SHORT COMMUNICATIONS

The Effect of Fluoroacetate on the Growth of the Facultative Methylotrophs Bacterium 5H2, *Pseudomonas* AM1 and Bacterium 5B1

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(Received 1 October 1975; revised 26 November 1975)

INTRODUCTION

Three groups of facultative methylotrophs which use the serine pathway for growth on C_1 compounds (Anthony, 1975; Quayle, 1972) may be distinguished by the following criteria: (i) organisms synthesizing isocitrate lyase to a high specific activity during growth on C_1 substrates and on acetate, e.g. bacterium 5H2 (Cox & Zatman, 1973) and *Pseudomonas* MA (Bellion & Hersh, 1972); (ii) organisms not apparently synthesizing isocitrate lyase during growth on any substrate, e.g. *Pseudomonas* AM1 (Large & Quayle, 1963; Dunstan, Anthony & Drabble, 1972a) and *Pseudomonas* 3A2 (Colby & Zatman, 1972); (iii) organisms in which isocitrate lyase is synthesized to a high specific activity during growth on acetate, but not apparently during methylotrophic growth, e.g. bacterium 5B1 (Colby & Zatman, 1972) and organism PAR (Bellion, 1975). All pathways which have been proposed for the conversion of acetyl-CoA to glyoxylate in group (i) methylotrophs involve the enzymic sequence citrate synthase-aconitate hydratase-isocitrate lyase (Bellion & Hersh, 1972; Cox & Zatman, 1973; Harder, Attwood & Quayle, 1973; Bellion & Woodson, 1975). The mechanism of the conversion of acetyl-CoA to glyoxylate in methylotrophs of group (ii) and in C_1 -grown methylotrophs of group (iii) is, in view of the apparent lack of isocitrate lyase, obscure. The present experiments were done to seek additional evidence that the failure to detect isocitrate lyase in these latter two groups is not spurious.

Fluoroacetate gives rise *in vivo* to fluorocitrate, a potent inhibitor of aconitate hydratase (Kun, 1969). We therefore anticipated that organisms which form isocitrate lyase during methylotrophic growth would be more sensitive to fluoroacetate than organisms that do not. We investigated the sensitivity of bacterium 5H2, bacterium 5B1 and *Pseudomonas* AM1 to fluoroacetate during growth on C_1 and non- C_1 substrates.

METHODS

The chelated mineral base E medium of Owens & Keddie (1969) was used for growth. Filter-sterilized growth substrates were added to a final concentration of 0.2 % except for sodium acetate which was at 0.15 %. Thiamin hydrochloride (0.5 mg l^{-1}) was also added for the growth of bacterium 5B1. Organisms were grown on an orbital shaker at 30 °C in 2 l conical flasks containing 1 l medium. Portions (50 ml) of these cultures were quickly transferred to pre-warmed 100 ml conical flasks when the culture was growing exponentially and had an E_{420} (10 mm light-path) of about 0.5. At zero time, dilutions (1 to 2 ml) of

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Fig. 1. Effect of fluoroacetate on the growth of bacterium 5H2, *Pseudomonas* AMI and bacterium 5BI on C₁ and non-C₁ compounds. Growth substrates: ●, methylamine hydrochloride; ■, trimethylamine hydrochloride; ▲, methanol; ○, succinate; ▽, glycerol; □, acetate; △, L-glutamate. (a) Inhibition of growth of bacterium 5H2. (b) Inhibition of growth of *Pseudomonas* AMI (—) and bacterium 5BI (---).

100 mM-sodium monofluoroacetate (Fluka Chemische Fabrik, Buchs, Switzerland) were added to the cultures; in the controls, water replaced fluoroacetate solution. Cultures were incubated at 30 °C in a shaking water bath (100 oscillations/min) for periods in excess of one doubling time of the control culture (2 to 8 h). Samples (1 ml) were removed at intervals, the E_{420} measured, and the bacterial dry wt/ml calculated using a previously determined calibration curve. Growth curves were constructed and mean doubling times calculated. A measure of the inhibition of growth rate was obtained using the following formula:

Percentage inhibition of growth rate

$$= 100 - \left(\frac{\text{mean doubling time of control culture}}{\text{mean doubling time of test culture}} \times 100 \right).$$

RESULTS

The sensitivity of bacterium 5H2 to fluoroacetate during growth on C₁ and non-C₁ compounds

The mean doubling times (h) of the control cultures of bacterium 5H2 growing on the different substrates were as follows: methylamine, 5.5; trimethylamine, 5.8; glycerol, 4.5; succinate, 7.5; L-glutamate, 7.0; acetate, 5.0. Figure 1(a) shows the sensitivity of this organism to fluoroacetate during its growth on each of these compounds. Inhibition of growth by fluoroacetate occurred without any significant lag period and growth rates were exponential in the inhibited cultures during the experimental period. There was a significant difference in sensitivity between C₁ and non-C₁-grown cells. Whilst the growth of bacteria on methylamine or trimethylamine was completely inhibited by the presence of 2 μM and 10 μM-fluoroacetate respectively, even 1 mM-fluoroacetate failed to inhibit completely the growth of cultures on non-C₁ substrates. The concentration of fluoroacetate required for 50 % inhibition of the growth rate (i.e. a doubling of the mean doubling time) is at least 10 times higher for bacteria growing on non-C₁ compounds compared with those growing on C₁ compounds. The relative insensitivity of bacterium 5H2 to fluoroacetate during its growth on acetate was striking. During growth on acetate, energy is obtained from the oxidation of acetyl-CoA via the tricarboxylic acid cycle and carbon is made available for biosynthesis

by the anaplerotic glyoxylate cycle (Cox & Zatman, 1973); in such circumstances a high sensitivity to fluoroacetate would be expected since both pathways involve aconitate hydratase. The marked insensitivity shown in Fig. 1(a) is probably due to a successful competition between the acetate (10 to 20 mM) and the fluoroacetate, either for their entry into the organism or during their subsequent metabolism. This suggestion is supported by the results of experiments (Cox, 1974) which showed that 1.0 mM-acetate gave 100 % protection against the inhibition by 1.0 μ M-fluoroacetate of the growth of bacterium 5H2 on methylamine.

The sensitivity of Pseudomonas AMI and bacterium 5B1 to fluoroacetate during growth on C₁ compounds and on succinate

The mean doubling times of the control cultures of *Pseudomonas* AMI growing on methylamine, methanol and succinate were 4.0, 3.5 and 3.5 h respectively, whilst those of bacterium 5B1 on trimethylamine and succinate were 4.0 and 1.8 h. Figure 1(b) shows the relative sensitivities of these organisms to fluoroacetate during their growth on these substrates; there was no significant difference in the sensitivity of either organism to fluoroacetate whether growth was occurring on a C₁ compound or on succinate, and this sensitivity was similar to that found for bacterium 5H2 growing on succinate (Fig. 1a).

DISCUSSION

The methylotrophic growth of bacterium 5H2 is at least 10 times more sensitive to inhibition by fluoroacetate than is its growth on non-C₁ compounds. This observation emphasizes the key role of aconitate hydratase, and hence isocitrate lyase, in the methylotrophic growth of this organism. It has been proposed (see Introduction) that citrate synthase, aconitate hydratase and isocitrate lyase are the enzymes involved in the conversion of acetyl-CoA to glyoxylate in bacterium 5H2 and similar organisms; this conversion is a key reaction in the anabolic metabolism of organisms utilizing the serine pathway for methylotrophic growth. Aconitate hydratase does not play such a key role in carbon assimilation during the growth of these organisms on non-C₁ compounds (other than acetate) and its inhibition would not be expected to have such a marked effect on growth. Glutamate synthesis will be inhibited in the presence of fluoroacetate but it seems probable that some carbon and energy would be available for growth from routes not involving aconitate hydratase, and growth would probably involve release from the cell of a metabolite such as acetate. These considerations thus explain the difference in sensitivity to fluoroacetate between C₁-grown and non-C₁-grown organisms, and the marked insensitivity of bacterium 5H2 to fluoroacetate observed during growth on glutamate.

The sensitivity of *Pseudomonas* AMI and bacterium 5B1 to fluoroacetate is different from that of bacterium 5H2, being virtually the same whether they are growing on a C₁ or non-C₁ compound and is similar to the low sensitivity observed with bacterium 5H2 during growth on succinate. A correlation thus exists between the ability to synthesize isocitrate lyase during growth of C₁ compounds and a high sensitivity to fluoroacetate under these conditions. The results obtained with *Pseudomonas* AMI thus support the conclusions of Large & Quayle (1963) for growth on C₁ compounds and Dunstan *et al.* (1972a) for growth on ethanol, that this organism does not form isocitrate lyase, and support the hypothesis that a novel mechanism exists for the conversion of acetyl-CoA to glyoxylate as postulated by Dunstan *et al.* (1972a, b).

The observation that bacterium 5B1 synthesizes isocitrate lyase to a high specific activity during growth on acetate but not during growth on trimethylamine is similar to the results of

Bellion (1975) for organism PAR (Leadbetter & Gottlieb, 1967) and Cox (1974) for bacterium ISI (Hampton, 1975). The ability of an organism to synthesize isocitrate lyase during growth on acetate clearly does not determine whether the isocitrate lyase-mediated route or the novel route of Dunstan *et al.* (1972*a, b*) is used for the conversion of acetyl-CoA to glyoxylate during its growth on C_1 compounds. Bellion & Woodson (1975) have shown that the isocitrate lyase enzyme induced during the growth of *Pseudomonas* MA on methylamine is distinct from the enzyme that is induced during growth on acetate. A similar situation may also exist in bacterium 5H2.

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