NAD⁺- and NADP⁺-Dependent Glutamate Dehydrogenases in *Nitrobacter agilis*

By SHARAD KUMAR AND D. J. D. NICHOLAS*

Department of Agricultural Biochemistry, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond 5064, Australia

(Received 9 August 1983; revised 31 November 1983)

Two isoenzymes of glutamate dehydrogenase located in the cytoplasmic fraction of *Nitrobacter agilis* were specific for NAD⁺ and NADP⁺, respectively. The NAD⁺-dependent enzyme functioned in both directions, i.e. amination and deamination, whereas the NADP⁺ enzyme was primarily for the amination of 2-oxoglutarate to glutamate. The NADP⁺ enzyme was purified 52-fold (free of the NAD⁺ enzyme) by affinity chromatography on 2',5'-ADP Sepharose 4B, and some of its properties studied. Substrate activation of the amination reaction of the NADP⁺ enzyme was observed with NH₄⁺ and NADPH. A comparison is made of the properties of the purified NADP⁺ enzyme from *Nitrobacter agilis* and *Nitrosomonas europaea*. The possible roles of two isoenzymes of glutamate dehydrogenase in *Nitrobacter agilis* are discussed.

INTRODUCTION

Glutamate dehydrogenases [L-glutamate:NAD(P) oxidoreductase] catalyse the oxidative amination of 2-oxoglutarate to glutamate as well as the reductive deamination of glutamate. Thus the enzyme can function in assimilating ammonia and in the catabolism of glutamate (Smith *et al.*, 1975). Bacterial glutamate dehydrogenases can utilize either NAD⁺ (EC 1.4.1.2: NAD⁺-GDH) or NADP⁺ (EC 1.4.1.4: NADP⁺-GDH) or both (EC 1.4.1.3) (Smith *et al.*, 1975). We have shown that the NO₂⁻-oxidizing chemolithotroph *Nitrobacter agilis* has two distinct pathways for assimilating ammonia, either involving glutamate dehydrogenase (GDH) or, alternatively, glutamine synthetase and glutamate synthase (Kumar & Nicholas, 1982). The GDH route for ammonia assimilation predominates when this bacterium is grown in cultures with NO₂⁻ or NO₂⁻ + NH₄⁺ as a nitrogen source. Under these conditions glutamate synthase has a relatively low activity (Kumar & Nicholas, 1982) and glutamine synthetase is largely adenylylated (i.e. biosynthetically inactive) (Kumar & Nicholas, 1984) thus restricting ammonia assimilation via this route. Unlike the GDH from the ammonia-oxidizing bacterium *Nitrosomonas europaea*, which is specific for NADP⁺ (Hooper *et al.*, 1967), the cell-free extracts of *Nitrobacter agilis* contain NAD⁺- and NADP⁺-dependent GDH activities (Wallace & Nicholas, 1968; Kumar & Nicholas, 1982). It was not clear whether there were two separate enzymes for NAD⁺ and NADP⁺ in *Nitrobacter agilis* or a single enzyme utilizing both nucleotides. We now report that there are two separate GDH enzymes in *Nitrobacter agilis* specific for either NAD⁺ or NADP⁺. The NADP⁺-specific GDH from *Nitrosomonas europaea* (Hooper *et al.*, 1967) is also considered in this comparative study.

METHODS

Bacteria and growth conditions. *Nitrobacter agilis* ATCC 14123 was grown in 40 l batches at 28 °C for 5 d with vigorous aeration in an inorganic medium as described by Aleem (1968) and Wallace & Nicholas (1969). Cells were harvested at 4 °C and 30000 g in a Sorvall RC-2B centrifuge fitted with a continuous-flow rotor, with a flow

Abbreviation: GDH, glutamate dehydrogenase.

rate of 12 l h^{-1} (Kumar & Nicholas, 1981). Cells were washed several times with 50 mM-Tris/HCl buffer (pH 7.5) and finally suspended in 50 mM-Tris/HCl, 1 mM- β -mercaptoethanol (pH 7.5). A culture of *Nitrosomonas europaea* was kindly supplied by Dr Jane Meiklejohn of Rothamsted Experimental Station, Harpenden, UK. Cultures were grown in either 8 or 40 l batches at 28 °C with vigorous aeration for 3 d in a medium described by Bhandari & Nicholas (1979). The pH was maintained at 7.8 throughout growth by titrating the medium with sterile 20% (w/v) K_2CO_3 using an automatic pH-stat unit (Radiometer). Cells were harvested and washed as for *Nitrobacter agilis*.

Preparation of cell extracts. Washed suspensions of *Nitrobacter agilis* and *Nitrosomonas europaea* in 50 mM-Tris/HCl, 1 mM- β -mercaptoethanol (pH 7.5) were disrupted with an ultrasonic probe (20 kHz) with 2 min bursts over a period of 30 min at 4 °C in an ice bath. Sonicated extracts were centrifuged at 30000 g (average) for 20 min at 4 °C in a Beckman L8-70 using a 50 Ti rotor. The supernatant fraction (S_{30}) was used in these studies.

Purification of NADP^+ -linked GDH. A similar procedure was used to purify glutamate dehydrogenase from both *Nitrobacter agilis* and *Nitrosomonas europaea*. These experiments were done in a cold room at 4 °C. The S_{30} fraction was further centrifuged at 110000 g (average) for 1 h at 4 °C and the supernatant (S_{110}) was loaded onto a 2',5'-ADP Sepharose 4B column ($0.8 \times 11\text{ cm}$) pre-equilibrated with buffer (50 mM-Tris/HCl, 1 mM- β -mercaptoethanol, pH 7.5). The column was then washed with buffer (flow rate 40 ml h^{-1}) until the absorbance (A_{280}) of the effluent was close to zero. The enzyme was then eluted with 2 mM-NADPH in the buffer and the fractions containing enzyme activity were pooled and dialysed overnight against fresh buffer. The enzyme was stable for at least 2 weeks when stored at -20 °C in the Tris/ β -mercaptoethanol buffer.

GDH assay. Activity of GDH was determined as described by Hooper *et al.* (1967) either from the rate of oxidation of NAD(P)H (amination reaction) or that of NAD(P) reduction (deamination reaction) at 340 nm at 30 °C. For the amination reaction the assay mixture in a total volume of 3 ml contained (mM): 2-oxoglutarate, 20; NH_4Cl , 240; NAD(P)H, 0.33; Tris/HCl buffer (pH 7.8–8.0), 50; and an appropriate quantity of the enzyme preparation. For the deamination reaction, the assay mixture in a final volume of 3 ml contained (mM): sodium glutamate, 17; NAD(P), 0.33; Tris/HCl buffer (pH 9.0), 50; and an appropriate quantity of the enzyme preparation. Both reactions were carried out at 30 °C in 1 cm cuvettes. The amination and deamination reactions were started by adding 2-oxoglutarate and glutamate respectively. The reaction rates, which were linear over a 10 min period, were corrected for endogenous oxidation/reduction of NAD(P)H/NAD(P).

Polyacrylamide gel electrophoresis. Discontinuous, non-denaturing PAGE was carried out in 5% (w/v) polyacrylamide tube gels (Davis, 1964). The stacking gel was 3% (w/v) polyacrylamide in 125 mM-Tris/HCl buffer (pH 6.8) and the running gel 5% (w/v) polyacrylamide in 375 mM-Tris/HCl buffer (pH 8.8). Electrophoresis was carried out at 2 mA per gel, constant current in 12.5 mM-Tris, 96 mM-glycine (pH 8.4) as electrode buffer. Specific staining for glutamate dehydrogenase in gels was done by the deamination reaction according to Tally *et al.* (1972). After electrophoresis the gels were incubated for 20 to 30 min in the dark at 37 °C in a reaction mixture containing: 40 ml 0.1 M-Tris/HCl buffer (pH 8.25); 3 ml 1 M-sodium glutamate; 1 ml 6.5 mM-phenazine methosulphate; 2 ml 5.7 mM-nitro blue tetrazolium and 1.3 ml 22 mM-NAD⁺ or -NADP⁺ or both. After the bands had appeared, the gels were rinsed in distilled water and stored in 7% (v/v) acetic acid until photographed.

Protein and ammonia determination. Protein was determined by the method of Bradford (1976) using bovine serum albumin fraction V, 99% pure (Sigma), as a standard. Intracellular ammonia was determined in S_{110} fractions by a modified Nessler's method (Ballantine, 1957) with NH_4Cl as a standard.

Materials. NAD⁺, NADH, NADP⁺, NADPH, ATP, Tris, sodium glutamate, 2-oxoglutaric acid and all carboxylic acids were from Sigma. All other chemicals used in the study were of the highest purity grade available. 2',5'-ADP Sepharose 4B was purchased from Pharmacia. Double glass-distilled water was used throughout.

RESULTS

Evidence for two isoenzymes of GDH in Nitrobacter agilis

Crude cell extracts of *Nitrobacter agilis* contain GDH which utilizes either NADH or NADPH for its amination reaction (Wallace & Nicholas, 1968; Kumar & Nicholas, 1982). To check whether the two activities are associated with two distinct proteins, electrophoresis of the crude extract (S_{30}) was carried out in 5% (w/v) polyacrylamide gels as described in Methods. The gels were stained for GDH activity using either NAD⁺ or NADP⁺ or both (Fig. 1). The activity bands stained in gels A (NAD⁺) and B (NADP⁺) had different electrophoretic mobilities and those in gel C (NAD⁺ + NADP⁺) also had two distinct bands. It is known that 2',5'-ADP Sepharose 4B binds enzymes that require NADP⁺. When crude extracts of *Nitrobacter agilis* were loaded onto a 2',5'-ADP Sepharose 4B column and washed with buffer as described in Methods, the effluent contained NAD⁺-linked GDH only (gel D). When the affinity column was eluted with 2 mM-NADPH, the eluate did not contain any NAD⁺-associated GDH (gel E) but NADP⁺ activity was detected (gels F and G). There were also two

Fig. 1. Detection of NAD^+ - and NADP^+ -dependent GDH activities from *Nitrobacter agilis*. To separate the two enzyme activities, the S_{30} fraction was loaded onto a 2',5'-ADP Sepharose 4B column (0.8×11 cm) and washed with 50 mM-Tris/HCl (pH 7.5), then eluted with 2 mM-NADPH in the buffer. Samples of S_{30} 2',5'-ADP Sepharose 4B buffer washings and NADPH-eluted proteins were dialysed overnight at 4°C against the same buffer and then loaded onto 5% (w/v) polyacrylamide tubes. Electrophoresis and GDH-specific staining were carried out as described in Methods. Gels A to D were loaded with about 250 μg protein and gels E to G, with about 5 μg protein. Gel A, (S_{30} + NAD^+); gel B, (S_{30} + NADP^+); gel C, (S_{30} + NAD^+ + NADP^+); gel D, (2',5'-ADP Sepharose 4B buffer washings + NAD^+ + NADP^+); gel E, (2',5'-ADP Sepharose 4B, NADPH eluate + NAD^+); gel F, (2',5'-ADP Sepharose 4B, NADPH eluate + NADP^+); gel G, (2',5'-ADP Sepharose 4B eluate + NAD^+ + NADP^+).

minor bands in gels F and G which may have been either aggregates or active dissociated subunits of NADP^+ -dependent GDH (see Smith *et al.*, 1975). Only the NADP^+ -specific enzyme was detected in crude extracts (S_{30}) of *Nitrosomonas europaea* analysed in polyacrylamide gels. The results indicate that *Nitrobacter agilis* has two distinct isoenzymes of GDH, one dependent on NAD^+ and the other on NADP^+ , while in *Nitrosomonas europaea* one single NADP^+ -dependent GDH is present.

Purification of NADP^+ -dependent GDH

The NADP^+ -associated GDH from *Nitrobacter agilis* was partially purified by affinity chromatography (Table 1a). The purified enzyme for *Nitrosomonas europaea*, reported to be dependent on NADP^+ only (Hooper *et al.*, 1967), was also prepared (Table 1b). The crude extracts (S_{30}) of *Nitrobacter agilis* contained both NAD^+ - and NADP^+ -dependent activities (Table 1a) while those of *Nitrosomonas europaea* had NADP^+ -linked GDH with detectable but slight NAD^+ -dependent activity (Table 1b). In S_{110} fractions the specific activity of the NADP^+ -requiring GDH of *Nitrosomonas europaea* was about 13-fold greater than that of the NADP^+ -dependent enzyme from *Nitrobacter agilis*. The NADP^+ enzymes from *Nitrobacter agilis* and *Nitrosomonas europaea* were purified 52-fold and 142-fold by affinity chromatography on 2',5'-ADP Sepharose 4B. Both purified preparations were free of detectable NAD^+ -dependent GDH activity.

Attempts to purify NAD^+ -linked GDH of *Nitrobacter agilis* by affinity chromatography on Blue Sepharose CL-6B were unsuccessful. Low activity of the NAD^+ enzyme as well as minimal cell yields of *Nitrobacter agilis* made it difficult to purify adequate amounts of the enzyme to study its properties and regulation.

Table 1. *Purification of NADP⁺-GDH from Nitrobacter agilis and Nitrosomonas europaea*

Enzyme purification and assay of enzyme activity were as described in Methods. Activity was determined by following the oxidation of either NADH or NADPH at 340 nm at 30 °C. One enzyme unit is defined as nmol NAD(P)H oxidized min⁻¹ and specific activity as units (mg protein)⁻¹ at 30 °C.

Purification step	Total protein (mg)	Total units		Specific activity		Purification (fold)		Percentage recovery	
		NADP ⁺	NAD ⁺	NADP ⁺	NAD ⁺	NADP ⁺	NAD ⁺	NADP ⁺	NAD ⁺
(a) <i>Nitrobacter agilis</i>									
S ₃₀	121	3111	2133	26	18	1	1	100	100
S ₁₁₀	59.5	2609	1649	44	28	1.7	1.5	84	77
Pooled 2',5'-ADP eluate of Sepharose 4B column	1	1353	3	1353	—	52	—	43	0
(b) <i>Nitrosomonas europaea</i>									
S ₃₀	1360	448000	131	330	0.1	1	1	100	100
S ₁₁₀	975	402000	0	412	0	1.3	—	91	0
Pooled 2',5'-ADP eluate of Sepharose 4B column	4.95	231000	0	46670	0	142	—	52	0

Table 2. *Amination and deamination reaction rates in extracts (S₁₁₀) of Nitrobacter agilis*

Amination and deamination reactions were determined in the S₁₁₀ fraction as described in Methods. Specific activity is expressed as nmol NAD(P)H oxidized or NAD(P)⁺ reduced min⁻¹ (mg protein)⁻¹ at 30 °C.

	Reaction	Specific activity
Amination:	NADPH → NADP ⁺	48.6
	NADH → NAD ⁺	25.0
Deamination:	NADP ⁺ → NADPH	≤ 2.0
	NAD ⁺ → NADH	22.5

Amination and deamination reactions in the S₁₁₀ fraction

The NADP⁺-requiring GDH from *Nitrosomonas europaea* functions in either direction, i.e. amination of 2-oxoglutarate to form glutamate and deamination of glutamate to 2-oxoglutarate (Hooper *et al.*, 1967). It is clear from Table 1 that the amination activity of NADP⁺-requiring GDH from *Nitrobacter agilis* was about twice that of NAD⁺-dependent GDH at the pH optimum of 8. On the other hand, the intensity of the enzyme activity bands in Fig. 1 indicates that NAD⁺-associated GDH activity was much higher than that observed for NADP⁺-dependent GDH. Since the gels were stained following deamination reaction, it is clear that the NADP⁺-linked GDH of *Nitrobacter agilis* predominantly operates in the direction of glutamate production. That this is indeed the case is supported by the data in Table 2. In the S₁₁₀ fraction of *Nitrobacter agilis* the amination and deamination activities of the NAD⁺-requiring enzyme were approximately equal; however, the deamination activity of the NADP⁺-associated GDH was only about 4% of the amination activity (Table 2).

Properties of NADP⁺-dependent GDH from Nitrobacter agilis

Since the NADP⁺-linked GDH from *Nitrosomonas europaea* has been studied in detail (Hooper *et al.*, 1967), this section will deal with some properties of partially purified (52-fold) NADP⁺-associated GDH from *Nitrobacter agilis*. The amination reaction had a sharp pH optimum at 8, whereas the deamination reaction was less specific, but with an optimum at 9. The rate of NADPH oxidation (amination reaction) was maximal with 10 mM-2-oxoglutarate. A double reciprocal plot of the rate of NADPH oxidation against substrate concentration gave an apparent *K_m* value of 3.57 for 2-oxoglutarate. The apparent *K_m* for 2-oxoglutarate did not

Fig. 2

Fig. 3

Fig. 2. Effects of NH_4^+ on the amination reaction of NADP^+ -GDH from *Nitrobacter agilis*. The amination reaction of partially purified NADP^+ -GDH (Table 1) was determined as described in Methods except that NH_4Cl concentration was varied over the range 0–200 mM. The data are plotted as the reciprocal of reaction velocity [$1/v$, (nmol NADPH oxidized min^{-1}) $^{-1}$] against the reciprocal of NH_4Cl concentration ($1/[S]$, mM^{-1}).

Fig. 3. Effects of NADPH on the amination reaction of NADP^+ -GDH from *Nitrobacter agilis*. The amination reaction of partially purified NADP^+ -GDH (Table 1) was determined as described in Methods except that NADPH concentration was varied over the range of 0–200 μM . The data are plotted as the reciprocal of reaction velocity [$1/v$, (nmol NADPH oxidized min^{-1}) $^{-1}$] against the reciprocal of NADPH concentration ($1/[S]$, mM^{-1}).

change with increasing concentrations of NH_4Cl . The NADPH oxidation rate increased with increasing concentrations of NH_4Cl (0–100 mM); it increased rapidly up to 20 mM- NH_4Cl and slowly thereafter so that the system appeared to be biphasic. Double reciprocal plots of the data (Fig. 2) produced two distinct apparent K_m values of 33 mM above 20 mM- NH_4^+ and 6.3 mM below 20 mM- NH_4^+ . This change in apparent K_m for NH_4Cl was not a salt effect since NaCl did not affect the value. A similar type of substrate stimulation of enzyme activity (Cleland, 1970) was observed with NADPH (Fig. 3). Two apparent K_m values of approximately 100 μM and 7 μM were recorded for NADPH concentrations above 50 μM and below 50 μM , respectively.

The amination reaction of NADP^+ -associated GDH from *Nitrosomonas europaea* is known to be inhibited by carboxylic acids and nicotinamide adenine nucleotides (Hooper *et al.*, 1967). The effects of some of these compounds on the amination reaction of NADP^+ -linked GDH of *Nitrobacter agilis* were studied as described in Methods. The organic acids used were first adjusted to pH 8.0 before addition to the standard reaction mixture. Thus fumaric acid inhibited the amination reaction appreciably (45% at 20 mM), while malic and pyruvic acids (20 mM) and 2-oxoglutarate (100 mM) were without effect. *cis*-Oxaloacetic acid slightly stimulated the reaction (18% at 5 mM), but this effect was not enhanced by increasing the concentration of oxaloacetic acid to 20 mM. None of the nucleotides (NAD^+ , NADP^+ , NADH , NADPH , and ATP) affected enzyme activity.

DISCUSSION

The results reported in this paper clearly show that the NAD^+ and NADP^+ -dependent GDH activities in *Nitrobacter agilis* are associated with two distinct protein fractions which differ in their electrophoretic mobilities. These data are similar to those reported for *Thiobacillus novellus* (Le'John *et al.*, 1968), *Hydrogenomonas* H16 (Kramer, 1970) and *Micrococcus aerogenes* (Kew & Woolfolk, 1970; Johnson & Westlake, 1972) where NAD^+ - and NADP^+ -associated GDH are also associated with separate proteins.

The NAD^+ -dependent GDH of *Nitrobacter agilis* appears to be capable of functioning in either direction, i.e. amination of 2-oxoglutarate to glutamate and deamination of glutamate to 2-oxoglutarate, whereas the NADP^+ -specific GDH functions mainly in the direction of

glutamate (ammonia assimilation). It is of interest that the amination reaction of NADP^+ -linked GDH from *Nitrobacter agilis* was stimulated by NH_4^+ and NADPH (substrate stimulation: Cleland, 1970) so that two distinct apparent K_m values were obtained for either substrate.

Studies of the kinetics of purified bovine GDH have been complicated by the polymerization-depolymerization phenomena involving the disaggregation or unfolding of the crystalline enzyme in dilute solutions (Smith *et al.*, 1975). This may also be an explanation of the appearance of two major electrophoretic bands and one minor one associated with NADP^+ -dependent GDH activities in purified preparations from *Nitrobacter agilis* compared with a single band in crude extracts (Fig. 1). The biphasic kinetics of the purified NADP^+ -linked GDH (Fig. 2) together with the substrate stimulation effects (Figs 2 and 3) are more likely to be associated with changes in aggregation of the same enzyme (Smith *et al.*, 1975) modified in some way during purification rather than with the presence of two distinct NADP^+ -dependent enzymes.

Nitrobacter agilis invariably contained high concentrations of NH_4^+ (approx. 30 mM) and this may explain why (a) glutamine synthetase is highly adenylylated (Kumar & Nicholas, 1984) and (b) NADP^+ -dependent GDH (K_m NH_4^+ , 6.3 to 33 mM) operates in the direction of glutamate production even when no exogenous NH_4^+ is available to the bacterium. Although the NADP^+ -associated GDH of *Nitrosomonas europaea* can function in either direction, i.e. amination and deamination, it is likely to be unidirectional since the deamination reaction was almost completely inhibited (80 to 90%) by 10 mM- NH_4Cl (Hooper *et al.*, 1967). No such regulation of NADP^+ -linked GDH from *Nitrobacter agilis* seems necessary because the deamination reaction of the enzyme is only about 4% of the amination reaction. Unlike the NADP^+ -dependent GDH of *Nitrosomonas europaea* (Hooper *et al.*, 1967) the amination reaction of NADP^+ -linked GDH from *Nitrobacter agilis* was unaffected by high concentrations (100 mM) of 2-oxoglutarate and nucleotides (NADPH, NADP^+); in fact, NADPH stimulated the activity, as shown in Fig. 3.

The high intracellular NH_4^+ concentrations observed under laboratory growth conditions support the view that GDH is the main pathway for ammonia assimilation in *Nitrobacter agilis*. Accumulation of NH_4^+ via the assimilatory NO_2^- reductase (Wallace & Nicholas, 1968) appears to be under minimal regulatory control. Under natural growth conditions in the soil the availability of N source (NO_2^- and/or NH_4^+) for *Nitrobacter agilis* can vary greatly. When NO_2^- is rate limiting, the nitrite oxidase for generating ATP would predominate, resulting in a minimal reduction of NO_2^- to NH_4^+ . Under these conditions glutamine synthetase would be unadenylylated (active form) and would readily assimilate the small amounts of NH_4^+ available (Kumar & Nicholas, 1984). On the other hand, under conditions where an ample supply of NO_2^- is available (e.g. culture growth conditions), NO_2^- reductase would probably produce sufficient amounts of NH_4^+ that would result in adenylylation of glutamine synthetase. The NH_4^+ thus produced would then be predominantly assimilated by the NADP^+ -linked GDH enzyme. The NAD^+ -associated GDH may be important for 2-oxoglutarate production required primarily for transamination reactions.

REFERENCES

- ALEEM, M. I. H. (1968). Mechanism of oxidative phosphorylation in chemoautotroph *Nitrobacter agilis*. *Biochimica et biophysica acta* **162**, 338–347.
- BALLANTINE, R. (1957). Determination of total nitrogen and ammonia. *Methods in Enzymology* **3**, 984–995.
- BHANDARI, B. & NICHOLAS, D. J. D. (1979). Ammonia and O_2 uptake in relation to proton translocation in cells of *Nitrosomonas europaea*. *Archives of Microbiology* **122**, 249–255.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- CLELAND, W. W. (1970). Steady state kinetics. In *The Enzymes*, vol. II, pp. 1–61. Edited by P. D. Boyer. New York: Academic Press.
- DAVIS, B. (1964). Disc electrophoresis II. Method and application to human serum proteins. *Annals of the New York Academy of Sciences* **121**, 404–427.
- HOOVER, A. B., HANSEN, J. & BELL, R. (1967). Characterization of glutamate dehydrogenase from the ammonia oxidising chemoautotroph *Nitrosomonas europaea*. *Journal of Biological Chemistry* **242**, 288–296.
- JOHNSON, W. H. & WESTLAKE, D. W. S. (1972). Purification and characterization of glutamic acid dehydrogenase and α -ketoglutaric acid reductase

- from *Peptococcus aerogenes*. *Canadian Journal of Microbiology* **18**, 881–892.
- KEW, O. M. & WOOLFOLK, C. A. (1970). Preparation of glutamate dehydrogenase from *Micrococcus aerogenes*. *Biochemical and Biophysical Research Communications* **39**, 1126–1133.
- KRAMER, J. (1970). NAD- and NADP-dependent glutamate dehydrogenase in *Hydrogenomonas* H16. *Archiv für Mikrobiologie* **71**, 226–234.
- KUMAR, S. & NICHOLAS, D. J. D. (1981). Oxygen-dependent nitrite uptake and nitrate production by cells, spheroplasts and membrane vesicles of *Nitrobacter agilis*. *FEMS Microbiology Letters* **11**, 201–206.
- KUMAR, S. & NICHOLAS, D. J. D. (1982). Assimilation of inorganic nitrogen compounds by *Nitrobacter agilis*. *Journal of General Microbiology* **128**, 1795–1801.
- KUMAR, S. & NICHOLAS, D. J. D. (1984). Purification, properties and regulation of glutamine synthetase from *Nitrobacter agilis*. *Journal of General Microbiology* **130**, 959–966.
- LE'JOHN, H. B., SUZUKI, I. & WRIGHT, J. A. (1968). Glutamate dehydrogenases of *Thiobacillus novellus*. Kinetic properties and a possible control mechanism. *Journal of Biological Chemistry* **243**, 118–128.
- SMITH, E. L., AUSTIN, B. M., BLUMENTHAL, K. M. & NYC, J. F. (1975). Glutamate dehydrogenases. In *The Enzymes*, vol. XI, pp. 293–367. Edited by P. D. Boyer. New York: Academic Press.
- TALLY, D. J., WHITE, R. H. & SCHMIDT, R. R. (1972). Evidence for NADH and NADPH specific isozymes of glutamate dehydrogenase and the continuous inducibility of the NADPH-specific isozyme throughout the cell cycle of eucaryotic *Chlorella*. *Journal of Biological Chemistry* **247**, 7927–7935.
- WALLACE, W. & NICHOLAS, D. J. D. (1968). Properties of some reductase enzymes in the nitrifying bacteria and their relationship to the oxidase systems. *Biochemical Journal* **109**, 763–773.
- WALLACE, W. & NICHOLAS, D. J. D. (1969). The biochemistry of nitrifying micro-organisms. *Biological Reviews* **44**, 359–391.