Fluoroacetate Metabolism in *Gloeocapsa* sp. LB795 and its Relationship to Acetylene Reduction (Nitrogen Fixation)

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(Received 13 December 1977; revised 6 February 1978)

Sodium fluoroacetate (1 mM) caused an accumulation of citrate and altered the lipid composition in cells of *Gloeocapsa* sp. LB795. It also inhibited acetylene reduction (nitrogen fixation) by the alga – markedly under aerobic conditions, but much less so in the absence of oxygen. This inhibition is largely the result of the conversion of fluoroacetate to fluorocitrate which, by inhibiting aconitate hydratase (EC 4.2.1.3), interrupts the synthesis of the 2-oxoglutarate required for the assimilation of NH_4^+ . The consequent accumulation of NH_4^+ within the cells of *Gloeocapsa* sp. inhibits nitrogenase synthesis and, since oxygen rapidly inactivates pre-existing nitrogenase, nitrogen fixation by *Gloeocapsa* sp. decreases under aerobic conditions.

INTRODUCTION

Gloeocapsa sp. LB795 is a nitrogen-fixing, unicellular blue-green alga. Because it contains its nitrogen-fixing enzyme, nitrogenase, in the same undifferentiated cell as its photosynthetic apparatus, it seemed likely that the ATP and reducing power necessary to support nitrogen fixation might be supplied directly by photosynthesis. However, *Gloeocapsa* sp. continues to fix nitrogen, as measured by the acetylene reduction technique, when photosynthetic electron transport is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, suggesting that an alternative source of reducing power for nitrogen fixation might exist. *In vitro* studies indicated that this alternative source of reductant might be NADPH produced by isocitrate dehydrogenase (NADP^+) (EC 1.1.1.42) (Gallon, Kurz & LaRue, 1973). Furthermore, since 10 mM-sodium fluoroacetate inhibited acetylene reduction by whole cells of *Gloeocapsa* sp. (Gallon, LaRue & Kurz, 1975*b*), it seemed possible that isocitrate dehydrogenase might be a major source of reductant *in vivo*, even when photosynthesis was not inhibited.

However, this conclusion was based on the assumption that fluoroacetate inhibited acetylene reduction by depriving nitrogenase of reducing power. Thus, fluoroacetate was assumed to be converted to fluorocitrate which, in turn, inhibited aconitate hydratase (EC 4.2.1.3) and prevented the formation of isocitrate. In the absence of isocitrate, isocitrate dehydrogenase would not produce NADPH. Since it has not positively been demonstrated that fluoroacetate inhibits acetylene reduction in this manner, the aim of the present work was to examine the metabolism of fluoroacetate by *Gloeocapsa* sp. and the effect of this metabolism on acetylene reduction.

METHODS

Growth of cultures. *Gloeocapsa* sp. LB795 (University of Texas Culture Collection) was grown in 15 l of sterile medium free of combined nitrogen. The medium, which was a modification of ASM-1 medium (Gorham *et al.*, 1964) had the following composition ($\mu\text{mol l}^{-1}$): NaCl, 2000; MgSO_4 , 150; MgCl_2 , 145; CaCl_2 , 190; K_2HPO_4 , 100; Na_2HPO_4 , 100; FeCl_3 , 9; H_3BO_3 , 6; MnCl_2 , 6; ZnCl_2 , 3; CoCl_2 , 0.08; CuCl_2 , 0.0008; MoO_3 , 0.1; Na_2EDTA , 20; and was adjusted to pH 7.5 with solid NaHCO_3 . Conditions of growth were as previously described (Gallon, LaRue & Kurz, 1972).

Enzyme assays. Nitrogen fixation by cultures of *Gloeocapsa* sp. (0.2×10^8 to 2×10^8 cells ml^{-1}) was measured by the acetylene reduction technique after 4 h incubation with or without the addition of other compounds (Tözüm *et al.*, 1977). Unless otherwise stated, assays were performed aerobically at 25 °C under illumination at 2500 lx.

Isocitrate dehydrogenase (NADP^+) was assayed by the method of Ochoa (1955), using a broken cell preparation of *Gloeocapsa* sp. (Gallon *et al.*, 1973).

$^{14}\text{CO}_2$ fixation. Cells taken during the exponential phase of growth (up to 10 d after inoculation) were concentrated 20- to 50-fold by settling under gravity. Samples (1 ml) were then incubated, with or without 1 mM-sodium fluoroacetate (Fluka, Buchs, Switzerland), for 4 h at 25 °C and 2500 lx in sealed 7 ml glass bottles. Sodium [^{14}C]carbonate (20 μCi ; sp. act. 50 to 60 Ci mol^{-1} ; The Radiochemical Centre, Amersham) was then added and after another 1 h incubation, 1 ml ethanol was added to prevent further reaction and also to disrupt the cells. Cell debris was removed by centrifugation and the ethanol-soluble products were separated and identified as described by Huggins (1966). The radioactivity in individual compounds was measured in a scintillation spectrophotometer after transferring radioactive spots from chromatographs to vials containing 20 ml 2-(4'-*tert*-butylphenyl)-5-(4'-biphenyl)-1,3,4-oxadiazole in toluene (5 g l^{-1}).

[2- ^{14}C]Fluoroacetate metabolism. Cells were collected as described above and incubated for 4 h with 2.5 μCi sodium [2- ^{14}C]fluoroacetate (sp. act. 10 Ci mol^{-1} ; ICN Corporation, Irvine, California, U.S.A.). Ethanol-soluble radioactive products were isolated, separated and identified as above or by chromatography in propanol/ammonia/water (6 : 3 : 1, by vol.) according to the method of Buffa, Peters & Wakelin (1951). Lipids were extracted as described by Folch, Lees & Sloane-Stanley (1957) after termination of the incubation by adding excess (6 ml) methanol or 2-propanol. They were then separated, identified and their radioactivity was determined as described by Abreu-Grobois, Billyard & Walton (1977).

Lipid synthesis. Cells collected as described above were incubated for 4 h with 2.5 μCi sodium [1- ^{14}C]acetate (sp. act. 57 Ci mol^{-1} ; The Radiochemical Centre) and with or without 1 mM-sodium fluoroacetate. Lipids were then extracted, separated, identified and their radioactivity was determined as described by Abreu-Grobois *et al.* (1977).

RESULTS AND DISCUSSION

Metabolism of fluoroacetate

Cultures of *Gloeocapsa* sp. LB795 metabolize fluoroacetate only to a limited extent. The only ethanol-soluble product formed from [2- ^{14}C]fluoroacetate was [^{14}C]fluorocitrate, and only 0.16% of the radioactivity added was found in this compound after 4 h incubation (Table 1). There was also an equivalent incorporation of radioactivity from [2- ^{14}C]fluoroacetate into the lipid of *Gloeocapsa* sp. Radioactivity was found in all the polar lipid fractions and analysis of their alkaline hydrolysis products showed that 70% of this radioactivity was located in their fatty acid moieties. The possible presence of fluorine in these lipids was not investigated.

The metabolism of fluoroacetate by *Gloeocapsa* sp. is therefore apparently simple. It is converted to fluorocitrate, presumably by enzymes of the tricarboxylic acid cycle; there is also some metabolism of fluoroacetate to fatty acids, though whether this involves a preliminary defluorination reaction is not known.

Fluoroacetate and carbon metabolism

Sodium fluoroacetate (1 mM) had no effect on photosynthetic oxygen evolution by *Gloeocapsa* sp., but increased the total incorporation of radioactivity from $\text{Na}_2^{14}\text{CO}_3$. The radioactivity in identified individual ethanol-soluble products of $^{14}\text{CO}_2$ fixation increased by similar amounts, with the exceptions of citrate, in which radioactivity accumulated to a greater extent, and glutamate, in which a decreased incorporation of radioactivity was

Table 1. *Metabolism of [¹⁴C]fluoroacetate by Gloeocapsa sp. LB795*

Cells were collected during the exponential phase of growth, concentrated and incubated aerobically at 25 °C and 2500 lx for 4 h with 2.5 µCi Na [2-¹⁴C]fluoroacetate. Ethanol-soluble radioactive products and lipid were isolated and identified as described in Methods.

Radioactive product	Radioactivity incorporated [d.p.s. (ml cells) ⁻¹]	% incorporation
Fluorocitrate	151	0.16
Total lipid	167	0.18
Lipid component		% (w/w) of total lipid
Origin	17	10.3
Sulpholipid	18	10.7
Phosphatidylglycerol	21	12.5
Digalactosyldiglyceride	15	9.1
Unidentified polar lipid	15	8.9
Monogalactosyldiglyceride	48	28.7
'Neutral lipid' (solvent front)	33	19.8

Table 2. *Incorporation of radioactivity from Na₂¹⁴CO₃ into citrate and glutamate by Gloeocapsa sp. LB795*

Cells were collected during the exponential phase of growth, concentrated and incubated aerobically at 25 °C and 2500 lx for 1 h with 20 µCi Na₂¹⁴CO₃ after 4 h preincubation with or without Na fluoroacetate (1 mM). Ethanol-soluble radioactive products were isolated and identified as described in Methods. The incorporation of radioactivity into ethanol-soluble products (in the absence of fluoroacetate) was 12.8 % of that added.

Radioactive product	Radioactivity incorporated [d.p.s. (ml cells) ⁻¹]		% of uninhibited incorporation
	No addition	Na fluoroacetate	
Total incorporation	94393	156691	166
Citrate	566	4273	755
Glutamate	7608	2920	38

found (Table 2). These observations are consistent with the view that in *Gloeocapsa* sp., as in many other organisms, fluoroacetate undergoes 'lethal synthesis' to fluorocitrate, which inhibits aconitate hydratase and blocks the metabolism of citrate to isocitrate, 2-oxoglutarate and glutamate (Buffa *et al.*, 1951). In addition, the NADP⁺-dependent isocitrate dehydrogenase of *Gloeocapsa* sp. was also inhibited by fluorocitrate. In the presence of 0.35 mM-fluorocitrate (equimolar with isocitrate), isocitrate dehydrogenase activity in cell extracts was 16 % of that in the absence of inhibitors.

The incorporation of radioactivity into lipids from [1-¹⁴C]acetate was inhibited by 50 % by 1 mM-fluoroacetate (Table 3). This decrease is consistent with the effect of isotope dilution as the result of the metabolism of fluoroacetate into lipid, demonstrated above, although it is possible that fluoroacetate may inhibit some step in lipid biosynthesis. In contrast, however, fluoroacetate increased the accumulation of radioactivity into an unidentified polar lipid. Although the nature of this lipid is not known, it may be an alkali-stable glycolipid which is occasionally found in extracts of *Gloeocapsa* sp. and which has an *R_F* value similar to that of the heterocyst cell wall glycolipid of *Anabaena cylindrica*. If so, it is possible that fluoroacetate stimulates the synthesis of a specific cell wall glycolipid. Such an increased synthesis could represent an early step in the response of *Gloeocapsa* sp. to unfavourable conditions.

Table 3. *Effect of fluoroacetate on lipid synthesis by Gloeocapsa sp. LB795*

Cells were collected during the exponential phase of growth, concentrated and incubated aerobically at 25 °C and 2500 lx for 4 h with 2.5 µCi Na [1-¹⁴C]acetate and, where indicated, Na fluoroacetate (1 mM). Lipids were extracted and identified as described by Abreu-Grobois *et al.* (1977). The incorporation of radioactivity into lipid (in the absence of fluoroacetate) was 6.0 % of that added.

	Radioactivity incorporated [d.p.s. (ml cells) ⁻¹]		% of uninhibited incorporation
	No addition	Na fluoroacetate	
Total lipid	2219	1132	51
Lipid component			
Origin	202	38	19
Sulpholipid	317	133	42
Phosphatidylglycerol	601	247	41
Digalactosyldiglyceride	337	121	36
Unidentified polar lipid	109	336	308
Monogalactosyldiglyceride	546	235	43
'Neutral lipid'	104	63	61

Table 4. *Inhibition of acetylene reduction in Gloeocapsa sp. LB795 by fluoroacetate and related compounds*

Cells were collected during the exponential phase of growth and 50 ml samples were incubated aerobically in 150 ml Erlenmeyer flasks at 25 °C and 2500 lx for 4 h with the compounds indicated. The flasks were then sealed, acetylene was added to 1 % (v/v) and after a further 1 h incubation the ethylene produced was measured by gas-liquid chromatography. The protein content of the cells was 0.098 µg per 10⁸ cells.

The rates of acetylene reduction are typical but the values in parentheses, which show the rates as percentages of those in the absence of any additions, are the means of at least 20 observations.

Addition	Acetylene reduction [pmol min ⁻¹ (ml cells) ⁻¹]
None	27.0 (100)
1 mM-Na fluoroacetate	4.6 (17)
1 mM-Na chloroacetate	27.0 (100)
1 mM-Na acetate	27.3 (101)
1 mM-Na fluoride	22.1 (82)
1 mM-Na fluoroacetate + 2 mM-Na acetate	26.2 (97)
1 mM-Na fluoroacetate + 5 mM-CaCl ₂	5.7 (21)
1 mM-Na fluoroacetate + 5 mM-MgCl ₂	2.4 (9)

The following compounds (at 2 mM) were without significant effect in reversing the inhibition caused by 1 mM-fluoroacetate, either in the presence or absence of 5 mM-CaCl₂: acetamide, fumarate, glutamate, glycollate, glyoxylate, 2-hydroxyglutarate, isocitrate, malate, 2-oxoglutarate and pyruvate.

Fluoroacetate and acetylene reduction

Acetylene reduction by cultures of *Gloeocapsa sp.* in the exponential phase of growth (up to 10 d after inoculation) was completely inhibited after 4 h exposure to 10 mM-fluoroacetate (Gallon *et al.*, 1975*b*) and markedly inhibited by 1 mM-fluoroacetate (Tözüm *et al.*, 1977). On the other hand, very old cultures of *Gloeocapsa sp.* (more than 17 d after inoculation) frequently showed less than a 50 % inhibition of acetylene reduction by 1 mM-fluoroacetate. The effect of 1 mM-fluoroacetate was similar whether cells were incubated in the light or in the dark.

The inhibition of acetylene reduction by fluoroacetate was specific since neither 1 mM-acetate nor 1 mM-chloroacetate inhibited acetylene reduction by *Gloeocapsa sp.* (Table 4). Furthermore, 1 mM-sodium fluoride was only slightly inhibitory after 4 h incubation, suggesting that the effect of fluoroacetate was not caused by fluoride arising from any defluorination of the inhibitor.

Of the carbon compounds tested, only 2 mM-acetate prevented the inhibition caused by

Table 5. *Effect of various gas phases on acetylene reduction by Gloeocapsa sp. LB795 and the inhibition by fluoroacetate*

Cells were incubated for 4 h under an atmosphere of the composition indicated and, where appropriate, with Na fluoroacetate (1 mM). Their ability to reduce acetylene was then measured as described in Table 4. The cells incubated under CO₂-free air were previously suspended in medium adjusted to pH 7.5 with NaOH rather than NaHCO₃. The protein content of the cells was 0.116 µg per 10⁸ cells.

The rates of acetylene reduction are typical; the percentages are the means of at least five observations.

Gas phase	Acetylene reduction [pmol min ⁻¹ (ml cells) ⁻¹]		% of uninhibited activity
	No addition	Na fluoroacetate	
Air	20.4	3.5	17
N ₂	25.7	18.4	72
N ₂ /O ₂ (0.7 : 0.3, v/v)	14.1	1.4	10
Air/CO ₂ (0.99 : 0.01, v/v)	17.3	2.8	16
CO ₂ -free air	2.9	0.1	3
Ar/O ₂ (0.8 : 0.2, v/v)	34.3	10.3	30

fluoroacetate (Table 4), presumably by competitively inhibiting the conversion of fluoroacetate to fluorocitrate. Acetamide, which was reported by Gitter (1956) to prevent the conversion of fluoroacetate to fluorocitrate in rats, was without effect on *Gloeocapsa* sp. Moreover, neither isocitrate nor 2-oxoglutarate reversed the inhibition of acetylene reduction by fluoroacetate, even though endogenous pools of these compounds in *Gloeocapsa* sp. would be depleted as a result of the inhibition of aconitate hydratase by fluorocitrate. However, isocitrate may be ineffective in reversing the inhibition because, as reported above, isocitrate dehydrogenase, as well as aconitate hydratase, was inhibited by fluorocitrate; and there is some doubt whether 2-oxoglutarate can enter the algal cells since exogenous 2-oxo[5-¹⁴C]glutarate was apparently not metabolized by *Gloeocapsa* sp. (unpublished observations).

CaCl₂ (5 mM), but not MgCl₂, slightly reversed the inhibition caused by fluoroacetate after 4 h (Table 4). Since citrate and other chelating agents inhibited acetylene reduction by *Gloeocapsa* sp. and their inhibitory effects were also reversed by CaCl₂ but not by MgCl₂, Tözüm *et al.* (1977) suggested that part of the inhibition caused by fluoroacetate may be the result of the observed accumulation of citrate in the algal cells.

Under anaerobic conditions, the inhibition of acetylene reduction by fluoroacetate was markedly reversed (Tözüm *et al.*, 1977), though fluoroacetate metabolism was apparently unaffected since citrate still accumulated. However, on no occasion was it possible to demonstrate complete reversal of the inhibition of acetylene reduction by this means, even when photosynthetic oxygen evolution was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (10 µM).

When cells of *Gloeocapsa* sp. were exposed to an increased concentration of oxygen, or when the CO₂ concentration was decreased, acetylene reduction became more sensitive to inhibition by fluoroacetate (Table 5). Conditions of high oxygen or low CO₂ concentration stimulate photorespiration, which, in the heterocystous blue-green alga *Anabaena cylindrica*, may compete with nitrogenase for reducing power (Lex, Silvester & Stewart, 1972). In *Gloeocapsa* sp., acetylene reduction decreased under conditions which favour high rates of photorespiration (Table 5), consistent with competition between photorespiration and acetylene reduction in this organism also. If so, acetylene reduction by *Gloeocapsa* sp. was apparently more sensitive to competition from photorespiration in the presence of fluoroacetate than in its absence. A possible explanation for this is that fluoroacetate, by interrupting one source of reductant to acetylene reduction (NADPH from isocitrate dehydrogenase), has made the process more dependent upon alternative sources with which photorespiration may, perhaps, compete more efficiently.

Table 6. *Effect of L-methionine-DL-sulphoximine on acetylene reduction by Gloeocapsa sp. LB795 in the presence of fluoroacetate or NH₄Cl*

Cells were collected, incubated for 4 h with the compounds shown and their ability to reduce acetylene was measured as described in Table 4. The protein content of the cells was 0.103 µg per 10⁸ cells.

The rates of acetylene reduction are typical, but the values in parentheses, which show the rates as percentages of those in the absence of any additions, are the means of at least 12 observations.

Addition	Acetylene reduction [pmol min ⁻¹ (ml cells) ⁻¹]
None	19.8 (100)
2 mM-methionine sulphoximine	17.4 (88)
2 mM-NH ₄ Cl	5.1 (26)
2 mM-NH ₄ Cl + 2 mM-methionine sulphoximine	12.3 (62)
2 mM-NH ₄ Cl + 5 mM-CaCl ₂	8.5 (43)
1 mM-Na fluoroacetate	2.2 (11)
1 mM-Na fluoroacetate + 2 mM-methionine sulphoximine	6.1 (31)

However, if there were competition between nitrogenase and photorespiration for reducing power, conditions which decrease the level of photorespiration in *Gloeocapsa* sp. should increase the rate of acetylene reduction. Such conditions include atmospheres with a low concentration of oxygen or a high concentration of CO₂. Whilst acetylene reduction was increased by a low concentration of oxygen, there was no stimulation under 0.01 atm CO₂ in air. Furthermore, fluoroacetate was no less inhibitory under air plus 0.01 atm CO₂ than under air alone (Table 5). These observations suggest that photorespiration may not play a major role in the inhibition of acetylene reduction by fluoroacetate, though this cannot definitely be established in the absence of a demonstration that photorespiration in *Gloeocapsa* sp. is active in air and inhibited by 0.01 atm CO₂ in air.

Gloeocapsa sp. nitrogenase, the enzyme responsible for acetylene reduction, is rapidly inactivated by oxygen both *in vitro* and *in vivo* (Gallon, Kurz & LaRue, 1975*a*). In *Gloeocapsa* sp., as in *Anabaena flos-aquae* (Bone, 1972), a constant level of nitrogenase activity may be maintained by a balance between synthesis and inactivation by oxygen. Hence, any compound which inhibits the synthesis of nitrogenase will, under an atmosphere containing oxygen, result in a decrease in nitrogenase activity. Such compounds include chloramphenicol, NH₄Cl and, in view of its effects on acetylene reduction under air and under N₂, possibly fluoroacetate (Tözüm *et al.*, 1977). Furthermore, because fluoroacetate inhibited acetylene reduction less under Ar/O₂ (0.8 : 0.2, by vol.) than under air (Table 5), the conversion of N₂ to NH₄⁺, catalysed by nitrogenase, may be involved in fluoroacetate inhibition. Ammonium salts repress nitrogenase synthesis in nitrogen-fixing organisms and fluoroacetate would probably cause an accumulation of NH₄⁺ in cells of *Gloeocapsa* sp. by interrupting the synthesis of 2-oxoglutarate which is required for the assimilation of newly-fixed NH₄⁺. The effect of fluoroacetate on acetylene reduction by *Gloeocapsa* sp. could therefore be the result of an accumulation of NH₄⁺ to a repressive level for nitrogenase synthesis. Because no oxygen inactivation of pre-existing nitrogenase would occur under anaerobic conditions, an apparent reversal of this inhibition of acetylene reduction is observed under these conditions (Table 5).

Gloeocapsa sp. almost certainly assimilates the NH₄⁺ produced by nitrogen fixation by the pathway involving glutamine synthetase (EC 6.3.1.2) and glutamate synthase (EC 2.6.1.53) though the *Gloeocapsa* sp. enzyme is ferredoxin-dependent rather than NADP⁺-dependent [see Mifflin & Lea (1976)]. L-Methionine-DL-sulphoximine (2 mM), an inhibitor of glutamine synthetase, reversed the inhibitory effect of 2 mM-NH₄Cl on acetylene reduction by *Gloeocapsa* sp. under aerobic conditions. In *Gloeocapsa* sp. therefore, as in *A. cylindrica* (Stewart & Rowell, 1975), NH₄⁺ apparently does not inhibit nitrogenase synthesis directly; rather the inhibitor is, or is regulated by, a product of NH₄⁺ assimilation. The exact nature of this

product has not been identified, though in *A. cylindrica* it may be carbamoyl phosphate (Stewart, Rowell & Tel-Or, 1975). The addition of 0.5 mM-carbamoyl phosphate to cultures of *Gloeocapsa* sp. inhibited acetylene reduction to 42% of its control level after 4 h aerobic incubation, but had virtually no effect under N_2 . It is therefore possible that carbamoyl phosphate may also be involved in the inhibition of nitrogenase synthesis in *Gloeocapsa* sp.

Whatever the mechanism, in *Gloeocapsa* sp. the inhibition of acetylene reduction by NH_4Cl was reversed by methionine sulfoximine which also partially relieves the inhibition caused by fluoroacetate (Table 6), further implicating NH_4^+ in this inhibition.

$CaCl_2$ (5 mM) partially reversed the inhibition of acetylene reduction by NH_4Cl (Table 6) as well as that caused by fluoroacetate (Table 4). Therefore, the chelating agent which accumulates in the presence of fluoroacetate and inhibits acetylene reduction may not only be citrate. Others, perhaps linked to NH_4^+ assimilation, may also cause part of the inhibition. In this context, the observation that the inhibition of acetylene reduction by 0.5 mM-carbamoyl phosphate was also markedly reversed by 5 mM- $CaCl_2$ may be significant.

In conclusion, fluoroacetate does not inhibit acetylene reduction by *Gloeocapsa* sp. solely by depriving nitrogenase of reductant generated by isocitrate dehydrogenase, as was first believed, though it is still possible that part of the inhibition might be exerted in this manner (Tözüm *et al.*, 1977). Indeed, it appears that fluoroacetate does not inhibit acetylene reduction by any single mechanism. A major part of the inhibition is dependent upon oxygen and is most probably the result of the inhibition of nitrogenase synthesis. This could be caused directly or indirectly by NH_4^+ which accumulates in the algal cells because fluoroacetate has interrupted the synthesis of the 2-oxoglutarate required for NH_4^+ assimilation. A decrease in the ability of *Gloeocapsa* sp. to reduce acetylene would then be dependent upon the inactivation by oxygen of pre-existing nitrogenase. On the other hand, since *Gloeocapsa* sp. nitrogenase may be membrane-bound (Gallon *et al.*, 1972) and fluoroacetate may inhibit the synthesis of lipids, which are major membrane components, it is possible that the oxygen-sensitive part of the fluoroacetate inhibition could result from a disruption of membranes by the inhibitor, thereby rendering nitrogenase more sensitive to oxygen inactivation. Furthermore, photorespiration might be involved in the oxygen-dependent part of the inhibition caused by fluoroacetate. The evidence presented here, however, favours the involvement of NH_4^+ in this part of the inhibition rather than these alternative explanations.

A further part of the inhibition of acetylene reduction caused by fluoroacetate is reversed by $CaCl_2$ and is distinct from the oxygen-dependent part of the inhibition since $CaCl_2$ has the same effect in the absence of oxygen as in air. This part of the inhibition may result from an accumulation of chelating agents within cells of *Gloeocapsa* sp., caused by fluoroacetate. Citrate is probably one of these chelators, but there appear to be others also.

Thus, although the metabolism of fluoroacetate by *Gloeocapsa* sp. is limited, sufficient fluorocitrate is formed to inhibit aconitate hydratase. This inhibition then results in a dramatic decrease in the ability of the alga to fix nitrogen but the mechanism by which nitrogen fixation is inhibited is more complex than has previously been suggested.

The contributions of Mr T. Billyard, Mr A. Kumar and Miss D. Tözüm to this work are gratefully acknowledged. The authors also thank Miss J. Link for her skilled technical assistance and Dr T. J. Walton for his help and advice in connection with lipid metabolism in *Gloeocapsa* sp.

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