SHORT COMMUNICATION

Effect of 2-Oxoglutarate on Glutamic Acid Excretion by *Citrobacter intermedius* C3

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There was no correlation between glutamate dehydrogenase activity and the ability to excrete glutamic acid in the wild type and two auxotrophic mutants of *Citrobacter intermedius*. Differences in the ability to excrete glutamic acid were attributed to mechanisms which regulated the availability of 2-oxoglutarate, as this intermediate induced the synthesis of glutamate synthase.

INTRODUCTION

Excretion of glutamic acid in *Citrobacter intermedius* strain C3 appeared to depend on the presence of an extrachromosomal element (Parés *et al.*, 1974). In this communication we describe the effect of 2-oxoglutarate on glutamic acid excretion and compare the activities of glutamate dehydrogenase (EC 1.4.1.4) and glutamate synthase (EC 1.4.1.13) in *C. intermedius* C3 and in auxotrophic mutants of it which differ with respect to glutamic acid excretion.

METHODS

Media. Minimal medium M1 contained (g l⁻¹): glucose, 20; NH₄Cl, 7; KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; the pH was adjusted to 7.2 with 1 M-NaOH. In medium M1 α , the carbon and energy sources were glucose (16 g l⁻¹) and 2-oxoglutarate (3 g l⁻¹). Media were supplemented with the appropriate amino acids (20 mg l⁻¹) when auxotrophic mutants were grown.

Bacterial strains. *Citrobacter intermedius* strain C3 (see Clotet *et al.*, 1968), when growing on M1 medium, undergoes reversible dissociation at the colonial level with respect to the ability to excrete glutamic acid (Parés *et al.*, 1974). Mutant strain CBC315 requires proline for growth and was obtained after treatment of strain C3 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG; 100 μ g ml⁻¹ in 0.2 M-Tris/maleate buffer, pH 6, for 30 min at 37 °C). Mutant strain CBC356 requires both proline and histidine for growth and was obtained after treatment of a histidine-requiring mutant of C3 with NTG.

Growth of bacteria and preparation of extracts. Bacteria were grown in 150 ml medium in 500 ml Erlenmeyer flasks in a water-bath shaker (100 strokes min⁻¹) at 30 °C. (The inoculum was 5 ml of a culture taken from the exponential growth phase.) When the turbidity reached an *A*₅₀₀ of about 0.38, the bacteria were harvested by centrifuging (7500 g, 4 °C, 10 min) and washed once with 200 mM-Tris/HCl buffer (pH 8.0). Resuspended bacteria were disrupted ultrasonically using a Braun Labsonic-1510 fitted with a needle probe (four 1 min periods at 90 W in an ice bath). After centrifuging again (17000 g, 20 min, 4 °C), the supernatants were used without purification.

Enzyme assays. Glutamate synthase was assayed by a method modified from that of Meers *et al.* (1970). The reaction mixture (3 ml) contained 100 mM-Tris/HCl buffer (pH 8.0), 2.5 mM-2-oxoglutarate, 2.5 mM-glutamine, 0.1 mM-NADPH (Sigma) and 0.1 to 0.3 ml crude extract. The difference in rate of NADPH oxidation in the presence and absence of glutamine was used to calculate enzyme activity. Glutamate dehydrogenase was assayed by measuring the oxidation of NADPH at 340 nm at room temperature (19 to

Table 1. *Glutamate dehydrogenase and glutamate synthase activities in strains of Citrobacter intermedius*

Enzyme activities were measured using crude extracts as described in Methods; each result (mean $\pm$ standard deviation) is the average for eight experiments. Glutamic acid excretion was measured in at least 30 experiments for each strain with about 250 colonies per experiment.

Medium	Strain	Enzyme activities [nmol min ⁻¹ (mg protein) ⁻¹]		Percentage of colonies excreting glutamic acid
		Glutamate dehydrogenase	Glutamate synthase	
M1	C3	237 $\pm$ 11	48 $\pm$ 1	55–65
M1	CBC315	230 $\pm$ 16	44 $\pm$ 3	0
M1	CBC356	241 $\pm$ 12	48 $\pm$ 4	100
M1 α	C3	247 $\pm$ 6	80 $\pm$ 5	100
M1 α	CBC315	262 $\pm$ 16	82 $\pm$ 9	100
M1 α	CBC356	293 $\pm$ 28	71 $\pm$ 3	100

23 °C) in a reaction mixture similar to that used for glutamate synthase except that the glutamine was substituted by 100 mM-NH₄Cl. Endogenous NADPH oxidase activity was invariably negligible.

Enzyme activities are expressed as nmol coenzyme converted min⁻¹ (mg protein)⁻¹. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma) as a standard.

Glutamic acid excretion. This was assayed using colonies grown for 24 h on solid M1 or M1 α media containing 1.2% (w/v) agar, with *Leuconostoc mesenteroides* P60 as the indicator strain (Parés *et al.*, 1974).

RESULTS AND DISCUSSION

When the parent strain C3 was grown on medium M1 α , all the colonies excreted glutamic acid; this contrasted with the reversible dissociation observed on M1 medium, where only 55 to 65% of the colonies excreted glutamic acid (Table 1). The same percentage of excreting and non-excreting colonies was obtained irrespective of whether excreting or non-excreting colonies were used as inocula. No colonies of strain CBC315 excreted glutamate on M1 medium, but on M1 α medium all the colonies did so. With strain CBC356, however, all the colonies excreted glutamic acid on either M1 or M1 α medium.

Despite their different patterns of glutamic acid excretion on solid M1 medium, all three strains had the same activities of glutamate dehydrogenase and glutamate synthase after growth in liquid M1 medium (Table 1). Thus, the biochemical basis of glutamic acid excretion in *C. intermedius* C3 seems to be different from that described for some other glutamic acid-producing bacteria in which excretion has been associated with the activity of either glutamate dehydrogenase or glutamate synthase (Vandecasteele *et al.*, 1975).

Addition of 2-oxoglutarate to the medium did not significantly change the activity of glutamate dehydrogenase in any of the strains (Table 1), thus confirming that there is no correlation between glutamate dehydrogenase activity and the ability to excrete glutamic acid.

Glutamate synthase had similar activity in all strains when growing in medium M1 (Table 1) and so cannot be involved in the reversible dissociation in the parental strain C3 or in the different behaviour between strains CBC315 and CBC356. However, glutamate synthase activity was almost doubled when strains C3, CBC315 and CBC356 were grown in medium M1 α ; this may indicate that 2-oxoglutarate induces the synthesis of glutamate synthase in *C. intermedius*.

Induction of glutamic acid excretion in strain CBC315 and suppression of dissociation in the parent strain C3 were not achieved when 2-oxoglutarate was substituted by equimolar concentrations of citrate, isocitrate, fumarate or DL-malate (J. Guinea & R. Parés, unpublished observations).

Our results suggest that differences in the ability to excrete glutamic acid may be due to mechanisms regulating the availability of intracellular 2-oxoglutarate. Since S factor can be transferred by conjugation with the consequent recovery of glutamic acid excretion (Parés *et al.*, 1974), we suggest that S factor carries either structural or regulatory genes for isocitrate dehydrogenase.

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