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Some properties of glutamate dehydrogenase, glutamine synthetase and glutamate synthase from *Corynebacterium callunae*

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Abstract. Characteristics of the three major ammonia assimilatory enzymes, glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT) in *Corynebacterium callunae* (NCIB 10338) were examined. The GDH of *C. callunae* specifically required NADPH and $NADP^+$ as coenzymes in the amination and deamination reactions, respectively. This enzyme showed a marked specificity for α-ketoglutarate and glutamate as substrates. The optimum pH was 7.2 for NADPH-GDH activity (amination) and 9.0 for $NADP^+$-GDH activity (deamination). The results showed that NADPH-GDH and $NADP^+$-GDH activities were controlled primarily by product inhibition and that the feedback effectors alanine and valine played a minor role in the control of NADPH-GDH activity. The transferase activity of GS was dependent on Mn^{+2} while the biosynthetic activity of the enzyme was dependent on Mg^{2+} as essential activators. The pH optima for transferase and biosynthetic activities were 8.0 and 7.0, respectively. In the transfer reaction, the K_m values were 15.2 mM for glutamine, 1.46 mM for hydroxylamine, 3.5×10^{-3} mM for ADP and 1.03 mM for arsenate. Feedback inhibition by alanine, glycine and serine was also found to play an important role in controlling GS activity. In addition, the enzyme activity was sensitive to ATP. The transferase activity of the enzyme was responsive to ionic strength as well as the specific monovalent cation present. GOGAT of *C. callunae* utilized either NADPH or NADH as coenzymes, although the latter was less effective. The enzyme specifically required α-ketoglutarate and glutamine as substrates. In cells grown in a medium with glutamate as the nitrogen source, the optimum pH was 7.6 for NADPH-GOGAT activity and 6.8 for NADH-GOGAT activity. Findings showed that NADPH-GOGAT and NADH-GOGAT activities were controlled by product inhibition caused by $NADP^+$ and NAD^+, respectively, and that ATP also had an important role in the control of NADPH-GOGAT activity. Both activities of GOGAT were found to be inhibited by azaserine.

Abbreviations: GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase

Key words: *Corynebacterium callunae* – Glutamate dehydrogenase – Glutamine synthetase – Glutamate synthase – Ammonia assimilation

Glutamate and glutamine are the most important intermediates in the biosynthesis of nitrogen-containing cell materials, and in the majority of organisms glutamate formation appears to be most widely utilized route of ammonia assimilation. In most bacteria, ammonia can be incorporated into glutamate either by the use of glutamate dehydrogenase or by a combination of glutamine synthetase and glutamate synthase.

Glutamate dehydrogenase [GDH; L-glutamate: $NAD(P)^+$ oxidoreductase] catalyzes the reductive amination of α-ketoglutarate reversibly in the presence of either NADH or NADPH, as shown by the following equation:

$$\alpha\text{-ketoglutarate} + NH_3 + NAD(P)H + H^+ \rightleftharpoons \text{L-glutamate} + NAD(P)^+ + H_2O$$

Many bacteria possess NADPH-dependent GDH (EC 1.4.1.4) that usually plays an anabolic role (glutamate synthesis) whereas NAD^+-dependent GDH (EC 1.4.1.2) serves a catabolic function (Tyler 1978; Janssen et al. 1980). Glutamine synthetase [GS; L-glutamate: ammonia ligase; EC 6.3.1.2] and glutamate synthase [GOGAT; L-glutamine (amide): 2-oxoglutarate amino transferase oxidoreductase $NAD(P)^+$] pathway is ATP dependent and physiologically irreversible, as follows:

$$\text{L-glutamate} + NH_3 + ATP \xrightarrow{GS} \text{L-glutamine} + ADP + P_i$$

$$\alpha\text{-ketoglutarate} + \text{L-glutamine} + NAD(P)H + H \xrightarrow{GOGAT} 2\ \text{L-glutamate} + NAD(P)^+$$

Most bacteria possess an NADPH-dependent GOGAT (EC 1.4.1.13) while some bacteria contain an NADH-dependent GOGAT (EC 1.4.1.14) and others possess a GOGAT enzyme that uses both of NADPH and NADH as coenzyme.

There are few published studies concerning these

ammonia assimilatory enzyme from the glutamic acid-producing coryneform bacteria. These microbes are used in the fermentation industry for the production of amino acids, including glutamic acid, lysine and threonine. In this study, *Corynebacterium callunae* (NCIB 10338) has been examined because this strain is a glutamic acid producer and it is a member of the same genus as other representative strains of glutamic acid-producing bacteria (Hirose and Okada 1979; Kinoshita 1985). The goal of the research was to determine if the ammonia assimilatory enzymes of this bacterium have unique properties that allow the cell to excel in glutamic acid production. Herein, some aspects of the properties of glutamate dehydrogenase, glutamine synthetase and glutamate synthase enzymes of *C. callunae*, and studies on the regulation of the activity of these enzymes have been described.

Materials and methods

Bacteria and growth conditions

Corynebacterium callunae (NCIB 10338) was acquired as a lyophilized culture from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland and was maintained at 4 °C on nutrient agar plates by monthly subculture.

To prepare crude extracts, *C. callunae* was grown in the minimal salt medium (T-salts) described by Phibbs and Bernlohr (1971) with addition of 0.02% yeast extract as growth factor. Control experiments showed that *C. callunae* cannot use 0.02% yeast extract as sole carbon or nitrogen source for growth. Glucose (15 mM) was added as sole source of carbon and, unless otherwise indicated, 30 mM glutamate or 5 mM glutamate as sole source of nitrogen for cells used for preparation of extracts for the GDH assay or GS/GOGAT assay, respectively. Unless otherwise specified, cells were grown at 30 °C in 500 ml sidearmed Erlenmeyer flasks containing 100 ml medium on a rotary shaker (Certomat U, Braun Melsungen, FRG) at a 120 rpm. Cells grown overnight in the test medium were used as inocula for final cultures.

Preparation of crude extracts

Cells were harvested in late logarithmic phase of growth by centrifugation (MSE) at $7000 \times g$ for 20 min at 4 °C, washed twice with 20 mM potassium phosphate buffer pH 7.0, containing 1 mM EDTA and 2 mM β-mercaptoethanol and stored at −20 °C overnight. Cells were thawed and suspended at about 35 mg dry weight cells per ml in the same buffer and sonicated 15 times for 30 s each, with 30 s intervals for cooling using an MSE (Soniprep 150, Crawley, West Sussex, UK 23 kHz) sonic disintegrator set at 8-microns amplitude. Cell debris was removed by centrifugation at $12000 \times g$ for 30 min at 4 °C. The supernatant solution was used as the source of enzyme on the same day. Dialysis of enzyme solutions did not affect apparent activities of the enzymes.

The optimal substrate concentrations were determined for use in each of the following assay mixtures. GDH and GOGAT activities were measured spectrophotometrically (Shimadzu-UV 160, Kyoto, Japan) from the rate of NADPH oxidation or $NADP^+$ reduction at 340 nm. The final volume of the reaction mixtures was 1 ml. The reactions of GDH and GOGAT were linear for at least 3 min at 30 °C. Low activities of NADPH and NADH oxidases present in the crude extract were determined with appropriate reagent blanks and were subtracted.

The NADPH-GDH (reductive amination reaction) assay mixture contained 50 mM Tris-HCl buffer, pH 7.6, 150 mM NH_4Cl, 7 mM α-ketoglutarate (potassium salt-pH was adjusted to 7.2 with 1 N KOH), 0.12 mM NADPH and crude extract. The $NADP^+$-GDH (oxidative deamination reaction) assay mixture contained 100 mM Tris-HCl buffer, pH 9.2, 200 mM glutamate (sodium salt-pH was adjusted to 9.2 with 1 N NaOH), 0.2 mM $NADP^+$, 50 mM KCl and crude extract. NADPH-GDH and $NADP^+$-GDH reactions were started by adding ammonium chloride or glutamate, respectively.

The NADPH-GOGAT activity was measured using a reaction mixture containing 50 mM Tris-HCl buffer, pH 7.6, 15 mM glutamine, 3 mM α-ketoglutarate (potassium salt-pH was adjusted to 7.6 with 1 N KOH), 0.05 mM NADPH and crude extract. The NADH-GOGAT assay mixture contained 50 mM potassium phosphate buffer, pH 6.8, 5 mM glutamine, 3 mM α-ketoglutarate (potassium salt-pH was adjusted to 6.8 with 1 N KOH), 0.05 mM NADH and crude extract. GOGAT reactions were initiated by adding glutamine to the reaction mixtures.

One unit of enzyme activity is defined as the amount of enzyme which catalyzes the oxidation of 1 µmol of NADPH or NADH or the reduction of 1 µmol of $NADP^+$ per min, respectively. Specific activity was expressed in units per mg protein in the crude extract.

Glutamine synthetase in crude extracts of *C. callunae* was measured by its transferase and biosynthetic activities. These assays are based on the methods of Farnden and Robertson (1980). Transferase activity was followed by measuring the formation of γ-glutamyl hydroxamate in the reaction mixture which contained: 50 mM imidazole-HCl buffer, pH 8.0, 40 mM glutamine, 1 mM $MgCl_2$, 30 mM potassium arsenate (pH was adjusted to 8.0 with 1 N KOH), 0.1 mM sodium-ADP, 30 mM hydroxylamine. HCl (neutralized with 1 N NaOH) and crude extract.

Biosynthetic activity of GS was assayed in reaction mixtures containing: 50 mM imidazole-HCl buffer, pH 7.0, 15 mM $MgCl_2$, 55 mM glutamate (sodium salt-pH was adjusted to 7.0 with 1 N NaOH), 2.5 mM ATP, 25 mM NH_4Cl and enzyme. The final volumes in both reaction mixtures of GS were 1 ml.

One unit of GS activity is defined as the amount of enzyme which catalyzes the formation of 1 µmol γ-glutamyl hydroxamate or inorganic phosphate per min in the transferase or biosynthetic assays, respectively. Specific activity was expressed in units per mg of protein in the crude extract.

All results in this paper are the means of activity values from at least two independent experiments which differed from each other by no more than 10%. Protein in the crude extracts was estimated by the method of Lowry et al. (1951) with bovine serum albumin as the standard and using a wavelength of 578 nm.

Materials

Nucleotides, amino acids, keto acids, azaserine, L-methionine-S-sulfoximine, glutaric acid, bovine serum albumin, γ-glutamyl hydroxamate and all other components of the reaction mixtures were obtained from Sigma Chemicals, St. Louis, Mo., USA. All other chemicals were from Merck, Darmstadt, FRG. Double glass distilled water used to prepare all solutions. All amino acids used were the L-forms.

Results

Coenzyme and substrate specificity of GDH and GOGAT

The GDH of *Corynebacterium callunae* requires NADPH as reductant for its activity. Efforts to substitute NADH in the reaction mixture failed to give any activity. Similarly, pyruvate and oxaloacetate in the amination reaction or glutamine, aspartic acid and alanine in the deamination reaction could not replace a-ketoglutarate or glutamate, respectively.

In addition to an NADPH-dependent activity, the GOGAT of *C. callunae* shows an NADH-dependent

Table 1. Effect of azaserine on glutamate synthase activity of *Corynebacterium callunae* grown on 5 mM glutamate as nitrogen source

Experiment condition[b]	Inhibition (%)[a]			
	NADPH-GOGAT		NADH-GOGAT	
Azaserine (1 mM) + crude extract	71	78[c]	20	32[c]
Azaserine (2 mM) + crude extract	89	93[c]	35	47[c]
Azaserine (4 mM) + crude extract	91	95[c]	46	60[c]

[a] Results are expressed as % inhibition of control (without azaserine). Control activity was 0.085

[b] Inhibitor and crude extracts were incubated for 5 min at 30 °C prior to initiation of reaction

[c] The concentrations of α-ketoglutarate and glutamine in standard reaction mixtures of GOGAT were decreased 4-fold

activity, but the rates were only about 25% of the NADPH-dependent activity. Also, this activity of GOGAT was not detected in ammonium chloride-grown (2 mM) cells in contrast to glutamate-grown (5 mM) cells. The NADH-dependent activity of GOGAT was measured at different pHs and substrate concentrations. Moreover, this activity of GOGAT was confirmed by using azaserine as a inhibitor of GOGAT (Table 1). In contrast to the coenzyme specificity, the GOGAT of *C. callunae* was shown to have a very narrow specificity for its other substrates. α-Ketoglutarate could not be replaced by either pyruvate or oxaloacetate and asparagine and ammonium chloride could not be used as alternative substrates for glutamine (data not shown).

Metal and nucleotide specificity of GS

The transferase activity of GS from *C. callunae* was absolutely dependent on Mn^{2+}, showing a maximum activity at 1 mM in the assay mixture. No other divalent cations tested, including Mg^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} would support the transferase activity at six different concentrations between 0.5–10 mM. Biosynthetic activity was dependent on Mg^{2+} with a maximum activity at 30 mM concentration, although Mn^{2+} at the same concentration was partially effective (20%). Co^{2+}, Ni^{2+} and Zn^{2+} were ineffective at 5 mM, 10 mM and 30 mM concentrations (data not shown). GS showed maximum transferase activity with ADP and good activity with AMP (87%) and GDP (52%) at the same concentration (0.1 mM) as ADP. ATP was ineffective with 14% reactivity (data not shown).

Optimum pH and temperature

Under the conditions of assay used, the apparent optimum pH of GDH (NADPH-dependent) for the reductive amination reaction both in 50 mM Tris-HCl buffer and in 50 mM potassium phosphate buffer was 7.2, while the oxidative deamination ($NADP^+$-dependent) reaction of GDH in 50 mM Tris-HCl buffer had a broad pH profile with an optimal activity at 9.0. The apparent optimum temperatures for the GDH activities were 30 °C (data not shown). The apparent optimum pH for NADPH-GOGAT depended on the nitrogen source used in the growth medium. For instance, the pH optimum of the NADPH-GOGAT from glutamate-grown (5 mM) cells was 7.6 while it was 6.8 in cells grown with ammonium chloride (2 mM) as a sole source of nitrogen. NADH-GOGAT had optimum activity at pH 6.8. The apparent temperature optima for NADPH-GOGAT and NADH-GOGAT were 42 °C and 25 °C, respectively. The temperature-activity curve of NADPH-GOGAT has broad range from 20 °C to 45 C (data not shown). The optimum pH values for the transferase and biosynthetic activities of GS were pH 8.0 and pH 7.0, respectively. Optimum pH of transferase reaction of the enzyme was the same when measured with 100 mM Tris-HCl buffer or 100 mM imidazole-HCl buffer and the activity of the enzyme exhibited a broader pH profile (data not shown).

The optimum reaction temperatures for the transferase and biosynthetic activities of GS were 55 °C and 45 °C, respectively (data not shown). All subsequent assays were done under these optimum conditions except that the reaction temperature for GS and GOGAT activities was kept as 30 °C.

Apparent Michaelis-Menten constants of GS

For a determination of the apparent Michaelis-Menten constants of the GS for the substrates of the transferase activity, the concentrations of three of the substrates were kept constant while the concentration of the fourth was varied. Lineweaver-Burk plots were drawn from substrate saturation curves. Linear plots were obtained in the case of hydroxylamine, glutamine, arsenate and ADP and the apparent K_m values for these substrates were 1.46 mM, 15.2 mM, 1.03 mM, and 3.5×10^{-3} mM, respectively.

It was not possible to determine the apparent K_m values of the substrates of the biosynthetic activity of GS because Lineweaver-Burk plots were not linear (data not shown).

Stability of enzymes

In *C. callunae*, both activities of GDH were relatively stable to heat and lost about 35% of their activity when heated at 60 °C for 10 min. EDTA and β-mercaptoethanol did not protect these enzyme activities, but, NADPH-GOGAT activity was protected by these stabilizing agents when extracts were incubated at 50 °C or 60 °C for 20 min. At 80 °C, GOGAT was completely inactivated under all conditions (data not shown).

NADPH and NADP$^+$-dependent GDH activities in crude extracts, prepared with standard buffer and stored at 4 °C, were found to be quite stable. No loss of activity was observed after ten days of storage. Under the same conditions, NADPH-GOGAT lost 30% of its initial activity (data not shown).

The GS of *C. callunae* was quite stable against thermal denaturation. For example, when the extract was incubated at 60 °C for 20 min, only 20% of the activity was lost. Neither β-mercaptoethanol nor EDTA was effective in protecting the enzyme from denaturation at 60 °C.

Effects of amino acids and keto acids

NADPH-dependent activity of GDH from glutamate-grown cells was assayed in the presence of 2 mM and 10 mM concentrations of alanine, valine, serine, leucine, or aspartic acid, in a combination of these amino acids, and in 100 mM glutamate. Of the various amino acids tested, only glutamate inhibited the enzyme activity, probably by product inhibition. In addition to glutamate, alanine at 2 mM and 10 mM also inhibited, by 32% or 42%, respectively, the NADPH-GDH activity of cells grown with 3 mM ammonium chloride as a sole source of nitrogen. In general, it was found that GDH was not susceptible to significant inhibition by a mixture of amino acids. For example, the maximum inhibition obtained when 10 mM alanine, 2 mM valine and 2 mM aspartic acid were combined was only 38% in ammonia-grown (3 mM) cells, and this was not considered to be physiologically significant (data not shown).

Neither activity of GOGAT was inhibited by 2 mM, 10 mM or 30 mM of serine, valine, methionine, alanine or leucine, or by 1.5 mM, 4 mM, and 12 mM glutamate. No cumulative inhibition was detected when mixtures (binary, ternary, etc.) of amino acids were used. In addition, GOGAT activities were not affected by fumarate or malate at concentrations of 10 mM or 20 mM (data not shown).

GS was assayed in the presence of 2 mM, 5 mM and 30 mM concentrations of alanine, glycine, serine, glutamate, or aspartic acid and in a combination of these amino acids. The transferase activity of the enzyme was significantly inhibited by alanine, glycine and serine. For instance, alanine, at 2 mM or 5 mM inhibited activity by 65% or 76%, respectively. When two or more inhibitors were present in the assay, their effects were cumulative.

Effect of adenine nucleotides

The effect of AMP, ADP and ATP (at concentrations of 1.0 mM and 1 mM) on NADPH and NADP$^+$-dependent activities of GDH were investigated. Neither inhibition nor activation was observed with these purine nucleotides.

When the same purine nucleotides were tested (at concentrations of 1 mM, 5 mM and 25 mM) as potential effectors of the NADPH and the NADPH-dependent activities of GOGAT, only ATP caused an inhibition of 22%, 37% or 68%, respectively. A negligible effect was observed with the other nucleotides (data not shown).

The transferase activity of GS was not inhibited by AMP even at 25 mM concentration, while ATP inhibited activity at 1 mM or 5 mM concentrations by 39% and 66%, respectively (data not shown).

Effect of salts

The effect of $MgCl_2$, NaCl and KCl on the reductive and oxidative reactions of GDH were investigated at concentrations of 1 mM and 10 mM. There was no inhibition observed with these salts (data not shown).

In extracts of *C. callunae*, $MnCl_2$ inhibited the NADPH-GOGAT activity while NADH-GOGAT was inhibited by $MgCl_2$. The extents of inhibition were 32% and 92% by 10 mM and 50 mM of $MnCl_2$ and 23% and 91% by 10 mM and 50 mM of $MgCl_2$, respectively. KCl was ineffective (data not shown).

$MgCl_2$, $CoCl_2$, $NiCl_2$, $ZnCl_2$, between 0.5 mM–60 mM concentrations, showed significant inhibition of Mn^{2+}-supported GS-transferase activity about two to three fold with the exception that Mg^{2+} inhibited activity only at the highest concentration (60 mM) (data not shown). The effect of K_2SO_4 and Na_2SO_4 on GS activity was investigated by two types of experiments.

In the first type, these salts, at 400 mM concentration, were added to the dilution buffer (50 mM imidazole-HCl, pH 8.0) and crude extracts were incubated in this buffer

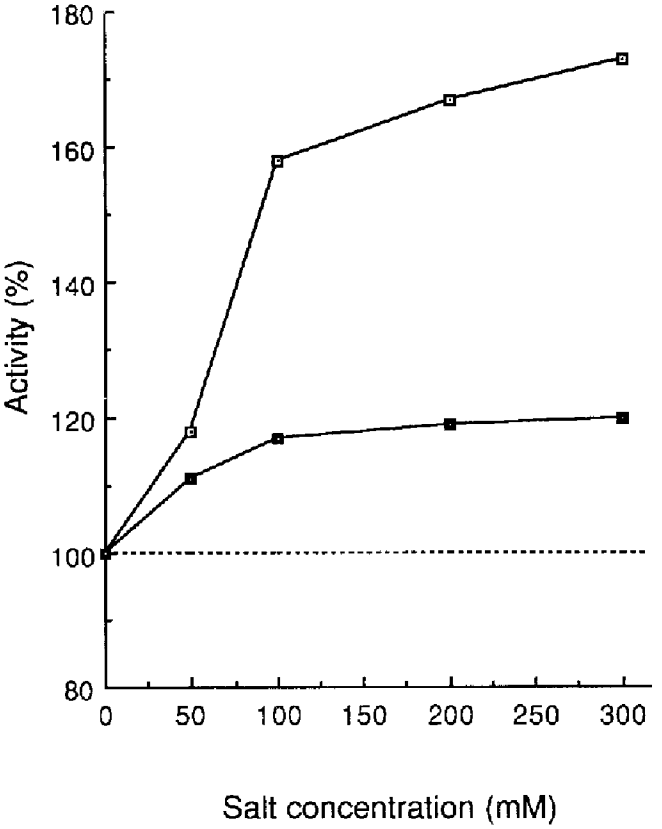

Fig. 1. Effect of K_2SO_4 (□) and Na_2SO_4 (■) on the transferase activity of GS. Control (– – – –) value was 14.8

Table 2. Effect of reaction products on the glutamate dehydrogenase activities of *Corynebacterium callunae* grown on 30 mM glutamate as nitrogen source

Reaction product	Concentration (mM)	Activity (%)			
		NADPH-GDH		$NADP^+$-GDH	
Control (without product)[a]	–	100		100	
Glutamate	100	12	22[b]	–	–
$NADP^-$	0.2	51	69[b]	–	–
α-Ketoglutarate	3.5	–	–	100	–
	10.5	–	–	98	97[b]
Ammonium chloride	75	–	–	0	7[b]
NADPH	0.12	–	–	27	20[b]

[a] The control (100%) value was 0.98
[b] These results were obtained from cells grown on 3 mM ammonium chloride as nitrogen source

Table 3. Effect of reaction products on the glutamate synthase activities of *Corynebacterium callunae* grown on 5 mM glutamate as nitrogen source

Reaction product	Concentration (mM)	Activity (%)	
		NADPH-GOGAT	NADH-GOGAT
Control (without product)[a]		100	100
Glutamate	1.5	96	100
	4	98	106
	12	97	109
$NADP^+$	0.1	86	–
	0.3	70	–
	0.5	53	72
NAD^+	0.1	–	71
	0.3	–	59
	0.5	–	49

[a] Control (100%) value was 0.091

for varying times up to 20 min. The salts significantly affected GS activity so that, at the end of the incubation time, K_2SO_4 addition had increased the activity by 75% while Na_2SO_4 addition increased it by 20%, in comparison to the control value.

In the other type of experiment, the same salts were added directly to the reaction mixtures of transferase assays at different concentrations up to 300 mM. Similar results were obtained as in the first group of experiments (Fig. 1).

These results showed that the transferase activity of the GS of *C. callunae* was strongly stimulated by potassium ions while Na^+ was less effective.

Effect of reaction products on GDH and GOGAT activities

The NADPH-dependent (reductive amination) and $NADP^+$-dependent (oxidative deamination) activities of GDH were significantly affected by some of the reaction products. The inhibition of GDH by glutamate was noted previously. $NADP^+$, another product of the amination reaction, inhibited the activity by 49% at a concentration of 0.2 mM while α-ketoglutarate was completely ineffective at 3.5 mM and 10.5 mM concentrations (Table 2). $NADP^+$-dependent activity of GDH was completelly inhibited by 75 mM NH_4Cl and by 73% with 0.12 mM NADPH. These data indicated that the primary mechanism of regulation of the *C. callunae* GDH is by product inhibition.

GOGAT activities were less sensitive to product inhibition than GDH. The inhibition at concentrations 0.1 mM, 0.3 mM and 0.5 mM with $NADP^+$ and NAD^+ were 16%, 30% and 47% on NADPH-GOGAT activity or were 29%, 41% and 51% on NADH-GOGAT activity, respectively, while glutamate was ineffective (Table 3).

Effect of inhibitors

Glutaric acid has been reported to be an inhibitor of bovine GDH (Caughey et al. 1954). To determine the effect of this substance on the activities of GDH of *C. callunae*, two types of experiments were done. In the first group of experiments, glutaric acid (1 mM, 3 mM and 5 mM) was added directly to the reaction mixtures of GDH but no inhibition was observed.

In the other type of experiment, crude extracts were incubated with and without glucaric acid (1 mM, 3 mM and 5 mM) for 15 min. $NADP^+$-GDH activity was not affected while the NADPH-GDH activity was inhibited by 30–40% with 5 mM glutaric acid (data not shown).

Azaserine is an analogue of glutamine which inhibits all glutamine-amide transfer reactions including GOGAT. When azaserine was added directly to the standard reaction mixtures of GOGAT at concentrations up to 4 mM, NADPH and NADH-dependent activities of GOGAT were inhibited to a maximum of 30% and 16%, respectively. However, the extent of inhibition of activity in crude extracts which were preincubated for 5 min with

azaserine (1 mM, 2 mM and 4 mM) were as great as 91% and NADH-GOGAT was inhibited up to 46% under the same conditions (Table 1).

Discussion

In *Corynebacterium callunae*, GDH is $NADP^+$ specific and the NAD^+ specific enzyme is absent. This finding is in agreement with those studying other glutamic acid producing coryneform bacteria such as *Brevibacterium flavum* (Shiio and Ozaki 1970) and *Corynebacterium* c91 (Vandecasteele et al. 1975). GDH activities were fairly stable to heat but were not protected by EDTA and β-mercaptoethanol. Similar results were obtained with *Mycobacterium smegmatis* (Singh and Venkitasubramanian 1977). Various amino acids, adenine nucleotides and several salts did not effect the GDH activities, but inhibition by the reaction products of the NADPH and $NADP^+$ activities of GDH were clearly demonstrated. Ammonium chloride completely inhibited $NADP^+$-GDH activity. This effect of ammonium chloride was also demonstrated with the GDHs of *B. flavum* (Shiio and Ozaki 1970), *Nitrosomonas europaea* (Hooper et al. 1967) and *Nitrobacter agilis* (Kumar and Nicholas 1984). Glutamate, NADPH and $NADP^+$ inhibited GDH activity of *B. flavum*, as of *C. callunae*. In contrast to the *C. callunae* enzyme, α-ketoglutarate inhibited the $NADP^+$-GDH activity of *B. flavum* (Shiio and Ozaki 1970), although to a small extent.

The dual coenzyme specificity of the GOGAT of *C. callunae* was demonstrated. Only a few bacteria have been reported to have the same property (Vairinhos et al. 1983; Matsuoka and Kimura 1986). Most bacteria, including *B. flavum* (Sung et al. 1984), possess only an NADPH-dependent GOGAT, although the existence of an NADH-dependent GOGAT was reported in *Corynebacterium* c91 (Vandecasteele et al. 1975). The pH optimum values for both activities of GOGAT agree well with those for the enzyme from other sources. The NADPH-GOGAT activity of *C. callunae* was greatest at 42 °C. This high value is comparable with the result (50 °C) obtained from *Clostridium pasteurianum* for the same activity of GOGAT (Singhal et al. 1989). In *B. flavum* (Tochikura et al. 1984) and *Gluconobacter suboxydans* (Tachiki et al. 1983a) the GOGAT enzyme was unstable to thermal inactivation. GOGAT activities in *C. callunae* were not susceptible to feedback inhibition by amino acids, in agreement with reports on the enzyme from *Bacillus licheniformis* (Schreier and Bernlohr 1984) and *Rhodobacter capsulatus* E1F1 (Caballero et al. 1989). NADPH and NADH-dependent GOGAT activities were quite sensitive to product inhibition by $NADP^+$ and NAD^+ coenzymes, respectively. Similar results were obtained with *Chlorobium vibrioforme* (Khanna and Nicholas 1983) and *Bacillus subtilis* PC1219 (Matsuoka and Kimura 1986). It is worth noting that product inhibition is the primary mechanism of regulation of GOGAT activity as well as GDH activities in *C. callunae*.

It was observed that the glutamine synthetase of *C. callunae* required Mn^{2+} for the transferase reaction and Mg^{2+} for the biosynthetic reaction. Similar metal ion specificity has been observed for the glutamine synthetase from other bacteria (Tachiki et al. 1981; Krishnan et al. 1986; Blanco et al. 1989). Sodium sulphate and especially potassium sulphate stimulate the transferase activity of GS. These findings agree with the results from *B. subtilis* (Deuel and Turner 1972; Matsuoka and Kimura 1985). The pH optimum value of the biosynthetic activity of GS of *C. callunae* is similar to the values from *Micrococcus glutamicus* (Tachiki et al. 1983b) and *Brevibacterium flavum* (Sung et al. 1984). The relatively high optimum reaction temperature for the GS activity is in agreement with the values from other sources (Tachiki et al. 1981; Murrel and Dalton 1983; Alvarez and McCarthy 1984). The similarity of the optimum reaction temperature values for both GS and GOGAT was also interesting. The transferase activity of GS is shown in the present investigation to be sensitive to feedback inhibition by alanine, glycine and serine, in accord with the results from other sources (Stacey et al. 1979; Khanna and Nicholas 1983; Florencio and Ramos 1985). The cumulative inhibition by various combinations of these amino acids reported herein implies that these modifiers act independently and that there are likely to be separate binding sites on the enzyme for these inhibitors. Similar inhibitory patterns have also been observed for GS from *C. vibrioforme* (Khanna and Nicholas 1983).

The results from this research showed that the overproduction of glutamate is not due to lack of control of GDH or GS enzymes in *C. callunae*. These enzymes are regulated by mechanisms that are common to many bacteria. Thus, it is logical to conclude that some other regulatory system(s) must be primarily responsible for over production of glutamate and other amino acids.

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