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GLUTAMATE DEHYDROGENASE FROM *ESCHERICHIA COLI*: INDUCTION, PURIFICATION AND PROPERTIES OF THE ENZYME

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Summary

When *Escherichia coli* was grown in a minimum medium with glucose as sole carbon source and a proper level of ammonia, $NADP^+$ specific glutamate dehydrogenase (L-glutamate: $NADP^+$ oxidoreductase (deaminating), EC 1.4.1.4) was induced.

The enzyme was solubilized by French press treatment and purified to homogeneity by $(NH_4)_2SO_4$ fractionation, heat treatment followed by DEAE-cellulose, hydroxylapatite and Bio-Gel chromatography with an overall yield of 30%.

The enzyme proved to be heat stable and relatively resistant to protein denaturants. The optimum of enzymic activity for the reductive amination is at pH 8 and at pH 9 for the oxidative deamination. The activity is affected by adenine nucleotides.

The molecular weight (about 250 000 for the native form and 46 000 for the inactive subunit) and amino acid composition, suggest strict similarities with the $NADP^+$ enzyme from fungal origin.

Introduction

Intensive studies have been carried out on glutamate dehydrogenase from many sources, vertebrates in particular, because of its biological significance and key role in metabolism. The enzyme serves as a link to the pathways of transamination and the tricarboxylic acid cycle and thus as a major pathway for the interconversion of the α-amino group nitrogen and ammonia.

The recent establishment of the primary structure of bovine [1] and chicken [2] enzymes has led to a new impetus to these studies because it is now possible to establish a more intimate correlation between structure and functions of the enzymes [3—7].

Studies are now being carried out in two laboratories to elucidate the molecular properties as well as the sequences of both the $NADP^+$- [8,9] and NAD^+- [10—12] dependent enzymes of the more primitive organism *Neurospora crassa*. It is in fact known that although the vertebrate enzyme does not show strict coenzyme specificity, in microorganism two different enzymes, NAD^+ and $NADP^+$ dependent, have been often isolated.

On the other hand only limited structural information is so far available on the glutamate dehydrogenases from bacteria [13,14].

In this context we report data regarding the production of the $NADP^+$-dependent glutamate dehydrogenase from *Escherichia coli* purification to homogeneity, and partial characterization, as a preliminary investigation for a structural study of the enzyme.

Materials and Methods

Bacteria. E. coli strains B and W were ATCC 14948 and 9637, respectively, strains CA 244, CA 265 and MRE 600 were kindly supplied by Dr K. Sargeant from the Microbiological Establishment of Porton Down (England), the strain K 12 S was from our collection.

Growth of E. coli cells and preparation of cell free extract. (A) In small scale experiments *E. coli* was grown under forced aeration at 37 °C in 1 l medium. After 48 h the cells were harvested by centrifugation and washed with 0.9% NaCl solution. For the extraction of proteins the cell paste was added to 10 ml of 0.05 M Tris · HCl, pH 7.2, buffer and the cells disrupted by ultrasonic treatment for 6 min using a Branson sonifier model B-12 with a 1-cm probe. The suspension was finally centrifuged at 46 000 × *g* for 20 min and the supernatant assayed.

(B) *E. coli* strain MRE 600 was grown in 20-l batches using the medium reported in Table I at 37 °C with vigorous aeration to reach late stationary phase. The inoculum was a culture of a 1 l at the beginning of the stationary phase. The cells were harvested by centrifugation in a Sorvall RC2-B continuous flow refrigerated centrifuge, washed twice with 0.9% NaCl solution and stored at —20 °C as frozen paste until processed.

Cells (200 g) were thawed, suspended in 400 ml of the above reported buffer and disrupted by passing the mixture through a Maulton Gaulin French press three times at 6000 lb/inch^2. The suspension was spun for 45 min at 40 000 × *g* at 2 °C and the supernatant was removed. The pellet was suspended in 100 ml of extracting buffer and, after 10 min of stirring, centrifuged again. The pooled supernatants represented the cell free extract. The extract may be directly processed or stored at —20 °C for some days without appreciable loss of activity.

Enzyme assays. Glutamate dehydrogenase was usually assayed by measuring spectrophotometrically the oxidation of NADPH at 340 nm at 22 °C. The reaction system (1 ml) consisted of 0.1 M Tris · HCl buffer (pH 8.0), 0.1 M NH_4Cl, 2.5 mM α-ketoglutarate and 0.1 mM NADPH. The reaction was initiated by the addition of $1 \cdot 10^{-5}$—$2 \cdot 10^{-4}$ units of the enzyme.

One enzyme unit was defined as the amount of enzyme that catalized in 1 min the conversion of 1 μmol of coenzyme under the assay conditions.

The $NADP^+$ reduction was determined using a system (1 ml) consisting of 10 mM sodium glutamate and 0.5 mM $NADP^+$ in 0.1 M Tris · HCl, pH 9.

Modification of enzyme. For experiments involving modification at thiol groups, β-mercaptoethanol was removed from enzyme solution by gel filtration on a Sephadex G-25 column (0.9 cm × 25 cm)) or by dialysis with 0.1 ionic strength phosphate buffer at the pH desired for the experiment. The buffer contained 1 mM EDTA, was freed of oxygen under vacuum and by bubbling a stream of N_2 for 1 h before use. For the modifications, reagents were added in concentrated solutions. All concentrations of reactants indicated below refer to final concentrations in the reaction mixtures.

Amino acid analysis. Analyses were performed with a Jeol model GAH automatic amino acid analyzer by the procedure of Hamilton [15] for the single column. Samples were hydrolyzed with 6 M HCl containing 0.1% mercaptoacetic acid and 0.05% phenol in evacuated sealed tubes, for 22 or 72 h at 110 °C.

Other procedures. Analytical polyacrylamide disc-gel electrophoresis was performed according to standard procedures. The molecular weight of the enzyme subunits was estimated by sodium dodecylsulphate-polyacrylamide gel electrophoresis by the procedure of Weber and Osborn [16]. Density gradient centrifugation was carried out by the method of Martin and Ames [17]. A Spinco model L ultracentrifuge with swinging bucket rotor SW-39 was used.

Protein concentration was determined by the turbidimetric method [18] using bovine serum albumin as reference protein. The concentration of the purified enzyme was estimated spectrophotometrically from $A^{0.1\%}_{280\,nm} = 0.95$.

S-Carboxymethylation and performic acid oxidation were performed according to established procedures [19].

Materials. NAD^+, $NADP^+$, NADH, NADPH, α-ketoglutarate, glutamate and other amino acids were purchased from Boehringer (Mannheim, Germany). Microgranular diethylaminoaethyl (DEAE)-cellulose (DE-32) was obtained from Reeve and Angel (Clifton, New Jersey) and Bio-Gel A-0.5 m from Bio-Rad (Richmond, California). Hydroxylapatite was prepared following the procedure described by Atkinson et al. [20]. The proteins used as markers for molecular weight determination were: chymotrypsinogen, hen egg and bovine serum albumin, rabit muscle aldolase, bovine liver catalase from Boehringer (Mannheim, Germany), jack bean urease from Miles—Seravac, *Candida utilis* 6-phosphogluconate dehydrogenase was a gift of Professor M. Rippa of the Department of Biochemistry, University of Ferrara, Italy.

Results

Studies on the control of glutamate dehydrogenase synthesis

Several strains of *E. coli* have been tested for the production of glutamate dehydrogenase. When the strains B, W, CA 244, CA 265, K 12 and MRE 600 were grown in a minimum medium with glucose as carbon source, the specific activity in the cell free extracts were between 0.22 and 0.28 μmol of NADPH oxidized per mg of protein per min. The strain MRE 600, giving slightly higher activity was chosen for further studies. In Table I are reported the dependence of enzyme activity upon the composition of the media employed.

TABLE I

GLUTAMATE DEHYDROGENASE LEVELS AFTER GROWTH ON MEDIA OF DIFFERENT COMPOSITION

The media a and b contained also Na_2HPO_4, 0.62 g, $NaH_2PO_4 \cdot 2H_2O$, 0.18 g, $MgSO_4 \cdot 7H_2O$, 0.1 g, NaCl, 0.3 g, per 100 ml. The media c—g contained also K_2HPO_4, 0.7 g, KH_2PO_4, 0.3 g, $MgSO_4 \cdot 7H_2O$, 0.01 g, per 100 ml. The final pH was 7.0.

Nitrogen and carbon sources (%)		Specific activity of the cell free extract (unit/mg)
(a) Casein hydrolyzate	2.5	0.02
(b) Glucose	1	0.03
glutamate	1	
(c) Glycerol	1	0.20
$(NH_4)_2SO_4$	0.2	
(d) Glucose	1	0.18
$(NH_4)_2SO_4$	0.05	
(e) Glucose	1	0.20
$(NH_4)_2SO_4$	0.1	
(f) Glucose	1	0.31
$(NH_4)_2SO_4$	0.2	
(g) Glucose	1	0.23
$(NH_4)_2SO_4$	0.4	

When L-glutamate or casein hydrolyzate was used as nitrogen and carbon source, the enzyme activity in the cell free extracts was very low. The repression was reversed by the addition of glucose or glycerol. These results are in agreement with the findings of Varricchio [21] and confirm the biosynthetic role of this enzyme. The NH_4^+ concentration controls the glutamate dehydrogenase synthesis to some extent, as observed by Muller and Stadtman [22], but in our experiments levels of NH_4^+ both lower or higher than 0.2% result in decreased enzyme production (see Table I). On the other hand, the glucose concentration does not affect the enzyme production but, as expected the yield of cells.

The specific activity of glutamate dehydrogenase does not vary appreciably either during the log phase or when the culture reaches or is at the stationary phase for some time.

Purification of the enzyme. All steps of purification were done at 4°C unless otherwise stated.

Step 1. $(NH_4)_2SO_4$ fractionation and heat treatment. The cell free extract from 200 g of cell paste (350 ml) was added with stirring of 50 ml of a 5% solution of streptomycin sulfate. After 30 min stirring, the mixture was centrifuged. The supernatant was subjected to $(NH_4)_2SO_4$ fractionation and the precipitate obtained at 35—47% saturation at 4°C, containing the enzyme activity was separated, dissolved in 20 ml of 0.2 M potassium phosphate buffer, 10^{-3} M EDTA and 10^{-3} M β-mercaptoethanol (Buffer A). The solution was heated at 60°C for 10 min with constant stirring under a N_2 stream. The precipitate was removed by centrifugation, washed twice with 10 ml of Buffer A and the pooled supernatants dialyzed overnight against Buffer A at a phosphate concentration of 0.05 M.

Step 2. DEAE-cellulose chromatography. The protein solution from Step 1 was adsorbed on a column (2.5 cm × 25 cm) of DEAE-cellulose (DE-32) equilibrated with 0.05 M phosphate Buffer A. Elution was performed at a flow rate of 30 ml/h with 50 ml of equilibrating buffer followed by a linear gradient of 600 ml of 0.05 M phosphate Buffer A and 600 ml of the same buffer containing 0.8 M NaCl. The effluent was analyzed for enzyme activity and for protein concentration at 280 nm. The glutamate dehydrogenase activity is eluted at about 0.3 M NaCl concentration in approximately 80 ml. The fractions containing the enzyme were pooled, and precipitated by dialysis against saturated $(NH_4)_2SO_4$ solution. The precipitate was recovered by centrifugation, redissolved in 0.005 M phosphate Buffer A and dialyzed against the same buffer.

Step 3. Hydroxyapatite column. The solution from Step 2 was applied to a column (1.5 cm × 10 cm) of hydroxyapatite. The column was eluted with a gradient of 150 ml of 0.005 M phosphate Buffer A and 150 ml of 0.2 M phosphate Buffer A. The fractions containing the enzyme activity were pooled and then precipitated by dialysis against saturated $(NH_4)_2SO_4$ solution.

Step 4. Bio-Gel A-0.5 m. The precipitate from Step 3 was dissolved in 1 ml of 0.05 M phosphate Buffer A and applied to a Bio-Gel A-0.5 m column (2.5 cm × 100 cm) eluted with the same buffer. The major peak contained homogeneous enzyme that was recovered by dialysis against saturated $(NH_4)_2SO_4$ solution.

In Table II a summary of the purification procedure is reported. The enzyme was purified about 1000-fold with an overall yield of 30%.

Alternative procedure. The pooled fractions from DEAE-cellulose could be alternatively dialyzed against 0.1 M phosphate buffer and applied to a column of DEAE-Sephadex (1.5 cm × 20 cm).

The column was eluted with a linear gradient of 200 ml of 0.1 M phosphate Buffer A and 200 ml of the same buffer plus 0.5 M NaCl at pH 7.2.

This column purifies from many foreign proteins, thus assuring better success in the hydroxyapatite fractionation of Step 3, a crucial point in the purification.

Criteria of purity. The enzyme migrated in analytical disc gels as a single

TABLE II

SUMMARY OF PURIFICATION OF *E. COLI* GLUTAMATE DEHYDROGENASE

Step	Volume (ml)	Total protein (mg)	Total activity (units × 10^{-3})	Specific activity (units/mg)	Yield (%)
1. Cell free extract	350	26000	7.6	0.29	
2. Streptomycin treatment	400	15000	7.6	0.50	100
3. $(NH_4)_2SO_4$ supernatant (0.35 saturation)	440	11000	6.4	0.58	84
4. $(NH_4)_2SO_4$ precipitate (0.35—0.47 saturation)	30	3000	6.0	2.0	76
5. Heat treatment	60	900	5.5	6.1	72
6. DEAE-cellulose	110	120	4.0	33	52
7. Hydroxyapatite	120	16	3.1	190	41
8. Bio-Gel A-0.5 m	60	8.8	2.2	250	29

protein band at pH 9.5, 8.5 and 7.5. A single band was also obtained when the enzyme was analyzed by gel electrophoresis with sodium dodecylsulphate by the procedure of Weber and Osborn [16].

A symmetrical peak of elution of both protein and enzyme activity was obtained at the 4th step of purification from the Bio-Gel column with a constant specific activity of 250 units/mg from several preparations.

pH effects. The pH optima determined in Tris or triethanolamine/HCl buffer were found to be near 8 for the reductive amination, whereas it is 9 for the glutamate oxidation.

Substrate specificity. Under standard conditions (0.1 M Tris · HCl buffer, pH 8, 0.1 mM coenzyme and 2.5 mM α-ketoglutarate) the specific activity measured with NADH as coenzyme was 0.5 unit/mg of protein; this value is 0.2% of the specific activity with NADPH as coenzyme. The possibility that this activity was due to NADPH as contaminant of NADH was ruled out by the observation that in the presence of large amount of enzyme a linear oxidation of NADH was observed, accounting for over the 70% of the starting amount.

The isolated enzyme was not contaminated by a NADH-dependent enzyme since (i) the same ratio of NADH/NADPH was obtained also in the earlier steps of the purification, (ii) after acrylamide gel electrophoresis followed by detection of the enzyme by coupling the enzyme-catalyzed reduction of $NADP^+$ or NAD^+ with a redox system [23] only one band coincident with the protein was revealed.

Several neutral, acidic and aromatic amino acids as well as γ-aminobutyric acid were tested for their ability to undergo oxidative deamination. None appeared to undergo appreciable oxidation, their efficiency as substrates being of the order of less than five per thousand compared to glutamate (Table III).

Substrate inhibition and influence of effectors. Initial velocity measurements of the amination reaction were performed versus NH_4^+ and α-ketoglutarate concentration. NH_4Cl up to 400 mM does not inhibit the enzyme, whereas some inhibition was observed with α-ketoglutarate at a concentration higher than 2.5 mM.

The influence of the adenine nucleotides ATP and AMP in the reductive amination reaction was tested in the range of $0.5 \cdot 10^{-3}$—$5 \cdot 10^{-3}$ M. ATP at low concentration was proved to enhance the enzyme activity, thus a 50% increase was observed at $2 \cdot 10^{-3}$ M whereas at higher concentration the effect is less pronounced. On the other hand, AMP at concentration up to 10^{-3} M was mildly activatory while at higher concentration inhibition was observed. This figure of concentration dependence behaviour towards adenine nucleotides is similar to that recently reported for glutamate dehydrogenase isolated from *Salmonella thyphimurium* [14].

Glutamate up to 100 mM or $NADP^+$ up to 2 mM do not show inhibitory effects in the oxidative deamination.

Stability of the enzyme. The enzyme is stable at 4 °C in 0.1 M phosphate Buffer A for several days without appreciable loss of activity. As a suspension in 50% $(NH_4)_2SO_4$ it is stable for some days.

The effect of temperature on the enzyme was studied by heating the enzyme samples (0.1 mg/ml in 0.1 M phosphate buffer, pH 7.2, containing 10^{-3} M EDTA and dithiothreitol) for 5 min at different temperatures ranging

from 35 to 70°C and measuring the residual activity. The temperature of half denaturation is about 65°C. α-Ketoglutarate or $NADP^+$ do not have significative protective effect on the heat denaturation of the enzyme.

The enzyme exhibits a remarkable stability towards protein denaturants, having a denaturation half time at 23°C in Buffer A containing 8 M urea of 10 min, in 6 M urea of about 1 h and being unaffected by 4 M urea. It is instantaneously inactivated by 0.7% sodium dodecylsulphate, whereas its denaturation half time in 0.3% is 3 min.

Effect of temperature on the reaction rate. The effect of the temperature on the rate of the glutamate oxidation is shown in Fig. 1A. The assay mixture in the cuvette was previously equilibrated at the desired temperature and the reaction was initiated by the addition of the enzyme. The activity of the enzyme increases throughout the temperature range of 28—48°C. Fig. 1B shows the Arrhenius plot of the data obtained; from the slope an apparent activation energy of 14 300 cal/mol was calculated. This figure is about the same of that reported for the bovine enzyme (14 000 cal/mol) [24] whereas the pig heart enzymes exhibits lower activation energy (8400 cal/mol) [25].

Molecular weight. The molecular weight of the native form of the enzyme was evaluated from its elution volume in a column of Bio-Gel A-0.5 m (2.5 cm × 100 cm) calibrated with standard proteins (Fig. 2A). A molecular weight of 245 000 ± 5 000 was calculated.

Fig. 2B shows a typical pattern of sedimentation obtained by the sucrose density gradient centrifugation method of Martin and Ames [17], using catalase (molecular weight 232 400) [26] as known reference protein. By this method a value of 250 000 ± 4 000 daltons was obtained.

The molecular weight of the subunit was evaluated by the method of Weber and Osborn [16] by sodium dodecylsulphate-gel electrophoresis using marker proteins. From the plot of Fig. 3 an approximate molecular weight of 46 000 for the glutamate dehydrogenase subunit was obtained.

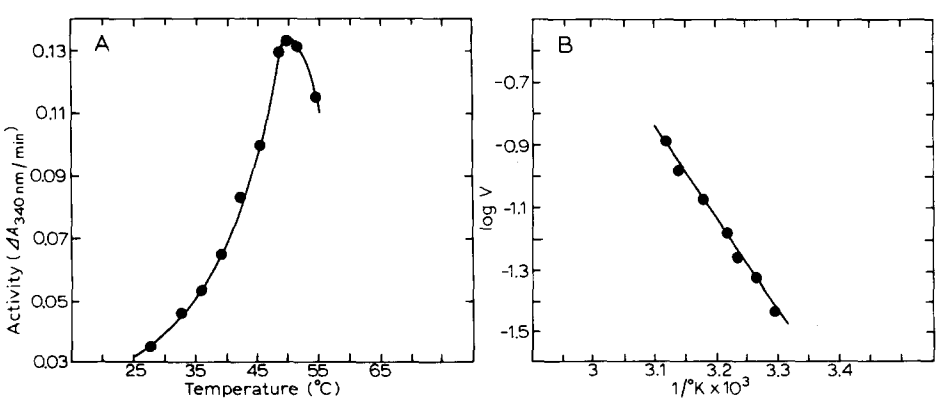

Fig. 1. (A) The effect of temperature on the enzymatic reaction of glutamate dehydrogenase. The assays were performed in 0.1 M Tris · HCl buffer, pH 8.0, with 20 mM glutamate and 0.5 mM $NADP^+$. The reaction was started by the addition of 10 μl of an enzyme solution of 250 units specific activity. (B) Arrhenius plot of the data of Fig. 1A.

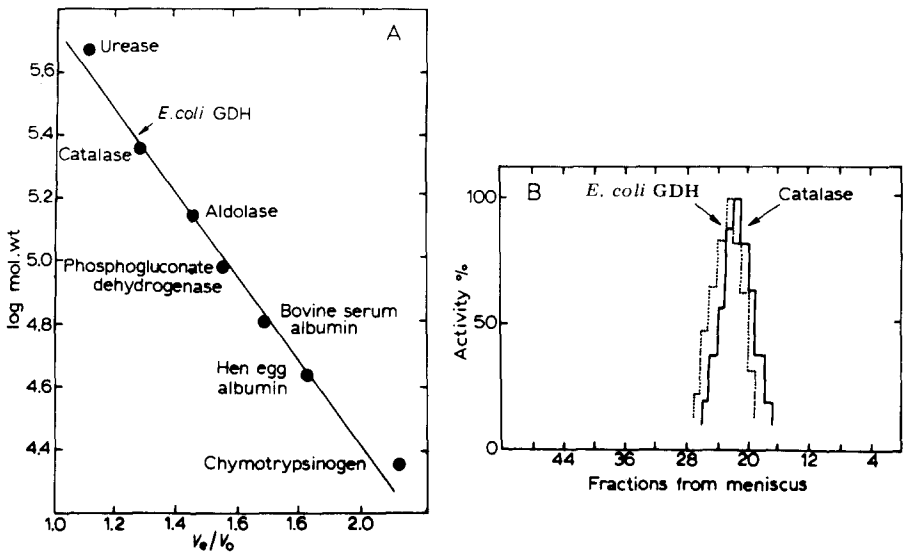

Fig. 2. Molecular weight estimation of native glutamate dehydrogenase (GDH). (A) By gel filtration on a Bio-Gel A-0.5 m column. The molecular weight assumed for the marker proteins were Jack bean urease, 483 000; catalase, 232 000; aldolase, 149 000; *C. utilis* 6-phosphogluconate dehydrogenase, 101 000; bovine serum albumin, 67 000; hen egg albumin, 45 000, and chymotrypsinogen, 25 000. (B) Sucrose gradient centrifugation of glutamate dehydrogenase; catalase, molecular weight 232 000, was used as internal standard. The ordinates represent enzyme activities (in arbitrary units) of the fractions collected from the bottom of the 5—20% sucrose gradient. Meniscus was at tube 50.

Amino acid composition. The theoretical amino acid composition calculated for a subunit molecular weight of 46 000 is reported in Table III together with the composition of the related $NADP^+$-dependent glutamate dehydrogenase from *Neurospora* [8], the beef liver [1] and the chicken [2] liver enzymes. Linear extrapolation to zero time was used to obtain values for serine, threonine and tyrosine. The values obtained on the 72-h sample were

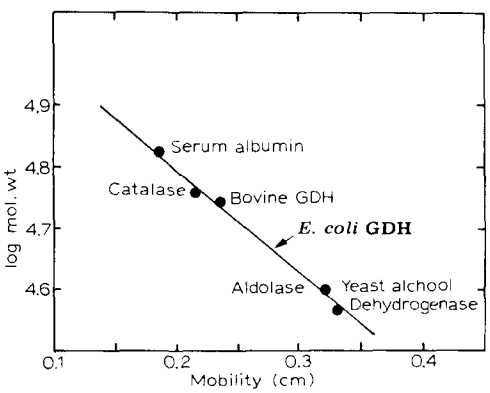

Fig. 3. Determination of the subunit molecular weight of glutamate dehydrogenase (GDH) by sodium dodecylsulphate disc-gel electrophoresis (for reference proteins were used: serum albumin, 67 000; catalase, 57 500; bovine glutamate dehydrogenase, 56 000; aldolase, 42 000; yeast alcohol dehydrogenase, 37 000.

TABLE III

RELATIVE RATE OF OXIDATIVE DEAMINATION OF AMINO ACIDS AND OF REDUCTIVE AMINATION OF α-KETOACIDS BY *E. COLI* GLUTAMATE DEHYDROGENASE

Substrate	Relative rate	
	$NADP^+$ reduction*	NADPH oxidation**
L-Glutamate	1000	
L-Valine	3.0	
L-Aspartic acid	1.5	
L-Alanine	0.5	
L-Threonine	0.5	
L-Tyrosine	0.5	
γ-Aminobutyric acid	0.1	
α-Ketoglutaric acid		1000
Pyruvic acid		5.0

* All the amino acids were tested at a concentration of 5—100 mM in 0.1 M Tris–HCl buffer, pH 9, containing 0.5 mM $NADP^+$. When glutamate was used as substrate less than 0.5 μg of enzyme was used, with the other substrates up to 30 μg of enzyme were necessary.

** The reductive amination was performed in 0.1 M Tris·HCl buffer, pH 8, 0.1 M NH_4Cl, 0.1 mM NADPH, 2.5 mM α-ketoglutarate and 5—100 mM pyruvic acid. With α-ketoglutarate as substrate 0.1 μg of enzyme was employed whereas with pyruvic acid up to 50 μg of enzyme was used.

TABLE IV

COMPARISON OF AMINO ACIDS COMPOSITION OF *E. COLI* GLUTAMATE DEHYDROGENASE WITH THE BOVINE, CHICKEN AND *NEUROSPORA* $NADP^+$ DEPENDENT ENZYMES

	Bovine*	Chicken**	*Neurospora***	*E. coli*†
Aspartic acid	50	48	41	41
Threonine	28	27	14	22
Serine	30	28	29	29
Glutamic acid	45	48	55	54
Proline	21	20	15	16
Glycine	47	48	56	51
Alanine	37	40	54	45
Cysteine	6	7	6	6
Valine	34	33	35	34
Methionine	13	15	9	10
Isoleucine	37	35	17	15
Leucine	31	32	40	31
Tyrosine	18	17	15	12
Phenylalanine	23	23	16	15
Tryptophan	3	3	7	4
Lysine	33	35	30	22
Histidine	14	16	9	9
Arginine	30	28	16	18

* From K. Moon and E. L. Smith [1].

** From K. Moon et al. [2].

*** From K. Blumenthal et al. [8].

† Calculated for an assumed molecular weight of 46 000.

used for valine and isoleucine. Tryptophan was estimated spectrophotometrically according to the equation of Goodwin and Morton [27] as modified by Beaven and Holliday [28]. The cysteine value was the mean figure obtained from the performic acid-treated enzyme and the colorometric titration with 5,5′-dithiobis-(2-nitrobenzoic acid) in 8 M urea.

Behaviour towards thiol group reagents. The enzyme activity was proved to be unaffected by *p*-hydroxy mercuribenzoate (10^{-3} M), iodoacetamide ($4 \cdot 10^{-3}$ M), 5,5′-dithiobis-(2-nitrobenzoic acid) ($2 \cdot 10^{-3}$), and *N*-ethylmaleimide (10^{-4} M) in 0.1 ionic strength phosphate buffer and pH 8.3.

The accessibility of the SH groups in the native enzyme was evaluated by colorimatric titration with 5,5′-dithiobis-(2-nitrobenzoic acid) in 0.1 M Tris · HCl buffer, pH 8.2 [30]; a value of 0.7 residue/subunit molecule was obtained.

The reaction with *N*-ethylmaleimide was studied also with a 400:1 molar ratio of reagent to enzyme subunit at pH 7.2; a very slow reaction takes place with 50% inactivation after 2 h of incubation.

Discussion

This investigation has been carried out in order to explore the possibility of induction and purification of glutamate dehydrogenase from *E. coli* in sufficient amount for chemical and structural studies, and as preliminary characterization of the enzyme.

When the microorganism is grown in a minimal medium with glucose as the sole carbon source and a proper level of ammonia, this enzyme represents about 0.1% of the soluble proteins. The purification procedure reported herein permits the isolation of the enzyme with an overall yield of 30%.

In these conditions of growth the organism produces only one $NADP^+$-dependent glutamate dehydrogenase; a weak NAD^+-dependent activity being related to a slight coenzyme non-specificity rather than to the presence of a second NAD^+-dependent enzyme.

Amino acids other than glutamic acid are oxidized at a rate of less than 0.5% compared to glutamic acid; conversely pyruvic acid is aminated to a negligeable extent compared to α-ketoglutaric acid. In this respect the *E. coli* enzyme exhibits higher specificity than the mammalian enzyme.

The pH vs activity profile shows different rate dependences for the NADH oxidation and the $NADP^+$ reduction. Whereas identical pH optima for both directions were often reported for glutamate dehydrogenase extracted from higher organisms, in the case of the enzyme from microorganisms this is not a general behaviour. In fact, whereas identical pH optima for both reactions are observed in the $NADP^+$ enzyme extracted from *Neurospora* [31], different rate pH dependences were observed in the NAD^+ enzyme from *Thiobacillus Novellus* [32], *Clostridium* SB_4 [33] as well as in the $NADP^+$-dependent enzyme from *T. novellus* [32].

Preliminary investigations indicate that analogous to the enzyme recently isolated from *S. thyphimurium* [14], the enzyme activity is affected by ATP and AMP. However, modulation of enzyme activity by pyridine nucleotides is not generally observed for glutamate dehydrogenase; for example, only the NAD^+ enzyme from *T. novellus* and not the $NADP^+$ one is so affected [32].

The enzyme is reasonably thermostable and relatively stable to denaturing

agents such as urea and sodium dodecylsulfate, the stability being of the same order of that reported for the enzyme isolated from *S. typhimurium* [13]. From our data, the enzyme appears much more thermostable than reported by Savageau et al. [34].

The molecular weights of the native enzyme and of the inactive subunits approximately 250 000 and 46 000, respectively, indicate similarity between the *E. coli* and the $NADP^+$ specific enzyme from *Neurospora* (288 400 and 48 800 for the intact enzyme and the subunit, respectively) [8]. Similarities are also evident from the amino acid composition of the two enzymes (Table III).

The enzyme is not inactivated by cysteine reagents like the related *Neurospora* $NADP^+$ glutamate dehydrogenase [8], and only one residue appears accessible to 5,5′-dithiobis-(2-nitrobenzoic acid) titration in the native form.

All these data suggest homology between the $NADP^+$ enzyme of bacterial and fungal origin.

In this context it may be of interest to note that the NAD^+ enzyme from *Neurospora* appears instead quite different from the $NADP^+$ one from the same source, on the base of molecular weight (480 000 for the native enzyme and 116 000 for the subunits) sequence data so far obtained and behaviour towards thiol group reagents [10—12].

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