SHORT COMMUNICATIONS

**Pathways and Regulation of Glutamate Synthesis in a
Corynebacterium sp. Overproducing Glutamate**

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INTRODUCTION

Since the discovery of glutamate synthase (Tempest, Meers & Brown, 1970) and the demonstration of its existence in many bacteria (Meers, Tempest & Brown, 1970; Miller & Stadtman, 1972) glutamate dehydrogenase is no longer assumed to be the general pathway for glutamate synthesis. Current evidence suggests that in several bacterial species glutamate is synthesized by a NADP-dependent glutamate synthase [L-glutamine:2-oxoglutarate aminotransferase (NADPH-oxidizing, EC. 2.6.1.53)] when the ammonia concentration is low, and by a NADP-dependent glutamate dehydrogenase (EC. 1.4.1.4) when the ammonia supply is not limiting (Brown, Macdonald-Brown & Stanley, 1973). Some bacterial species accumulate large amounts of glutamate in the extracellular medium and various reasons have been proposed to explain this metabolic peculiarity (Kinoshita & Tanaka, 1972). Kinoshita & Tanaka (1972) found a NADP-dependent glutamate dehydrogenase in this type of bacteria but did not investigate whether glutamate synthase was also present. We investigated the presence of this enzyme in these bacteria and its possible relationships to their particular capacity for glutamate production.

METHODS

The strain used, *Corynebacterium* c91, was isolated from soil by Dr Ballerini, in this laboratory, by enrichment culture with *n*-hexadecane as a carbon source. It can accumulate large amounts of glutamic acid in the culture medium when grown on *n*-hexadecane (D. Ballerini, unpublished results) and resembles *Corynebacterium hydrocarboclastus* (Iizuka & Komagata, 1964).

The bacterium was grown in shaken flasks at 30 °C in a mineral medium (Robinson, 1964) with 1 % fructose as the carbon source and the indicated nitrogen source. Extracts were prepared by ultrasonic treatment (15 periods of 15 s with a Branson sonifier at a power output of 75 W) of bacterial suspensions (about 2.5 g bacteria in 4 ml of 50 mM potassium phosphate buffer pH 7.0), cooled by immersion in an ice bath. The extracts obtained after centrifuging (20 min at 15000 g) were dialysed at 4 °C against the same buffer before use. Glutamate synthase and glutamate dehydrogenase activities were determined according to Meers *et al.* (1970) except that the temperature was 25 °C and that NADH was substituted for NADPH in the assay of glutamate synthase (concentrations of L-glutamine, 2-oxo-glutarate and NADH or NADPH were 5, 5 and 0.08 mM, respectively). Glutamine synthetase (EC. 6.3.1.2) was assayed according to Rowe *et al.* (1970), using their procedure A for synthetase activity determination.

Table 1. *Enzyme activities in extracts of Corynebacterium c91 grown with various nitrogen sources*

For assay conditions, see Methods.

Nitrogen source for growth	Specific activity* (nmol/min/mg protein)		
	Glutamate dehydrogenase	Glutamine synthetase	Glutamate synthase
L-lysine (1.8 g/l)	< 1	85	82
KNO ₃ (2 g/l)	3.1	84.5	73
(NH ₄) ₂ SO ₄ (2 g/l)	106	6.75	22.5

* Specific activities of glutamate dehydrogenase, glutamine synthetase and glutamate synthase are expressed in nmoles of NADPH, glutamyl hydroxamate or NADH, respectively, per min per mg protein. Values indicated are the average of results representing at least two independent preparations.

RESULTS AND DISCUSSION

Relative glutamate synthase activities in extracts of *Corynebacterium c91* grown with nitrate as nitrogen source were: (i) complete assay medium, containing L-glutamine, 2-oxoglutarate and NADH, 100 %; (ii) without 2-oxoglutarate, 17 %; (iii) without L-glutamine, 15.9 %; (iv) without 2-oxoglutarate and L-glutamine, 16.3 %; (v) with NH₄Cl (40 mM) instead of L-glutamine, 17 %; (vi) with NADPH instead of NADH, 1.7 %; (vii) with NADPH instead of NADH, and HN₄Cl (40 mM) added, 7.3 %. The absolute rate observed in (i) was 50.6 nmol/min/mg protein. These results show that glutamate synthase activity is present and that the enzyme utilizes NADH specifically (i, ii, iii). In other bacteria the enzyme is usually NADPH-dependent. A NADH-specific glutamate synthase has, however, been reported in some nitrogen-fixing bacteria (Nagatani, Shimizu & Valentine, 1971) and in yeast (Roon, Even & Larimore, 1974). Slight activity of a NADP-dependent glutamate dehydrogenase was found in the extracts (vi, vii) but no NAD-dependent glutamate dehydrogenase was detectable (iv, v).

No loss of activity of glutamate synthase was observed during a three-week storage at -25 °C. At 4 °C, a similar preparation lost 60 % activity over the same period of time, but in the presence of 2 mM-2-oxoglutarate it lost only 25 % activity.

The effect of the activity of glutamate synthase on the nitrogen source used for growing the bacteria was studied (Table 1). When the nitrogen source was not an ammonium salt, high activities of glutamate synthase and glutamine synthetase were evident and glutamate dehydrogenase activity was barely detectable. Under these growth conditions, glutamate synthesis therefore proceeded through the glutamate synthase pathway. When ammonium salts were used a high activity of glutamate dehydrogenase (NADP-specific) was found, with the activities of glutamate synthase and glutamine synthetase being much decreased although not entirely suppressed. Under these growth conditions glutamate dehydrogenase was the predominant pathway for glutamate synthesis and this pattern resembles that of several other bacteria. In support of this view of the physiological roles of these two pathways, *K_m* values of glutamate synthase for glutamine and glutamate dehydrogenase for NH₄⁺ of 0.8 and 9 mM, respectively, have been found.

Since *Corynebacterium c91* excretes large amounts of glutamic acid in a medium containing ammonium salts as nitrogen source, glutamate dehydrogenase is probably the enzyme functional in glutamate synthesis in these conditions. Even so, glutamate synthase may contribute, as it was still synthesized in the presence of ammonium ions and was not inhibited by 50 mM-glutamate, whereas at this concentration of glutamate a 50 % inhibition

of glutamate dehydrogenase was observed. At 100 mM-glutamate (a value exceeded several-fold when the bacteria are excreting glutamate) glutamate dehydrogenase was inhibited by 75%. As the reverse reaction was negligible under the conditions utilized (described in Methods), this probably represented a true physiological control of glutamate synthesis.

Investigation of the presence and regulation of glutamate synthase in other types of glutamate producers, such as those of the *Corynebacterium glutamicum* group (Abe, Takayama & Kinoshita, 1967) could provide useful information. In particular, the presence of a NADH-linked instead of a NADPH-linked glutamate synthase, an unusual feature in bacteria, would be an interesting point to investigate.

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