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PROPERTIES OF GLUTAMATE DEHYDROGENASE FROM BACILLUS SUBTILIS

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SUMMARY

Bacillus subtilis strain 168 possesses an NAD-dependent glutamate dehydrogenase. The level of this enzyme is influenced by the stage of growth, the source of nitrogen, and a high rate of tryptophan biosynthesis. The enzyme appears to serve an anabolic function and, therefore, must be considered as a possible route for the incorporation of inorganic nitrogen into an organic form.

INTRODUCTION

In Bacillus subtilis, glutamate has been proposed to be produced by the concerted action of glutamine synthetase (E.C.6.3.1.2) and glutamate synthase (E.C.2.6.1.53) as follows:

1. glutamine synthetase

NH_3 + glutamate + ATP ------> glutamine + ADP + Pi

2. glutamate synthase

glutamine + α-ketoglutarate + NADPH -----> 2 glutamate + NADP

Another enzyme involved in glutamate formation, namely glutamate dehydrogenase (GDH; E.C. 1.4.1.2.),

3. glutamate dehydrogenase

NH_3 + α-ketoglutarate + NADH ----> glutamate + NAD

has been previously reported to be missing in the competent strain 168 of B. subtilis (1-3), although there have been reports (2-5) that this enzymatic activity exists in other strains and species of Bacillus. As a result the role of this enzyme has been ignored in several studies, particularly those that deal with the activity of the enzymes of nitrogen metabolism during the process of sporulation (14, 15).

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We have found an NAD-dependent GDH in strain 168 of B. subtilis. The activity of this enzyme is influenced by the following parameters: (i) the growth phase of the culture; (ii) the biosynthesis of tryptophan; (iii) the source of nitrogen used for growth; (iv) the presence of glutamine as a growth supplement in a medium containing ammonium ions as the source of nitrogen.

MATERIALS AND METHODS

Bacterial strain. A prototrophic derivative of B. subtilis 168, designated NP1 (6), was used as the parent of mutants NP100, I12 and I15 (6, 7).

Culture conditions and extract preparation. The bacteria were routinely grown in 200 ml of minimal glucose medium (8) and cellular extracts were prepared as previously described (7). When glutamate or glutamine was used as a nitrogen source, $(NH_4)_2SO_4$ was omitted from the minimal-salts medium and replaced with 0.2% of the amino acid.

Enzyme assays. GDH was assayed at 37°C by following the decrease in A_{340} in a recording Gilford spectrophotometer. The reaction mixture contained: 90 µmol of Tris buffer pH 7.5, 90 µmol of α-ketoglutarate, 100 µmol of NH_4Cl, and 500 nmol of NADH in a final volume of 1.0 ml. Enzyme (0.1 ml) was used to initiate the reaction. Subunits E and G (previously called X [7, 9]) of anthranilate synthase were assayed as previously described (10).

RESULTS

Effect of growth stage on the activity of GDH. The activity of GDH was assayed in the prototroph NP1 and the mtr mutant NP100 (the mtr locus codes for the presumed aporepressor of the tryptophan operon [11]) as a function of growth (Fig. 1). The level of GDH was found to increase sharply as the cultures entered the late log and early stationary phase of growth. In addition, the mtr mutant NP100, which synthesizes the tryptophan biosynthetic enzymes constitutively and excretes tryptophan into the growth medium, possessed a 5 to 10-fold higher specific activity for GDH than did the parent NP1.

Influence of the rate of tryptophan biosynthesis on the activity of GDH. The high specific activity of GDH in the mtr mutant NP100 may result from the high rate of tryptophan production in

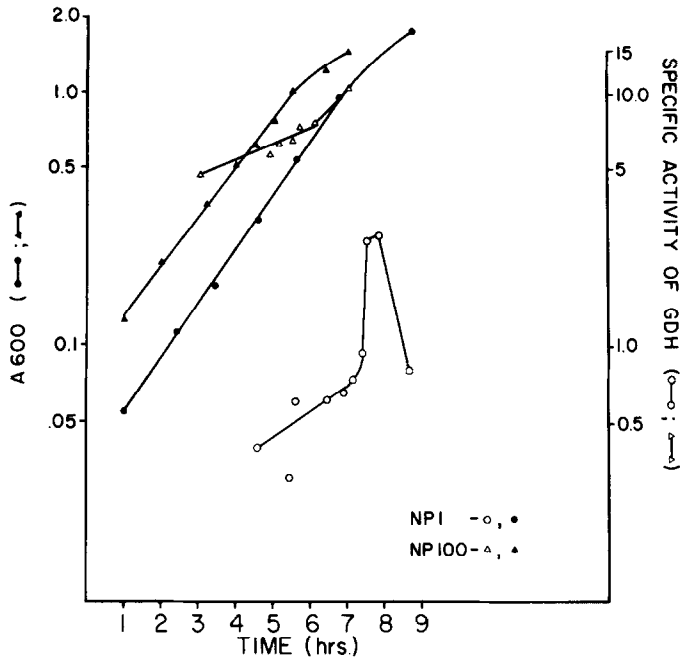

Figure 1. Relationship between GDH and stage of growth. The growth rates of NP1 in minimal-glucose medium (•---•) and NP100 in minimal-glucose medium containing 50 μg of phenylalanine/ml (▲--- ▲) represent the average rates of 10 and 7 cultures respectively. The open symbols represent the specific activity of GDH in NP1 (o --- o) or NP100 (Δ --- Δ).

this mutant (12), since one mole of glutamine is converted to 1 mole of α-ketoglutarate for every mole of tryptophan produced. Spontaneous mutants of NP100 defective in the anthranilate synthase complex (EG) [chorismate + glutamine $\xrightarrow{EG}$ anthranilate + pyruvate + glutamate] were therefore selected (13), and examined for GDH (Table 1). One mutant, designated I12, has a defect in the trpE locus which codes for the large subunit (E) of the anthranilate synthase complex (13). This mutant still contains the subunit G of the complex but lacks anthranilate synthase activity in vitro. As a result essentially no tryptophan is excreted into the medium, and no glutamine is utilized by the anthranilate synthase enzyme complex. The other mutant, I15, contains a defective subunit G and

TABLE I

Activity of GDH in mtr trpE and mtr gat mutants derived from mtr mutant NP100

Collection No.	Relevant Genotypes	Specific Activities[a] Anthranilate synthase		GDH
		E	G	
NP1	-	0.05	0.3	0.5
NP100	mtr2	5.0	10.8	4.5
I12	mtr2, trpE5	0	6.0	0.5
I15	mtr2, gat7	4.0	0	0.4

[a]The specific activities are as follows: subunit E and G - nmoles of anthranilate/min/mg protein; GDH - μmoles NADH oxidized/hr/mg protein.

uses ammonia instead of glutamine in the anthranilate synthase reaction (7, 13). In both mutants the level of GDH was reduced by about a factor of 10. When tryptophan (50 μg/ml) is supplemented in the growth medium of mutants I12, and I15 or the prototroph NP1, the level of GDH was unchanged. These results suggest that the increase in GDH in the mtr mutant is a physiological consequence of a high rate of glutamine utilization in the production of tryptophan, and is not induced by the presence of tryptophan in the medium.

Relationship between the source of nitrogen and the level of GDH. In the prototroph, NP1, the specific activity of GDH is reduced when the cells are grown in the presence of glutamate either with or without $(NH_4)_2SO_4$ (Table 2). This result is more consistent with a biosynthetic function for GDH than a catabolic one. Glutamine, either as a source of nitrogen or as an amino acid supplement, had little or no effect on the level of GDH.

In the mtr mutant NP100, however, glutamine was more effective in repressing GDH than was glutamate (Table 2). Presumably, this

TABLE II

Effect of the nitrogen source on GDH

Nitrogen source[a]	Growth Supplement[b]	Specific Activity of GDH	
		NP1	NP100
$(NH_4)_2SO_4$	None	0.60	5.2
$(NH_4)_2SO_4$	Glutamate	0.24	4.2
$(NH_4)_2SO_4$	Glutamine	0.45	2.6
Glutamate	None	0.24	2.6
Glutamine	None	0.60	1.1

[a]The concentrations of the nitrogen sources were as follows: $(NH_4)_2SO_4$-15mM; glutamate - 0.2%; glutamine - 0.2%.

[b]Glutamate and glutamine were added to a final concentration of 400 µg/ml.

is related to the high utilization of glutamine in the production of tryptophan in this mutant.

DISCUSSION

These results demonstrate that derivatives of B. subtilis strain 168 possess a GDH activity which has the following properties. First, the specific activity is a function of the growth stage with the activity reaching a maximum level in the early stationary phase of growth; this characteristic has also been reported for the GDH's from other species of Bacillus (2,5). This increase in GDH activity as the culture enters the stationary phase of growth may be a mechanism for fulfilling the cell's requirement for glutamate during sporulation. This possibility could be verified by isolating mutants which lack GDH and determining the effect of this lesion on ammonia assimilation during vegetative growth and sporulation.

Second, GDH appears to have a biosynthetic function since growth on glutamate represses the activity of this enzyme. In

this regard the role of GDH in nitrogen metabolism and the relationship of this enzyme to the process of sporulation in species of Bacillus cannot be ignored (13, 14).

Finally, the level of GDH appears to be responding to changes in the internal pool level(s) of some critical metabolite(s); this is indicated by the high level of GDH in the mtr mutant NP100. This mutant excretes large quantities of tryptophan (50 µg tryptophan/mg dry weight/hr for NP100 verses 0.9 µg tryptophan/mg dry weight/hr for the prototroph NP1). Since the biosynthesis of one mole of tryptophan is accompanied by the conversion of one mole of glutamine to one mole of α-ketoglutarate, a high rate of tryptophan biosynthesis might be expected to alter the pool levels of glutamine, glutamate and α-ketoglutarate with perhaps a compensatory effect on the enzymes of nitrogen metabolism. The altered GDH activity of NP100 suggests such a possible compensatory effect. That is, in the mtr mutant GDH is 5 to 10-fold higher than in the prototroph, and mutations at either the trpE (mutant I12) or gat (mutant I15) loci which decrease the rate of glutamine utilization and tryptophan excretion, also cause a drop in GDH. These results support the hypothesis that the increase in GDH is a physiological consequence of a high rate of tryptophan biosynthesis. The changes in the metabolites and the relationship of GDH to nitrogen metabolism is being pursued.

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