P. variabilis* was originally chosen because of the variable size of the organism (Foubert and Douglas 1948) which might very well depend on the nutritional status of the cells. When cultured in the presence of selenium, the size of ATCC 14956 was, however, small and unlike that typical for *P. magnus* and no hydrogen gas was detected as product. Thus, a hydrogen formation as observed in former studies might reflect unfavourable conditions, whereas in our studies hydrogen was taken up and an active glycine reductase was formed in the presence of selenium that acted as an efficient electron accepting and energy conserving system. Therefore, further studies are presently being performed on the ability of *P. glycinophilus* to grow mixotrophically in the presence of hydrogen gas or formate, CO₂, and glycine, carrying out a net synthesis of acetate from CO₂ via glycine (Dürre and Andreesen 1982b).

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