

GLUTAMATE DEHYDROGENASES OF UNICELLULAR GREEN ALGAE: EFFECTS ON NITRATE AND AMMONIUM IN VIVO

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SUMMARY

The composition and control of the synthesis of glutamate dehydrogenases by an inorganic nitrogen source have been studied in the following green unicellular algae species: *Chlorella* sp. K., *Chlorella ellipsoidea*, *Chlorella pyrenoidosa* 82, *Ankistrodesmus braunii* and *Scenedesmus obliquus*. *A. braunii* and *S. obliquus* cells were found to contain two glutamate dehydrogenases, one specific to NADP and the other non-specific for the cofactor; both enzymes are essentially constitutive. *Ch. ellipsoidea* and *Ch. pyrenoidosa* 82 cells contain only one glutamate dehydrogenase which is not specific for the cofactor, and its synthesis does not depend on the form of the nitrogen source in the medium. In the cells of the thermophilic strain *Chlorella* sp. K., ammonium induces de novo synthesis of NADP-specific glutamate dehydrogenase, while the other glutamate dehydrogenase, non-specific for the cofactor, is synthesized constitutively.

INTRODUCTION

In recent years, it was frequently reported that a definite molecular form (or isoenzyme) of glutamate dehydrogenase was synthesized de nova under the influence of ammonium in the cells of two thermophilic *Chlorella* strains, as well as in some higher plants [1-6]. Shatilov et al. were the first to show the induction of de novo synthesis of NADP-glutamate dehydrogenase under the influence of ammonium in the thermophilic strain of *Ch. pyrenoidosa* 82T

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[1,2]. Later, similar results were obtained for another thermophilic strain, *Ch. pyrenoidosa* 7-11-05 [3]. Subsequently, induction of de novo synthesis of NAD(P)-glutamate dehydrogenase was demonstrated in the roots of *Oryza sativa* [4], and that of NAD-glutamate dehydrogenase in the duckweed *Lemna gibba* [5] and in the leaves of *Avena sativa* L [6]. Kretovich and coworkers [7-9] were the first to note ammonium-induced intensified synthesis of glutamate dehydrogenase in the individual parts of some higher plants. These data were also confirmed by other authors [10,11].

Ammonium-induced NADP-glutamate dehydrogenase was isolated from *Ch. pyrenoidosa* 82T and characterized [1,12-14]. It proved to be an allosteric "hysteretic" enzyme, which differed clearly in properties from the constitutive NAD(P)-glutamate dehydrogenase [13-15]. The question arises whether the induction of the synthesis of such a form of glutamate dehydrogenase in response to ammonium is a unique property of individual *Chlorella* strains, or whether this phenomenon is characteristic of all unicellular green algae. Moreover, it appeared interesting to study glutamate dehydrogenases, their forms and control of the synthesis in unicellular green algae in general, since these aspects are obscure.

MATERIALS AND METHODS

The following unicellular green algae were used:

Ch. pyrenoidosa Chick 82, Czechoslovakia, Prague, Prat's collection, A-82 = *Ch. vulgaris* Beijer, var. *vulgaris*, strain Pringsheim, Prague, Ac-82); *Chlorella* sp. K., selective strain of K.V. Kosikov, Institute of Genetics, USSR Academy of Sciences = *Ch. vulgaris* Beijer, var. *vulgaris*, strain Kossikow, Prague, 132-2); *Ch. ellipsoidea* Gern., Czechoslovakia, Prague, Prat's collection, A-25; *A. braunii*, Fodder Institute, Moscow; *S. obliquus* (Turp)-Kütz, Czechoslovakia, Prague, Prat's collection, A-125.

All the species mentioned are kept also in Culture Collection of algal strains at Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences.

Chlorella sp.K. is a thermophilic strain; the other cultures are mesophilic

The cultures were grown to a density of 3-4 mg dry biomass per ml under sterile photoautotrophic conditions in Tamiya medium with KNO_3 as the nitrogen source [15], the cultivation was performed at 26-28°C, continuous illumination with luminescent lamps ($30 \cdot 10^3 \text{ erg/cm}^2$) and bubbling with a gas-air mixture containing 1-1.5% CO_2 . The grown cells were collected by centrifuging, and equal amounts of wet biomass were transferred into equal volumes of Tamiya medium containing KNO_3 or $(\text{NH}_4)_2\text{SO}_4$ (conc. 1 g/l) as nitrogen source and subsequently incubated for 5 h under the above conditions. In some cases, prior to introducing the nitrogen source, the cultures were incubated for 18 h in a medium without nitrogen under similar conditions in order to obtain nitrogen-starved cells. After incubation in a medium with the nitrogen source, the cells were separated by centrifugation and washed with phosphate buffer, pH 7.4. The cells were homogenized with quartz beads

(Chance ballotini beads, Czechoslovakia) in a special disintegrator with a cooling system at 14 000 rev./min in 0.07 M phosphate buffer, pH 7.4, containing 5 mM 2-mercaptoethanol, the wet biomass, buffer and bead ratio being 1 : 1 : 1.5 (wt : v : v). The homogenate was then centrifuged for 25 min at $18\,000 \times g$, frozen at -10°C for 12 h, quickly thawed in a water flow at 30°C , and ultracentrifuged for 1 h at $100\,000 \times g$ (over the centre of the tube). The supernatant was used to determine the total glutamate dehydrogenase activity in the amination reaction with NADH and NADPH as cofactors, and specifically to reveal the composition of the glutamate dehydrogenase (isoenzyme spectra) after electrophoresis in polyacrylamide gel [18,19]. Activity was determined spectrophotometrically in 3 ml at 20°C [12]. All the measurements were performed on a recording spectrophotometer (Hitachi EPS-3T) equipped with a recorder (QD-15). Protein was determined according to Lowry et al. [17]. Glutamate dehydrogenase bands in polyacrylamide gel columns after electrophoresis were developed in a reaction mixture (3 ml) containing 3 mM Na L-glutamate, 2.1 mM NAD(P)⁺, 0.1 mM 5-methylphenazine methosulphate and 0.6 mM nitroblue tetrazolium in 0.1 M phosphate buffer, pH 7.4. It should be noted that a small concentration of 5-methylphenazine methosulphate excludes rapid development of non-specific purple-blue colouring of the entire gel column. This is essentially important, since manifestation of low activity entails rather lengthy incubation in the reaction mixture, for instance when developing the activity of constitutive glutamate dehydrogenase with NADP⁺ as cofactor.

RESULTS AND DISCUSSION

Fig. 1 and 2 show data which characterize the changes of the specific glutamate dehydrogenase activities with both cofactors in the amination reaction and the zymograms, which were also developed with both cofactors, in the cell-free extracts from *A. braunii* and *S. obliquus*, depending on the conditions of nitrogen supply. A specific feature of these algae is the presence of two glutamate dehydrogenases, which differ in specificity for the cofactor irrespective of the nitrogen source, i.e. both glutamate dehydrogenases are constitutive enzymes. Judging by the zymograms, one glutamate dehydrogenase in the *A. braunii* and *S. obliquus* cells is strictly specific for NADP, while the other is not specific for the cofactor, being in this respect similar to the constitutive glutamate dehydrogenase isolated previously from the cells of the thermophilic strain *Ch. pyrenoidosa* 82T [20].

It is worthy of mention that, during nitrogen starvation, the *A. braunii* and *S. obliquus* cells were noted to have greatly increased NADP-dependent glutamate dehydrogenase activity (Fig. 1c); during subsequent assimilation of nitrate or ammonium, this activity gradually decreases. Cycloheximide (5 μg ml) partially suppresses the increase of activity in the course of starvation (Fig. 1c').

The cells of *Ch. pyrenoidosa* 82 and *Ch. ellipsoidea* (Figs. 3 and 4) contain only one glutamate dehydrogenase, whose activity does not change with con-

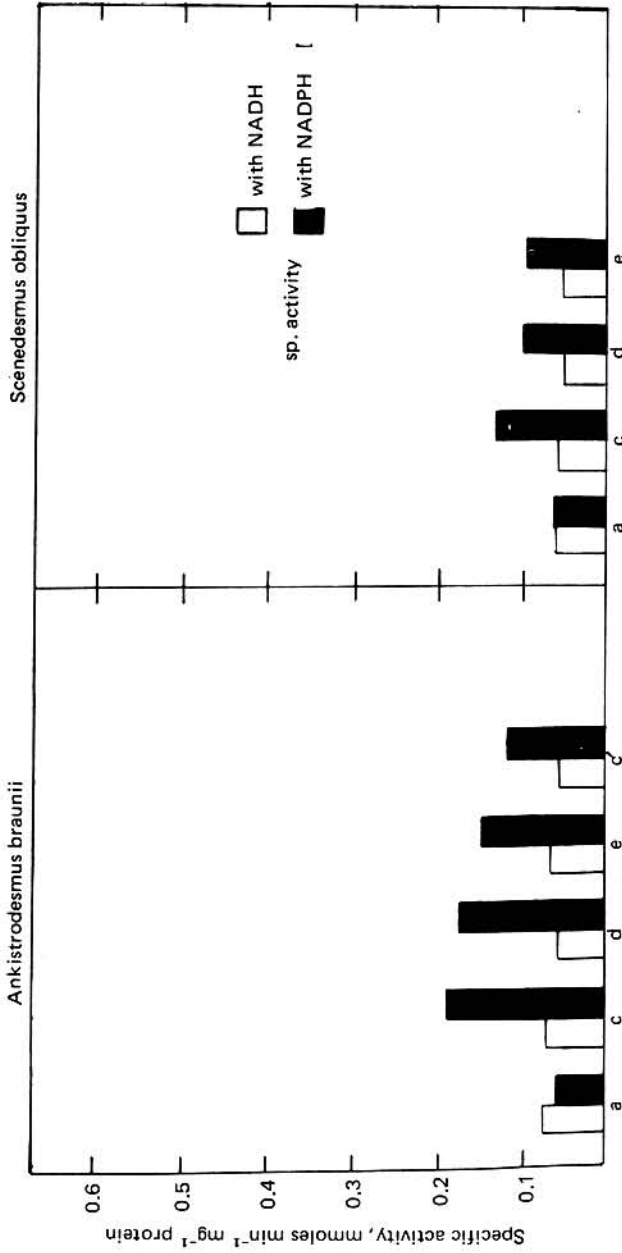


Fig. 1. Dependence of NADH- and NADPH-glutamate dehydrogenase activities in *A. braunii* and *S. obliquus* cells on conditions of nitrogen supply. a, KNO₃; b, (NH₄)₂SO₄ (absent here); c, after nitrogen starvation; d, KNO₃ after starvation; e, (NH₄)₂SO₄ after starvation; c', starvation in presence of cycloheximide. In the figures that follow, a,b,c,d and e denote the same.

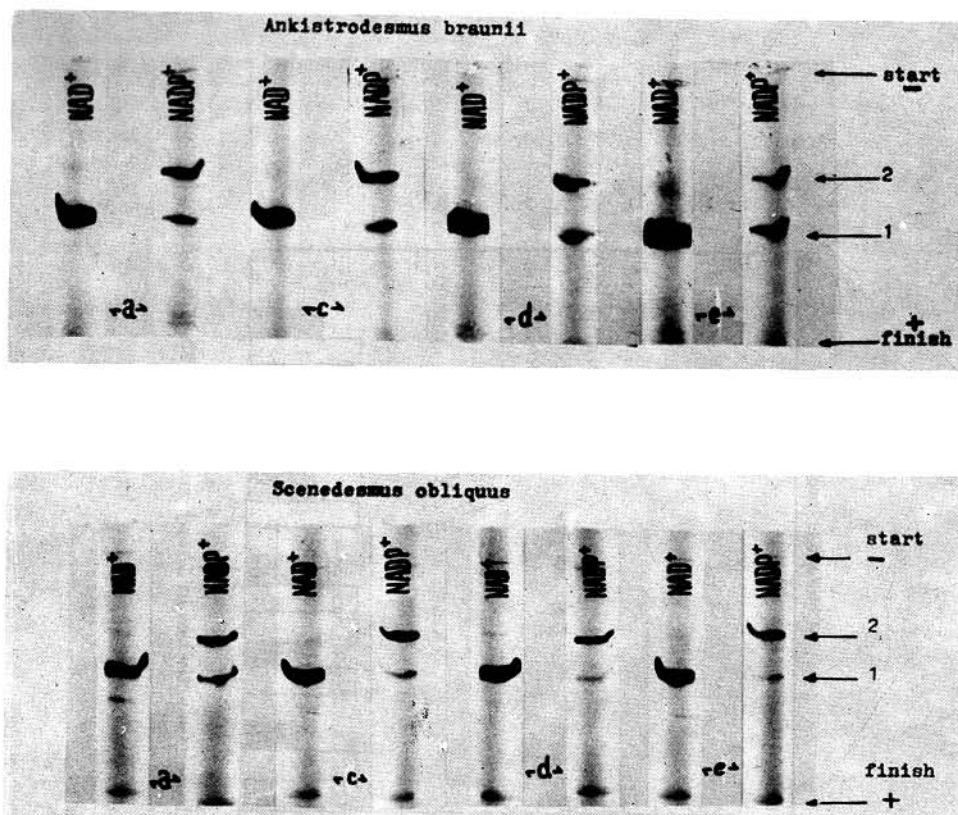


Fig. 2. Zymograms of *A. braunii* and *S. obliquus* glutamate dehydrogenases. 1, NAD(P)-glutamate dehydrogenase; 2, NADP-glutamate dehydrogenase.

ditions of nitrogen supply. As regards the cofactors, this glutamate dehydrogenase is also similar to the constitutive NAD(P)-glutamate dehydrogenase from *Ch. pyrenoidosa* 82T.

Figs. 5 and 6 show data for *Chlorella* sp. K. and also for *Ch. pyrenoidosa* 82T, an already known example of ammonium-induced NADP-glutamate dehydrogenase. This strain (variants d and e) was used in every experiment as a control for the inductive action of ammonium. In nitrate assimilation both normal and nitrogen-deficient cells of *Chlorella* sp. K. only one single glutamate dehydrogenase non-specific for the cofactor is present. Replacement of nitrate by ammonium results in induction of de nova synthesis of another glutamate dehydrogenase specific for NADP. Cycloheximide completely blocks the induction of this glutamate dehydrogenase. Thus, the picture of the influence of nitrate and ammonium on the synthesis of glutamate dehydrogenases in the cells of the thermophilic strain *Chlorella* sp. K. is exactly the same as that in the cells of the two other thermophilic strains *Ch. pyrenoidosa* 82T [1,2] and *Ch. pyrenoidosa* 7-11-01 [3].

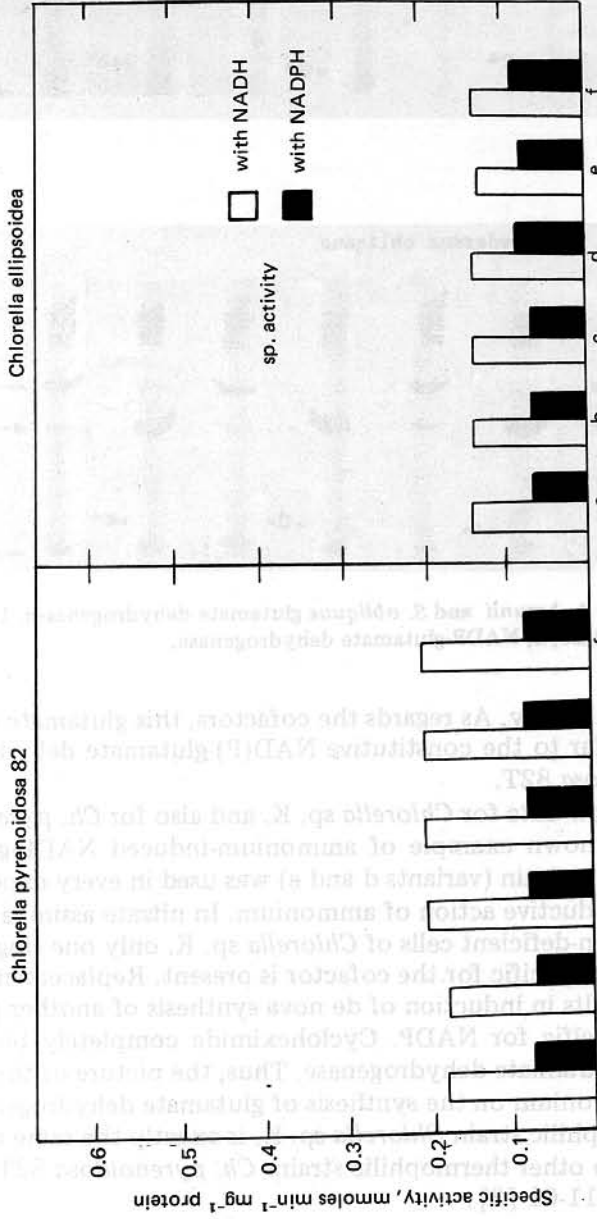


Fig. 3. Dependence of NADH- and NADPH-glutamate dehydrogenase activities in *Ch. pyrenoidosa* 82 and *Ch. ellipsoidea* cells upon conditions of nitrogen supply.

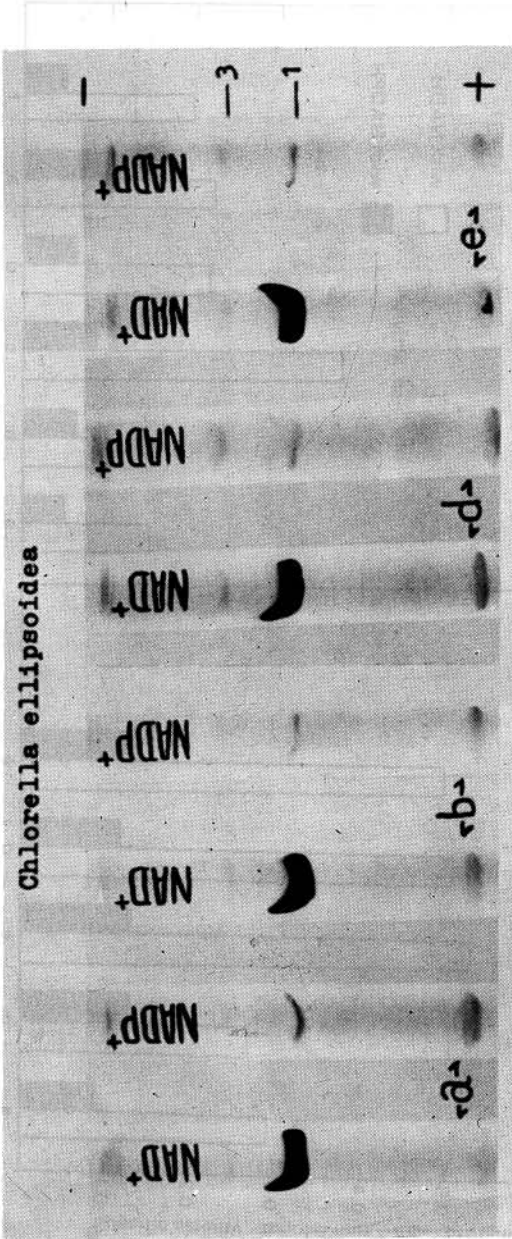


Fig. 4. Zymograms for glutamate dehydrogenase of *Ch. ellipsoidea*. Zymograms for glutamate dehydrogenase of *Ch. pyrenoidosa* are exactly the same and, for that reason, are not cited. 1, constitutive (NAD(P)-glutamate dehydrogenase; 3, see Fig. 6.

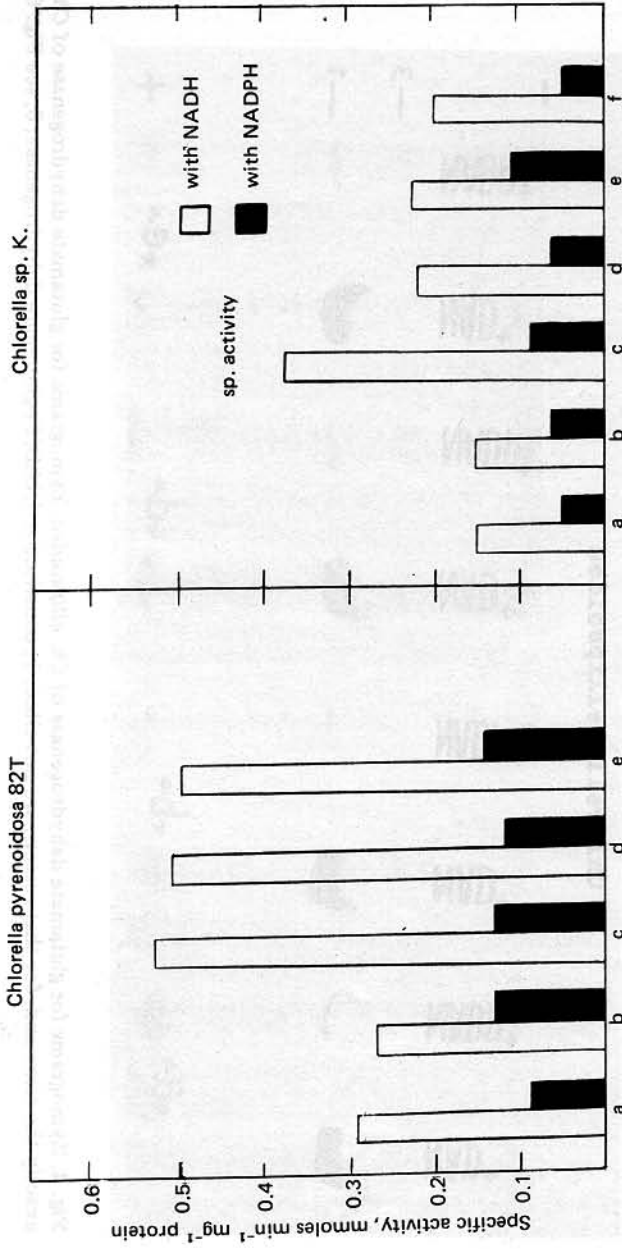


Fig. 5. Dependence of NADH- and NADPH-glutamate dehydrogenase activities in *Ch. pyrenoidosa* 82T and *Chlorella* sp. K. cells upon conditions of nitrogen supply; a,b,c,d,e, see Fig. 1; f, (NH₄)₂SO₄ + cycloheximide after starvation.

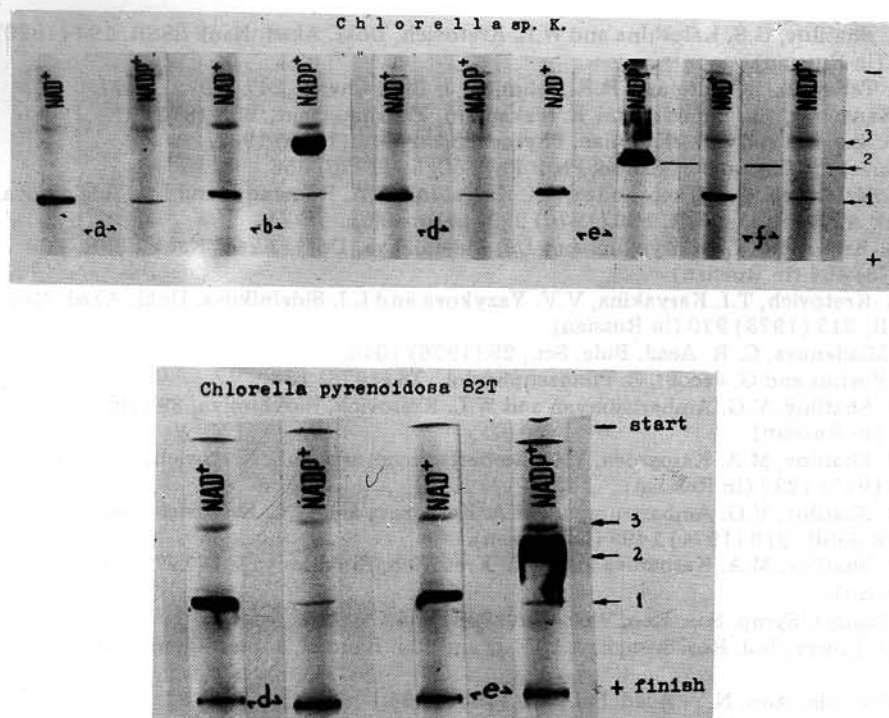


Fig. 6. Zymograms for glutamate dehydrogenases of *Chlorella* sp. K. and *Ch. pyrenoidosa* 82T. 1, constitutive NAD(P)-glutamate dehydrogenase; 2, ammonium-induced NADP-glutamate dehydrogenase; 3, light-brown band not associated with glutamate dehydrogenase activity.

It is not clear whether the induction of de novo synthesis of NADP-glutamate dehydrogenase under the influence of ammonium in the cells of the thermophilic *Chlorella* strains is associated with their thermophilic properties, or with other biochemical features.

The results obtained in the present work show the presence of a constitutive NAD(P)-glutamate dehydrogenase, non-specific for the cofactor, to be a common feature for all the unicellular green algae examined. The other glutamate dehydrogenase, specific for NADP, is altogether absent in some *Chlorella* species (*Ch. pyrenoidosa* 82 and *Ch. ellipsoidea*), and in other species is synthesized in response to ammonium (*Chlorella* sp. K., *Ch. pyrenoidosa* 82T [1,2] and *Ch. pyrenoidosa* 7-11-05 [3]). In *A. braunii* and *S. obliquus*, this glutamate dehydrogenase is constitutive.

REFERENCES

- 1 V.R. Shatilov, V.G. Ambartsumyan and W.L. Kretovich, Dokl. Akad. Nauk SSSR, 207 (1972) 1229-1232 (in Russian).

- 2 V.R. Shatilov, G.S. Kaloshina and W.L. Kretovich, Dokl. Akad. Nauk SSSR, 194 (1970) 964 (in Russian).
- 3 D.J. Talley, L.H. White and R.R. Schmidt, J. Biol. Chem., 247 (1972) 7927.
- 4 T. Kanamori, Sh. Kanishi and E. Takahashi, Physiol. Plant, 26 (1972) 1.
- 5 D.V. Shepard and D.A. Thurman, Phytochemistry, 12 (1973) 1937.
- 6 I. Barásh, H. Mor and T. Sadon, Plant Physiol., 56 (1975) 856.
- 7 W.L. Kretovich, G.S. Tkemaladze, T.I. Karyakina, E.A. Romanova and L.I. Sidelnikova, Dokl. Akad. Nauk SSSR, 190 (1970) 222 (in Russian).
- 8 W.L. Kretovich, T.I. Karyakina and L.I. Sidelnikova, Dokl. Akad. Nauk SSSR, 208 (1973) 464 (in Russian).
- 9 W.L. Kretovich, T.I. Karyakina, V.V. Yazykova and L.I. Sidelnikova, Dokl. Akad. Nauk SSSR, 213 (1973) 970 (in Russian).
- 10 J.I. Mladenova, C. R. Acad. Bulg. Sci., 29 (1976) 1043.
- 11 Ch. Postius and G. Jacobi, Z. Pflanzenphysiol., 78 (1976) 133.
- 12 V.R. Shatilov, V.G. Ambartsumyan and W.L. Kretovich, Biokhimiya, 39 (1974) 571 (in Russian).
- 13 V.R. Shatilov, M.A. Kasparova, V.G. Ambartsumyan and W.L. Kretovich, Biokhimiya, 40 (1975) 1237 (in Russian).
- 14 V.R. Shatilov, V.G. Ambartsumyan, M.A. Kasparova and W.L. Kretovich, Dokl. Akad. Nauk SSSR, 215 (1974) 1497 (in Russian).
- 15 V.R. Shatilov, M.A. Kasparova and W.L. Kretovich, Biokhimiya, 41 (1976) 1636 (in Russian).
- 16 M. Tamiya, Symp. Soc. Exp. Biol., 17 (1963) 188.
- 17 O. H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem., 193 (1951) 265.
- 18 L. Ornstein, Ann. N.Y. Acad. Sci., 121 (1964) 321.
- 19 B.J. Davies, Ann. N.Y. Acad. Sci., 121 (1964) 404.
- 20 V.R. Shatilov, Z.G. Evstigneeva and W.L. Kretovich, Biokhimiya, 34 (1969) 409 (in Russian).