Arch. Microbiol. 120, 151–153 (1979)

Archives of
Microbiology



# Aspects of Carbon Metabolism in *Chloroflexus*

Reidun Sirevåg[1,*] and Richard Castenholz[2]

[1] Botanical Laboratory, University of Oslo, Blindern, Oslo 3, Norway
[2] Department of Biology, University of Oregon, Eugene, Oregon 97403, U.S.A.

**Abstract.** 1. When fluoroacetate was added to aerobic, washed cells of *Chloroflexus*, $O_2$ uptake was strongly inhibited and citrate accumulated. Under anaerobic conditions in the light, fluoroacetate inhibited $CO_2$ uptake and caused citrate accumulation. The results are taken as evidence for the operation of a tricarboxylic acid cycle in *Chloroflexus* both under aerobic conditions in the dark and anaerobically in the light. 2. Organic compounds are assimilated into the storage materials polyglucose and poly-β-hydroxybutyric acid by washed cells of *Chloroflexus*. The type of storage product formed from acetate depends upon the availability of reducing power. 3. Low activities of the key enzymes of the reductive pentose phosphate cycle, ribulose-1,5-bisphosphate carboxylase and phosphoribulokinase were detected in cell free extracts of photoheterotrophically grown *Chloroflexus*.

**Key words:** *Chloroflexus* – TCA cycle – Organic compounds – Fluoroacetate – RuBP carboxylase – Phosphoribulokinase.

The photosynthetic, gliding bacterium *Chloroflexus* (Trüper, 1976) is similar to members of the Chlorobiaceae with regard to pigment content, fine structure and the utilization of reduced sulfur compounds (Pierson and Castenholz, 1974a, b; Madigan and Brock, 1975). However, in contrast to *Chlorobium*, a member of the Chlorobiaceae and an obligate photoautotroph, *Chloroflexus* appears to be nutritionally extremely versatile. Growth experiments performed by Madigan et al. (1974) showed that a variety of organic compounds stimulate growth in *Chloroflexus* both under aerobic conditions in the dark and anaerobically in the light.

Thus, *Chloroflexus* is similar to the purple nonsulfur bacteria in being both photoheterotrophic and facultatively chemoheterotrophic in addition to the ability to grow photoautotrophically. In the purple nonsulfur bacteria, organic compounds are photometabolized by resting cells into the cellular reserve materials PHB and polysaccharide. This reductive synthesis is coupled either with an oxidation of part of the organic substrate via the TCA cycle to provide reducing power (Elsden and Ormerod, 1956), or with an uptake of molecular hydrogen (Stanier et al., 1959).

Here we report some results from an investigation of the carbon metabolism in *Chloroflexus*, which suggest that the role of organic substrates in the metabolism of this organism is similar to that in purple nonsulfur bacteria. Furthermore, evidence is presented that the key enzymes of the reductive pentose phosphate cycle are present under photoheterotrophic conditions, as was also indicated by Madigan and Brock (1977).

## Materials and Methods

*Organism and Culture Conditions. Chloroflexus aurantiacus* strain OK-70 fl was grown photoheterotrophically under anaerobic conditions in the "Roux" modification of D-medium described by Pierson and Castenholz (1974b). The cultures, in 300 ml screw-capped bottles were incubated at 52° C and light intensity of 2000 lux. For aerobic growth, the medium contained half the normal amount of yeast extract and casamino acids plus 2 g Na-acetate/l. Aerobic cultures were grown in dim light at 55° C in 1 l bottles continuously bubbled with sterile air. Cell dry weight was determined as described by Pierson and Castenholz (1974a), but using G. E. Nucleopore filters (GE-80, 0.8 µm pore size).

*Experiments with Washed Cells.* Cells were harvested by centrifugation at 35° C, washed twice with and resuspended in D-medium containing 0.8 % glycyl-glycine, pH 8.2. Uptake of $CO_2$ anaerobically in the light and $O_2$ uptake in the dark were measured in volumetric respirometers (Scholander and Iversen, 1958) as described by Sirevåg and Ormerod (1970), except that the temperature was 45° C. The

* *Present address and address for offprint requests:* Dr. Reidun Sirevåg, Botanical Laboratory, P.O.-Box 1045, Blindern, Oslo 3, Norway

*Abbreviations.* RuBP, Ribulose-1,5-bisphosphate; TCA, tricarboxylic acid; PHB, poly-β-hydroxybutyric acid

0302-8933/79/0120/0151/$01.00

respirometer flasks contained in a total of 4 ml: washed cells, approximately 10 mg dry weight, medium of the same composition as that used to wash the cells, 20 µmol each of $NaHCO_3$ and the organic substrates employed, and fluoroacetate as indicated. The gas phase was $Ar:CO_2$ (95:5), $H_2:CO_2$ (95:5) or air as indicated. For $O_2$ uptake, $CO_2$ was absorbed by a filter paper disc and 10 N KOH. The reaction was stopped after 60 min by addition of 0.1 ml of 2 M $H_2SO_4$, and the cells and supernatant used for analysis.

*Analytical Methods.* The supernatant was analyzed for citric acid as described by Taylor (1953). Polyglucose content of the cells was determined as glucose by glucose oxidase after hydrolyzation as described by Sirevåg (1975). PHB in the cells was estimated by the method of Law and Slepecky (1961) as described by Herbert et al. (1971) after digestion of the cells in NaClO.

*Cell-Free Extracts and Enzyme Assays.* Cells were harvested by centrifugation in the cold, washed twice in 0.02 M Tris-HCl buffer, pH 8.0 and stored at −20° C as cell paste until the next day. Cell-free extracts were made according to Tabita et al. (1974). RuBP-carboxylase was assayed radiochemically as described by Buchanan and Sirevåg (1976). Phosphoribulokinase was assayed according to Hart and Gibson (1975) and protein was determined by the procedure of Lowry et al. (1951).

## Results and Discussion

In order to examine whether the TCA cycle played any role in the metabolism of *Chloroflexus*, fluoroacetate was used. In cells with a functional TCA cycle, this compound is converted to fluorocitrate which in turn inhibits aconitase and thus causes an accumulation of citrate (Morrison and Peters, 1954).

The results presented in Table 1 and 2 indeed indicate that this cycle operates in *Chloroflexus* both under aerobic and anaerobic conditions. As the data in Table 1 show, $O_2$ uptake in the presence of acetate is inhibited 83% by $10^{-4}$ M fluoroacetate. In cells without added substrate, the $O_2$ uptake was inhibited 45% by this concentration. Furthermore, the addition of fluoroacetate to aerobic cells caused an accumulation of citrate.

Also anaerobically in the light, the addition of fluoroacetate to washed cells causes an accumulation of citrate (Table 2), which in this case is increased in the presence of acetate. At the same time, $CO_2$ uptake under these conditions was inhibited 77% by $10^{-2}$ M fluoroacetate.

Together, these data are in agreement with similar observations with *Rhodospirillum rubrum* (Elsden and Ormerod, 1956) and strongly indicate that the role of the TCA cycle in *Chloroflexus* is the same as that in the purple nonsulfur bacteria, namely to provide reducing power for respiration under aerobic conditions and for the reductive assimilation of organic compounds under anaerobic conditions in the light. In this respect *Chloroflexus* is fundamentally different from *Chlorobium* where fluoroacetate, although inhibiting $CO_2$ uptake, never causes accumulation of citrate (Sirevåg and Ormerod, 1970).

**Table 1.** Effect of fluoroacetate on $O_2$ uptake and citrate accumulation in washed cells of *Chloroflexus* incubated aerobically with acetate in the dark

| Fluoroacetate conc. (M) | $O_2$ uptake µmol/10 mg cells | | Citrate accumulated µmol/10 mg cells | |
|---|---|---|---|---|
| | Endogenous | + acetate | Endogenous | + acetate |
| 0 | 3.3 | 13.2 | 0.10 | 0.0 |
| $10^{-4}$ | 1.5 | 2.2 | 0.22 | 0.23 |
| $10^{-3}$ | 1.5 | 1.4 | 0.23 | 0.23 |
| $10^{-2}$ | 0.4 | 1.9 | 0.20 | 0.23 |

**Table 2.** Effect of fluoroacetate on $CO_2$ uptake and citrate accumulation in washed cells of *Chloroflexus* incubated anaerobically with acetate in the light

| Fluoroacetate conc. (M) | $CO_2$ uptake µmol/10 mg cells | | Citrate accumulated µmol/10 mg cells | |
|---|---|---|---|---|
| | Endogenous | + acetate | Endogenous | + acetate |
| 0 | 1.9 | 14.8 | 0.0 | 0.0 |
| $10^{-4}$ | 1.7 | 7.2 | 0.11 | 0.15 |
| $10^{-3}$ | 2.0 | 7.1 | 0.14 | 0.15 |
| $10^{-2}$ | 3.1 | 3.4 | 0.15 | 0.27 |

**Table 3.** Storage products formed anaerobically in the light by washed cells of *Chloroflexus* in the presence of acetate with or without an inorganic electron donor

| Addition | Gas phase | | | | | |
|---|---|---|---|---|---|---|
| | $H_2/CO_2$ | | | $Ar/CO_2$ | | |
| | Gas uptake | Polyglucose | PHB | $CO_2$ uptake | Polyglucose | PHB |
| | µmol/10 mg cells | | | µmol/10 mg cells | | |
| None | 24.5 | 0.12 | 0.05 | 4.4 | 0.66 | 0.0 |
| Acetate | 30.0 | 0.09 | 2.55 | 3.9 | 1.55 | 0.0 |

Pierson and Castenholz (1974a) presented results which indicated that *Chloroflexus* produced PHB as reserve material during photoheterotrophic growth. We have demonstrated synthesis of this polymer from acetate and have also found that washed cells make polyglucose as reserve material, not only from acetate but also from other organic compounds such as pyruvate, malate and succinate. The data shown in Table 3 indicate that the synthesis of these reserve materials from acetate varies according to the conditions employed. When molecular hydrogen was present, signif-

icant amounts of PHB were formed but very little polyglucose, whereas in the absence of the inorganic hydrogen donor, polyglucose was the only storage product formed.

In order to obtain a more complete picture of the carbon metabolism in *Chloroflexus*, cell-free extracts from photoheterotrophically grown cells were examined for the presence of the key enzymes of the reductive pentose phosphate cycle, namely RuBP carboxylase and phosphoribulokinase. The activities of these two enzymes were found to be 13.3 nmol $CO_2$ fixed/min mg protein and 0.2 nmol ATP used/min mg protein, respectively. These low activities are probably due to the photoheterotrophic growth conditions employed, where the relative contribution of $CO_2$ assimilated via RuBP carboxylase to cell material probably is low. However, this may also be the case for photoautotrophically grown cells where growth rates are very low (Castenholz, unpublished data). The presence of a functional reductive pentose phosphate cycle in *Chloroflexus* is another feature by which this organism differs from *Chlorobium*, where several lines of evidence now strongly suggest that this mechanism of $CO_2$ fixation is totally absent (Chernyadiev et al., 1974; Buchanan and Sirevåg, 1976; Sirevåg et al., 1977; Takabe and Akazawa, 1977).

*Acknowledgements.* We thank Dr. John G. Ormerod for his valuable advice during this work. The work was supported by grant D.70.49-38 from Norges almenviteskapelige forskningsråd (NAVF).

## References

Buchanan, B. B., Sirevåg, R.: Ribulose 1,5-diphosphate carboxylase and *Chlorobium thiosulfatophilium*. Arch. Microbiol. **109**, 15–19 (1976)

Chernyadiev, I., Kontratieva, I., Doman, N. G.: Activity of ribulose diphosphate and phosphopyruvate carboxylase in phototrophic bacteria. Mikrobiologiya **43**, 949–954 (1974)

Elsden, S. R., Ormerod, J. G.: The effect of monofluoroacetate on the metabolism of *Rhodospirillum rubrum*. Biochem. J. **63**, 691–701 (1956)

Hart, B. A., Gibson, J.: Ribulose-5-phosphate kinase from *Chromatium*. In: Methods in enzymology. Vol. XLII. Carbohydrate metabolism, Part C (W. A. Wood, ed.). pp. 115–119. New York-London: Academic Press 1975

Herbert, D., Phipps, P. J., Strange, R. E.: Chemical analysis of microbial cells. In: Methods in microbiology. Vol. 5b (J. R. Norris, D. W. Ribbons, eds.), pp. 209–383. London-New York: Academic Press 1971

Law, J. H., Slepecky, R. A.: Assay of poly-β-hydroxybutyric acid. J. Bacteriol. **82**, 33–36 (1961)

Lowry, O. H., Rosebrough, M. S., Farr, A. L., Randall, R. S.: Protein measurements with the Folin phenol reagent. J. biol. Chem. **193**, 265–275 (1951)

Madigan, M. T., Brock, T. D.: Photosynthetic sulfide oxydation by *Chloroflexus aurantiacus*, a filamentous, photosynthetic, gliding bacterium. J. Bacteriol. **122**, 782–784 (1975)

Madigan, M. T., Brock, T. D.: $CO_2$ fixation in photosynthetically-grown *Chloroflexus aurantiacus*. FEMS Microbiol. Lett. **1**, 301–304 (1977)

Madigan, M. T., Petersen, S. R., Brock, T. D.: Nutritional studies on *Chloroflexus*, a filamentous, photosynthetic, gliding bacterium. Arch. Microbiol. **100**, 97–103 (1974)

Morrison, J. F., Peters, R. A.: The inhibition of aconitase by fluorocitrate. Biochem. J. **56**, XXXVI (1954)

Pierson, B. K., Castenholz, R. W.: A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. Arch. Microbiol. **100**, 5–24 (1974a)

Pierson, B. K., Castenholz, R. W.: Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacterium. Arch. Microbiol. **100**, 283–305 (1974b)

Scholander, P. F., Iversen, O.: New design of volumetric respirometer. Scand. J. clin. Lab. Invest. **10**, 429–431 (1958)

Sirevåg, R.: Photoassimilation of acetate and metabolism of carbonhydrate in *Chlorobium thiosulfatophilum*. Arch. Microbiol. **104**, 105–111 (1975)

Sirevåg, R., Buchanan, B. B., Berry, J. A., Troughton, J. H.: Mechanisms of $CO_2$ fication in bacterial photosynthesis studied by the carbon isotope fractionation technique. Arch. Microbiol. **112**, 35–38 (1977)

Sirevåg, R., Ormerod, J. G.: Carbon dioxide fixation in green sulphur bacteria. Biochem. J. **120**, 399–408 (1970)

Stanier, R. Y., Doudoroff, M., Kunisawa, R., Contopoulou, R.: The role of organic substrates in bacterial photosynthesis. Biochemistry **45**, 1246–1260 (1959)

Tabita, R. F., McFadden, B., Pfennig, N.: D-ribulose 1,5-diphosphate carboxylase in *Chlorobium thiosulfatophilum* Tassajara. Biochim. biophys. Acta (Amst.) **341**, 187–194 (1974)

Takabe, T., Akazawa, T.: A comparative study on the effect of $O_2$ on photosynthetic carbon metabolism by *Chlorobium thiosulfatophilum* and *Chromatium vinosum*. Plant Cell Physiol. **18**, 753–765 (1977)

Taylor, T. G.: A modified procedure for the microdetermination of citric acid. Biochem. J. **54**, 48–49 (1953)

Trüper, H.: Higher taxa of the phototrophic bacteria: Chloroflexaceae fam. nov., a family for the gliding filamentous, phototrophic "green" bacteria. Intern. J. Syst. Bacteriol. **26**, 74–75 (1976)

Received October 12, 1978