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Purification and Properties of Glutamate Synthase and Glutamate Dehydrogenase from *Bacillus megaterium*

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Bacillus megaterium N.C.T.C. no. 10342 exhibits glutamate synthase (EC 2.6.1.53) and glutamate dehydrogenase (EC 1.4.1.4) activities. Concentrations of glutamate synthase were high when the bacteria were grown on 3mM-NH_4Cl and low when they were grown on 100mM-NH_4Cl, whereas glutamate dehydrogenase concentrations were higher when the bacteria were grown on 100mM-NH_4Cl than on 3mM-NH_4Cl. Glutamate synthase and glutamate dehydrogenase were purified to homogeneity from *B. megaterium* grown in 10mM-glucose/10mM-NH_4Cl. The purified enzymes had mol.wts. 840000 and 270000 for glutamate synthase and glutamate dehydrogenase respectively. The K_m values for substrates with NADPH and coenzyme were (glutamate synthase activity shown first) 9μM and 360μM for 2-oxoglutarate, 7.1μM and 8.7μM for NADPH, and 0.2mM for glutamine and 22mM for NH_4Cl, similar values to those of enzymes from *Escherichia coli*. Glutamate synthase contained NH_3-dependent activity (different from authentic glutamate dehydrogenase), which was enhanced 4-fold during treatment at pH4.6. NH_3-dependent activity was generally about 2% of the glutamine-dependent activity. Amidination of glutamate synthase by the bi-functional cross-linking reagent dimethyl suberimidate inactivated glutamine-dependent glutamate synthase activity, but increased NH_3-dependent activity. A cross-linked structure of mol.wt. approx. 200000 was the main product formed.

In bacteria the biosynthesis of glutamate from NH_3 is catalysed by glutamate dehydrogenase and by the coupled functioning of glutamine synthetase and glutamate synthase. In bacilli glutamate is utilized for both growth and sporulation and thus the synthesis of amino acid cannot be readily explained, especially because bacilli are generally thought to lack glutamate dehydrogenase activity (Phibbs & Bernlohr, 1971). Meers *et al.* (1971) and Meers & Pedersen (1971) concluded that glutamate dehydrogenase is used to assimilate NH_3 under the growth conditions of excess of NH_3. The addition of glutamate to a carbon-limited chemostat culture results in direct deamination of the amino acid, thus preventing an excessive increase in the glutamate pool. Extremely low glutamate dehydrogenase activities were detected when glutamate was the only source of carbon and nitrogen (Phibbs & Bernlohr, 1971). Elmerich & Aubert (1971) reported that coupled reactions of glutamine synthetase and glutamate synthase represent the major pathway of glutamate synthesis in *Bacillus megaterium*.

Glutamate dehydrogenase has been purified 55-fold from *Bacillus licheniformis* (Phibbs & Bernlohr, 1971) and 250–300-fold from a thermophilic bacillus (Epstein & Grossowics, 1975). Glutamate synthase has been purified about 20-fold from *B. megaterium* (Elmerich & Aubert, 1971). The highly purified glutamate dehydrogenase from a thermophilic bacillus has a molecular weight (2×10^6) and K_m value quite different from those of the other bacterial sources.

Our data provide evidence that *B. megaterium* N.C.T.C. no. 10342 exhibits both glutamate dehydrogenase and glutamate synthase activities, and that the concentrations of the enzymes are regulated by the glucose/NH_3 ratio. Purified glutamate synthase and glutamate dehydrogenase are similar in enzymic and molecular properties to glutamate synthase and glutamate dehydrogenase from other bacterial sources.

Abbreviations used: SDS, sodium dodecyl sulphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Materials and Methods

Reagents

L-[U-^{14}C]Glutamine (sp. radioactivity 38mCi/mmol) and iodo[1-^{14}C]acetamide (sp. radioactivity 3.6mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Octanedinitrile was purchased from Aldrich Chemical Co., Beerse, Belgium. Ultrogel AcA 22 was a product of Industrie Biologique Française and was from LKB-Produkter A.B., Stockholm, Sweden. β-Galactosidase, alcohol dehydrogenase, glyceraldehyde 3-

phosphate dehydrogenase and bovine serum albumin were from Boehringer, Mannheim, Germany. Glutamate synthase from *Escherichia coli* was purified as previously described (Mäntsälä & Zalkin, 1976*a*).

Bacterial strain and cultivation

The bacterial strain used for the enzyme purification was *B. megaterium* N.C.T.C. no. 10342. The freeze-dried cells were cultured first in the Micro Inoculum Broth (Difco Laboratories, Detroit, MI, U.S.A.) medium, then in Micro Inoculum Broth (0.5%)/10mM-glucose/10mM-NH_3 medium and finally in 10mM-glucose/10mM-NH_3 medium. If the inocula were stored at 8°C for longer than 2 weeks there was no longer any growth in the minimal medium. The main cultures were grown aerobically at 35°C in a glucose/NH_3 minimal medium (Miller & Stadtman, 1972). The cells were harvested by centrifugation for 15min at 7000*g* in the late-exponential phase if not otherwise stated. For the extraction of proteins 280g of cells was disrupted in 10mM-potassium phosphate/10mM-2-mercaptoethanol/1mM-EDTA buffer, pH7.2 (buffer A) (3ml/g of cell paste), by French-press treatment as described by Mäntsälä & Zalkin (1976*a*). The suspension was finally centrifuged at 30000*g* for 1h at 4°C.

Enzyme assays

Glutamine- and NH_3-dependent glutamine synthase activities were assayed as previously described (Miller & Stadtman, 1972; Mäntsälä & Zalkin, 1976*a*). The glutamate dehydrogenase assay was as described by Mäntsälä & Zalkin (1976*b*), except that 4mM-2-oxoglutarate was used. In each case 1 unit of activity corresponds to the amount of enzyme catalysing the utilization of 1μmol of NADPH/min at 25°C. Glutaminase activity was determined by the method of Curthoys & Weiss (1974).

Electrophoresis

Discontinuous polyacrylamide-gel electrophoresis at pH8.5 was performed as described by Baker *et al.* (1972) and polyacrylamide-gel electrophoresis in the presence of 0.1% SDS at pH7.1 by the procedure of Shapiro *et al.* (1967), as modified by Weber & Osborn (1969).

Molecular-weight determinations

Molecular-weight estimations were performed as described by Martin & Ames (1961) by using sucrose-density-gradient centrifugation. Sucrose gradients from 5 to 20% (w/v) were used. Samples in 0.2ml of buffer B (buffer A containing 2mM-2-oxoglutarate and 100mM-KCl) were layered on 19.8ml gradients and centrifuged in an MSE Super Speed 50 ultracentrifuge at 100000*g* for 10h. *E. coli* β-galactosidase (mol.wt. 540000) and yeast alcohol dehydrogenase (mol.wt. 141000) served as the reference standards. SDS/polyacrylamide-gel electrophoresis was used to determine subunit molecular weights. β-Galactosidase (subunit mol.wt. 135000), bovine serum albumin (mol.wt. 67000), catalase (subunit mol.wt. 60000) and glyceraldehyde 3-phosphate dehydrogenase (subunit mol.wt. 37000) were used as the standards.

Incorporation of [^{14}C]carbamoylmethyl

Reaction mixtures contained glutamate synthase (0.64mg), 20mM-Hepes (potassium salt, pH7.2) and 0.180mM-iodo[1-^{14}C]acetamide in a final volume of 0.3ml. After incubation for 30min at 25°C the mixture was passed through a column (1cm×20cm) of Sephadex G-25. The column was eluted with 20mM-Hepes and the enzyme fractions were pooled and dialysed against 500vol. of the same buffer solution and counted for radioactivity as described by Nagano *et al.* (1970).

Preparation of apo-(glutamate synthase)

Glutamate synthase was precipitated with 0.2M-potassium acetate, pH4.6, as described by Mäntsälä & Zalkin (1976*a*). After incubation at 4°C for 3h the suspension was returned to neutral pH by addition of 1M-Tris/HCl (pH8.5). The precipitate was isolated by centrifugation for 15min at 5000*g* and suspended in 20mM-Hepes (potassium salt) buffer, pH7.2. The concentration of the released flavin was determined as described by Burch *et al.* (1948).

Cross-linking studies

Amidination of glutamate synthase was carried out in 0.2M-triethanolamine hydrochloride (pH8.5) as described by Davies & Stark (1970). Dimethyl suberimidate was prepared by the method of McElvain & Schroeder (1949). Dimethyl suberimidate and glutamate synthase were mixed to give 1.2mg of protein/ml and 3mg of suberimidate/ml in a volume of 150μl. Samples (30μl) were taken at intervals of 30min, denatured (Mäntsälä & Zalkin, 1976*a*) and electrophoresed as described above in the presence of 0.1% SDS. The gels (12cm×0.5cm) contained 3% (w/v) acrylamide.

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Amino acid analyses were carried out in a Perkin–Elmer KLA-5 amino acid analyser.

Results

Growth experiments

There was no significant difference in the concentrations of glutamate synthase when glutamate or lysine served as the nitrogen source (Table 1). In the presence of 3mM-NH_4Cl the cultures ceased growth at a Klett reading of 111, with filter no. 62. However, the concentrations of glutamate synthase were higher than in the 100mM-NH_4Cl medium, whereas glutamate dehydrogenase concentrations were high in the NH_3-rich medium.

Enzyme purifications

Nucleic acids were removed from the crude extract by the slow addition of 1% streptomycin sulphate (10ml of 10% streptomycin sulphate/100ml of crude extract). The supernatant was treated with a saturated solution of $(NH_4)_2SO_4$ adjusted with KOH to pH7.2. Proteins precipitated between 33 and 53% saturation were dissolved in 92ml of buffer B and heated at 62°C for 10min (Mäntsälä & Zalkin, 1976*a*). The supernatant was saturated with $(NH_4)_2SO_4$ to obtain a protein fraction precipitated between 37.5 and 49% saturation. The dissolved fraction (22ml) was applied to two tandem columns (3cm×100cm) containing Ultrogel AcA 22. Protein was eluted with buffer B. The activities of interest were eluted in two regions. The pooled fractions of glutamine-dependent glutamate synthase activity and of dehydrogenase activity were applied separately to a column (1.5cm×25cm) packed with DEAE-Sephadex A-50. The proteins were eluted with a linear gradient of 0.1–0.7M-KCl in buffer B. The pooled fractions were concentrated by ultrafiltration with an Amicon PM 30 membrane. The dialysed fractions were applied to a column (1.5cm×8cm) of hydroxyapatite. Elution was carried out with a linear 5–200mM-potassium phosphate gradient, pH7.0. The pooled fractions of highest activity were concentrated as above and the final purification of the dehydrogenase was obtained by using preparative polyacrylamide-gel electrophoresis. The equipment supplied by Stålproducter, Uppsala, Sweden, was used and the run was carried out at 4°C. About 16mg of protein was loaded on the column. Fig. 1 shows the elution profile of glutamate dehydrogenase. The activity was associated with the protein peak emerging at 95ml of washing buffer (25mM-asparagine/19mM-Tris, pH8.5; Miller & Stadtman, 1972). The gel (1cm×15cm) contained 5% (w/v) acrylamide, was run at 10mA and eluted with washing buffer at 0.5ml/min. The most active fractions were pooled and concentrated as above.

The overall yields were 30.5 and 23% and specific activities 23.8 and 45.0 units/mg of protein in glutamate synthase and glutamate dehydrogenase respectively.

Enzyme purity

Homogeneity of the two enzymes was established by polyacrylamide-gel electrophoresis at pH8.5, SDS/

Table 1. *Effect of growth conditions on the activities of glutamate synthase and glutamate dehydrogenase*

The samples were disrupted in a Raytheon DF 101 sonic oscillator in buffer A, centrifuged and the supernatants were assayed for glutamate synthase and dehydrogenase activities as previously described (Tempest *et al.*, 1970; Miller & Stadtman, 1972; Mäntsälä & Zalkin, 1976*a*). Klett units were read by using filter no. 62.

Medium	Klett units	Activity (units/mg of protein) Glutamate synthase	Glutamate dehydrogenase
Casamino acids (1%)	75	0.04	0.002
(Difco Laboratories, Detroit, MI, U.S.A.)	152	0.07	0.001
	265	0.06	0.001
Glucose (20mM)+ glutamate (10mM)	61	0.05	0.014
	148	0.09	0.028
	196	0.08	0.035
Glucose (20mM)+ lysine (10mM)	56	0.03	0.014
	121	0.06	0.026
	173	0.07	0.023
Glucose (20mM)+ NH_4Cl (100mM)	60	0.04	0.042
	139	0.06	0.147
	187	0.07	0.062
Glucose (20mM)+ NH_4Cl (3mM)	41	0.09	0.018
	89	0.11	0.073
	111	0.13	0.069

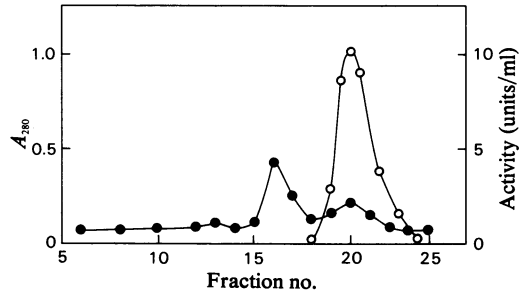

Fig. 1. *Purification of glutamate dehydrogenase on preparative polyacrylamide-gel electrophoresis*

The conditions for preparation and electrophoresis were described in the text. Electrophoresis was carried out at pH8.5 for 9h by using 25mM-asparagine/19mM-Tris buffer. Fractions (5ml) were collected and assayed for glutamate dehydrogenase activity. Symbols: ●, protein; ○, glutamate dehydrogenase activity.

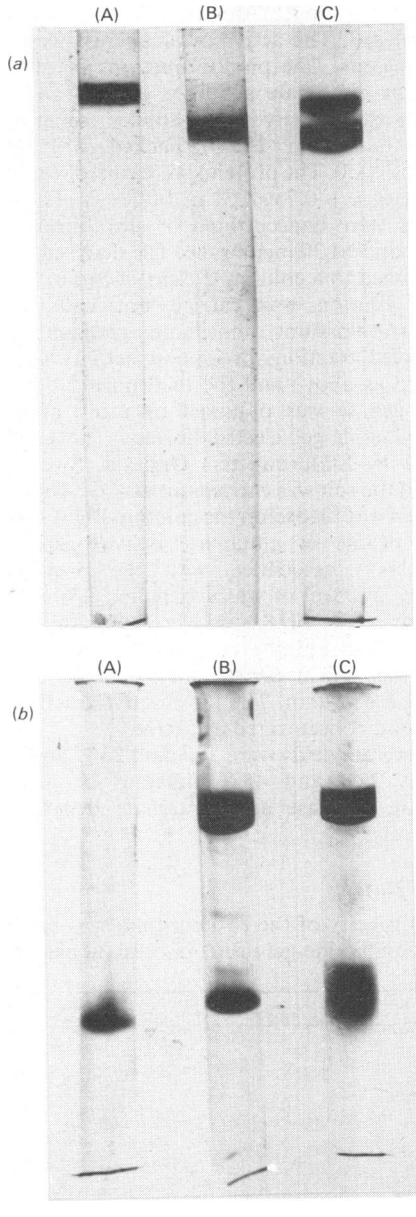

Fig. 2. *Polyacrylamide gels electrophoresed (a) at pH 8.5 and (b) in the presence of SDS at pH 7.1*
(A) With 26 μg of glutamate dehydrogenase; (B) with 44 μg of glutamate synthase; (C) with 33 μg of glutamate synthase + 18 μg of glutamate dehydrogenase.

polyacrylamide-gel electrophoresis at pH 7.1 (Fig. 2) and sucrose-density-gradient ultracentrifugation experiments as described by Martin & Ames (1961). The enzymes showed the same electrophoretic mobility as the enzymes from *E. coli* (Mäntsälä & Zalkin, 1976*b*). Sucrose gradients (5–20%) were prepared in the presence of buffer B. Samples (1.4 mg of glutamate synthase and 0.45 mg of glutamate dehydrogenase) in 0.2 ml of buffer B layered on 19.8 ml gradients were centrifuged as described in the Materials and Methods section. Only two peaks of protein were detected after protein and enzyme determinations, corresponding to glutamate synthase and dehydrogenase activities.

Table 2. *Amino acid composition of glutamate synthase and glutamate dehydrogenase from B. megaterium*
Samples (about 0.3 mg of protein) were hydrolysed in evacuated glass tubes at 110°C for 24 h with 6 M-HCl containing 10 μl of ethylene glycol. After hydrolysis the samples were evaporated in vacuum and analysed in a Perkin–Elmer KLA-5 amino acid analyser. Enzyme activities were calculated for 24 h hydrolysates (average of two determinations).

	Amino acid content	
	Glutamate synthase (mol/mol of enzyme of mol.wt. 210000)	Glutamate dehydrogenase (mol/mol of enzyme of mol.wt. 47000)
Tyr	35.2	8.1
Phe	58.7	19.0
Lys	71.9	16.9
His	33.4	12.6
Arg	99.5	19.4
Asp	175.3	34.1
Glu	176.7	42.1
Thr	86.4	21.3
Ser	81.6	22.9
Pro	71.3	16.2
Ala	156.4	46.1
Gly	162.3	48.6
Val	106.2	29.2
Met	34.2	12.3
Ile	74.0	17.4
Leu	166.9	38.8
Cysteic acid	16.7	6.2

Amino acid composition

The amino acid analyses of purified glutamate synthase and glutamate dehydrogenase are presented in Table 2.

Kinetic and molecular properties

Kinetic studies with the purified enzymes revealed that both enzymes are highly specific for NADPH. In the standard assay, NADH supported about 0.1 and 2.3% of the NADPH activity with glutamate synthase and glutamate dehydrogenase respectively. Table 3 summarizes some molecular and enzymic properties of the purified enzymes.

Two methods were used to determine the subunit structure of glutamate synthase. In one method, the

colour intensities of the small and the large subunit of the SDS-treated glutamate synthase after electrophoresis and staining with Coomassie Blue were compared. After densitometer tracing, the ratio of relative areas 1.00/2.70 (calculated 1.00/2.58) and the molecular weights of 840000 for the native enzyme, 142000 for the large subunit and 55000 for the small subunit (Table 3) suggest an $\alpha_4\beta_4$ structure for the native enzyme. In the other method, cross-linking of the polypeptides was investigated (Fig. 3), which also suggests an $\alpha_4\beta_4$ structure, since the estimated molecular weight of the cross-linked adduct is about 180000. After incubation for 1 h the most dominating combination was a protomeric $\alpha\beta$ structure, indicating that the small and the large polypeptide are tightly linked to each other in the native enzyme. Longer incubation time increased the number of high polymeric forms. A similar result was obtained when the highly purified glutamate synthase from *E. coli* was treated with dimethyl suberimidate.

Amidination of glutamate synthase with dimethyl suberimidate inactivated glutamine-dependent activity, but increased NH_3-dependent activity (Fig. 4). Inactivation of glutamine-dependent activity did not

Table 3. *Properties of glutamate synthase and glutamate dehydrogenase*

The mean values of the molecular weights were calculated from the results of three runs. The apparent K_m and $V_{max.}$ values were calculated from the double-reciprocal plots. Assay mixtures in 50mM-Hepes buffer (potassium salt) at pH7.5 and 8.0 for glutamate synthase and glutamate dehydrogenase respectively contained 1mM-EDTA, 3.4μg of enzyme and variable amounts of: $NADP^+$ (with 4mM-2-oxoglutarate and 100mM-NH_4Cl); NADPH (with 2mM- or 4mM-2-oxoglutarate and 2mM-L-glutamine or 100mM-NH_4Cl); 2-oxoglutarate (with 0.1mM-NADPH and 2mM-L-glutamine or 100mM-NH_4Cl); glutamate (with 0.1mM-$NADPH^+$); NH_4Cl (with 4mM-2-oxoglutarate, 0.1mM-NADPH) (Miller & Stadtman, 1972; Mäntsälä & Zalkin, 1976*b*).

	Enzyme properties	
	Glutamate synthase	Glutamate dehydrogenase
Molecular weights		
Native	840000±45000	270000±20000
Large subunit	142000± 8000	
Small subunit	55000± 3000	
Subunit		47000± 3000
Apparent K_m (M)		
2-Oxoglutarate	9.0×10^{-6}	3.6×10^{-4}
NH_4Cl		2.2×10^{-2}
Glutamate		2.9×10^{-2}
NADPH	7.1×10^{-6}	8.7×10^{-6}
$NADP^+$		5.0×10^{-5}
pH optima		
Amination	7.3	7.9
Deamination		9.0
$V_{max.}$ (units)		
2-Oxoglutarate	0.76	1.67
NH_4Cl		2.50
Glutamate		19.80
NADPH	0.71	2.38
$NADP^+$		11.70

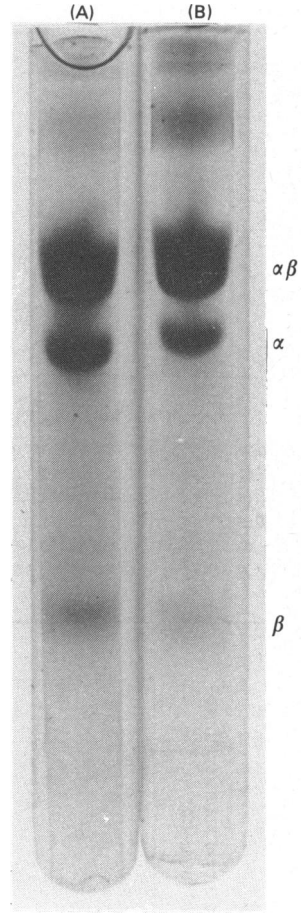

Fig. 3. *Amidination of glutamate synthase from B. megaterium with dimethyl suberimidate*

Glutamate synthase (1.2mg/ml) and dimethyl suberimidate (3mg/ml) were incubated in a volume of 150μl at room temperature. Samples (30μl) were taken at intervals of 30min and denatured as described by Mäntsälä & Zalkin (1976*a*). Electrophoresis was carried out at a current of 8mA/tube in the presence of SDS (0.1%). (A) Glutamate synthase from *B. megaterium* after incubation for 90min. (B) Glutamate synthase from *E. coli* after incubation for 90min. Abbreviations: α, the large subunit; β, the small subunit.

follow formation of the protomer. About 90% cross-linking resulted in 50% inactivation of the enzyme. The same treatment increased NH_3-dependent activity about 1.5-fold. Although we conclude that a structural change is responsible for the decrease in glutamine-dependent activity, it is not excluded that dimethyl suberimidate inactivates the enzyme reacting with a residue located at or close to the active site.

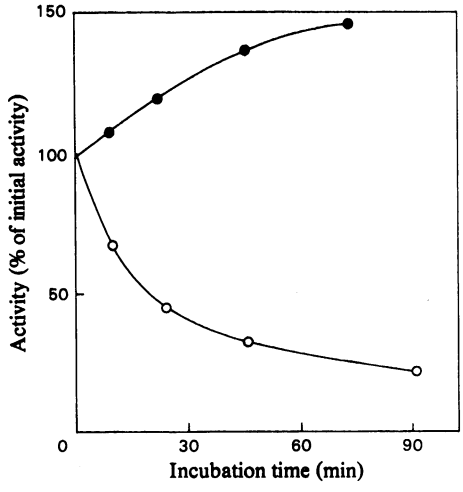

Fig. 4. *Effect of dimethyl suberimidate on glutamine- and NH_3-dependent activities*

Glutamate synthase and dimethyl suberimidate were incubated as described in the legend to Fig. 3. Samples (10μl) were taken as indicated and assayed for glutamine- (○) and NH_3-dependent (●) activities.

Enhancement of NH_3-dependent activity of apo-(glutamate synthase)

Purified glutamate synthase from *B. megaterium* exhibited NH_3-dependent activity similar to the enzymes from *E. coli* (Mäntsälä & Zalkin, 1976*b*) and *Aerobacter aerogenes* (Trotta *et al.*, 1974; Geary & Meister, 1977). The activity was normally 2–4% of the glutamine-dependent activity. Treatment with 0.2M-sodium acetate buffer, pH4.6 (Mäntsälä & Zalkin, 1976*a*), released non-haem iron and flavin into the supernatant solution and precipitated the apoenzyme. The supernatant did not contain any protein, but 7.1nmol of total flavin nucleotide (both FAD and FMN)/mg of native protein was released during this treatment. After neutralization the apoenzyme remained insoluble, but NH_3-dependent activity increased about 4-fold. A similar stimulation of NH_3-dependent activity during treatment at pH4.6 was also found when activity measurements were made before the neutralization of the precipitated apoenzyme.

Glutamine-dependent glutamate synthase and glutamate dehydrogenase activities decreased during the same treatment (Fig. 5). In some experiments glutamine-dependent activity disappeared immediately at pH4.6, though the activity measurements were made either before or after neutralization of the precipitated enzyme. However, the inactivation was reversible and activity appeared slowly

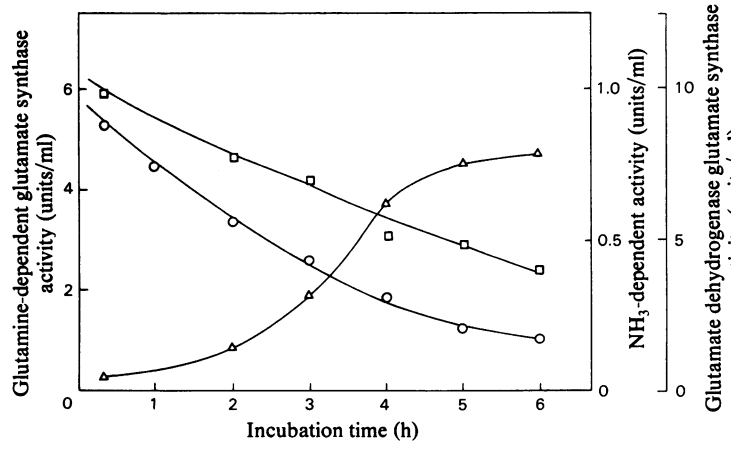

Fig. 5. *Effect of treatment at pH4.6 on enzyme activities*

Glutamate synthase (220μg) and glutamate dehydrogenase (90μg) were incubated at room temperature (25°C) in a mixture containing 0.1M-KCl and 0.2M-potassium acetate, pH4.6 (total volume of reaction mixture 0.1ml). Samples (0.01ml) were removed and assayed for enzyme activities. Symbols: ○, glutamine-dependent glutamate synthase; △, NH_3-dependent glutamate synthase; □, glutamate dehydrogenase.

within 30–60min. After this reactivation, slow inactivation of glutamine-dependent activity and stimulation of NH_3-dependent activity were similar to those shown in Fig. 5. Similar to many other glutamine amidotransferases, glutamate synthase exhibits glutaminase activity. This reaction is a partial one that hydrolyses the amido group of glutamine, uncoupled from amination. Glutaminase activity did not decrease equally with glutamine-dependent activity. Glutaminase activity decreased from 0.83 unit/mg (native enzyme) to 0.57 unit/mg (apoenzyme). To determine the effect of pH4.6 treatment on the incorporation of [^{14}C]carbamoylmethyl, samples were removed at selected times and incubated with iodo[1-^{14}C]acetamide. After incubation the samples were passed through a Sephadex column, dialysed and counted for radioactivity. Incorporation of [^{14}C]carbamoylmethyl decreased during treatment at pH4.6 from 1.45mol/mol of enzyme to 0.97mol/mol of enzyme.

Discussion

With an excess of nitrogen, even as glutamate, a carbon-limited culture of *B. licheniformis* synthesizes glutamate dehydrogenase. However, the addition of glutamate to the cells growing in excess of glucose/NH_3 medium results in strong repression of synthesis of this enzyme (Meers & Pedersen, 1971). Similarly the cells grown on media containing glutamate or lysine as the nitrogen source exhibited very low glutamate dehydrogenase activities compared with the activities obtained in cells grown on glucose/NH_3 medium (Table 1). Further, the specific activities of glutamate dehydrogenase were extremely low when *B. megaterium* was grown on casamino acids. These results suggest that the enzymes have a physiological significance similar to that found in many other bacteria; under conditions of low concentrations of NH_3 glutamate synthase functions, and the concentrations of glutamate dehydrogenase are very low, whereas in the presence of high concentrations of NH_3 glutamate dehydrogenase provides the main route for NH_3 assimilation.

The molecular structure and properties of glutamate synthase and glutamate dehydrogenase from bacilli are not well established. It is known that glutamate dehydrogenases from different sources vary greatly in their molecular structure. The molecular weight of glutamate dehydrogenase from a thermophilic bacillus is approx. 2×10^6, which is about 6–7 times greater than the molecular weight of the enzyme from many other bacterial sources. The molecular weight and physical properties of glutamate dehydrogenase from *B. megaterium* were similar to those of many bacterial glutamate dehydrogenases (Coulton & Kapoor, 1973; Johnson & Westlake, 1972; Veronese *et al.*, 1975; Sakamoto *et al.*, 1975), whereas those of glutamate synthase were similar to the enzyme from *E. coli* (Mäntsälä & Zalkin, 1976*a*) and *A. aerogenes* (Trotta *et al.*, 1974). Glutamate synthase from *E. coli* (Mäntsälä & Zalkin, 1976*a*) and *A. aerogenes* (Trotta *et al.*, 1974; Geary & Meister, 1977) exhibits glutamine- and NH_3-dependent activities. However, as in the case of carbamoyl phosphate synthetase from mammalian (Abrams & Bentley, 1959) and avian (Lagerkvist, 1958) tissues, glutamate synthase from bacterial sources exhibits higher activity with glutamine than with NH_4Cl as the amino donor. It seems very unlikely that NH_3 utilization by glutamate synthase is an artefact due to contamination by glutamate dehydrogenase, because no evidence of contamination was detected in SDS or discontinuous polyacrylamide gels and because the NH_3 activity of the enzyme increased about 4-fold during treatment at pH4.6, whereas glutamate dehydrogenase was inactivated during this treatment. When glutamate synthase was purified from *Klebsiella aerogenes* MK 270 (this mutant contains only about 1% NH_3-dependent activity), NH_3-dependent activity was associated with glutamine-dependent activity throughout the purification procedure (results not shown). The overall results favour the conclusion that the quaternary structure of glutamate synthase has a functional site for glutamine, but that a site for NH_3 is somehow blocked. After acid treatment apo(glutamate synthase) has a functional site for NH_3, but non-haem iron and flavin, which obviously are required for function of glutamine site, are released. The fact that the incorporation of [^{14}C]carbamoylmethyl and glutamine-dependent activity have not been decreased equally indicates that flavin is linked only to glutamine-dependent glutamate synthase. The isolation of *S*-carboxymethylcysteine after alkylation with iodo-[1-^{14}C]acetamide provided evidence that a cysteine residue may have an essential role in the binding of glutamine in glutamate synthase from *E. coli* (Mäntsälä & Zalkin, 1976*a*). On the basis of experiments with alkylating and thiol (results not shown) reagents we suggest that a cysteine residue could be essential also to the binding of [^{14}C]carbamoylmethyl in glutamate synthase from *B. megaterium*. Nearly 90% inactivation of glutamine-dependent glutamate synthase was found within 5min in the presence of 50μM-*p*-mercuribenzoate. Glutamine (20mM) provided substantial protection (60–70%) against inactivation. During treatment at pH4.6 the site for glutamine is partially destroyed and the incorporation of [^{14}C]carbamoylmethyl and glutaminase activity somewhat decreased. Mäntsälä & Zalkin (1976*a*,*b*) and Geary & Meister (1977) have shown that the mechanism of the glutamine-mediated reductive amination is fundamentally different from that of the NH_3-mediated reductive amination.

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