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Glutamate dehydrogenase of a green alga, *Bryopsis maxima*

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Glutamate dehydrogenase (GDH) (EC 1.4.1.3) was obtained from a marine green alga, *Bryopsis maxima* as an ammonium sulfate-salting out preparation, and the MICHAELIS constants for substrates and co-enzyme were determined. Effect of some chemicals on its activity was also investigated, and it was found that the GDH activity was inhibited by PCMB as in higher plants, but was not affected in contrast to the NAD(H)-dependent GDH of some higher plants.

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In contrast to a current view that the assimilation of ammonia in higher plants is brought about by glutamate synthetase (EC 6.3.1.2) (O'NEIL and JOY, 1973) coupled with another Chloroplast-enzyme, glutamate synthase (EC 2.6.1.53) (LEA and MIFLIN, L974: ARIMR and KUMAZAWA, 1977), it has generally been berieved so far that ammonia is incorporated first into keto-acid maninly into α-ketoglutarate, being catalyzed by glutamate dehydrogenase (GDH) (EC 1.4.1.3), to form glutamate. The GDH, therefore, has received many investigations in higher plants (e.g. BULEN, 1956; JOY, 1969, 1971; PAHLICH and JOY, 1971; LEA and THURMAN, 1972; MIFLIN, 1974; BARASH *et al.* 1973, 1974). In unicelluar algae also, some interesting results have been obtained (e.g. SMITH *et al.*, 1961; BASSHAM *et al.*, 1964; KRETOVICH *et al.*, 1970; DHARMAWARDENE *et al.*, 1973; HAYSTEAD *et al.*, 1973), while there are only few reports in multicellular algae such as in *Ulva lactuca* (JACOBI, 1957) and *Porphyra yezoensis* (SATO *et al.*, 1975). In addition, both the NAD and NADP served as the electron acceptor with the *Ulva* GDH (JACOBI, 1957) while that of *Porphyra* utilized NADP only (SATO *et al.*, 1975).

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For this reason, we investigated the enzymatic properties of GDH of another green alga, *Bryopsis maxima,* to compare the results with those from the former marine algae. The reason for choosing *Bryopsis maxima* based mainly on the fact that we have recently used this alga as the experimental material for the investigation of nitrite reductase* and ribulose 1.5-diphosphate carboxylase (YAMADA *et al.*, 1978).

Materials and Methods

Algal material: *Bryopsis maxima* was collected at the coast of Inubo Cape, Choshi, Chiba Prefecture, in March, July and September, 1977. Fresh fronds were quickly brought back to laboratory in an ice box and cleaned for a short time by removing from macroscopic contaminants in running tap water. They were used for extraction of enzyme either immediately after collection or after having been stored at −40°C.

Chemicals: NADP(H) and NAD(H) are the products of the Sigma Chemical Company. All other chemicals were purchesed from Wako Pure Chemical Industries, Ltd.

Extraction of enzyme: Fresh or defrosted

* Umpublished observation.

algal fronds (60 g) were pestled by hands in an ice-cooled mortar for 10 min using 0.05 M Tris-HC*l* buffer, pH 7.5 (30 m*l*), as extraction medium. The minced frond mass was squeezed by hands through four layers of gauze. The residue was treated again in the same way as above and the extracts were combined.

Ammonium sulfate fractionation: To the extract obtained above, was added solid ammonium sulfate to a 25% saturation, and the precipitate formed was removed by centrifugation at 10000×g for 30 min. Solid ammonium sulfate was further added to a 75% saturation. The precipitate formed was collected by centrifugation at 10000×g for 50 min and dissolved in 10 m*l* of 0.2 M Tris-HCl buffer, pH 8.0, to dialyze against two changes of nearly hundred-fold volume of the same buffer for 24 hr each. The dialyzed solution was then diluted to 20 m*l* and used as GDH solution for experiments. When frozen fronds were used as the starting materials, a greenish viscous substance often deposited after the dialysis and it was removed by centrifugation before dilution.

Enzyme assay: Two kinds of standard reaction mixtures were prepared. The one (a) is for the reaction of glutamate synthesis, and it consisted of 0.2 m*l* of 0.2 M α-ketoglutarate, 0.2 m*l* of 0.4 M ammonium sulfate, 0.5 m*l* of 1.13×10^{-3} M NADPH (or 1.4×10^{-3} M NADH), 2.0 m*l* of 0.25 M Tris-HCl buffer, pH 7.65, and 0.1 m*l* of enzyme solution. Another (b) is for the reverse reaction, and it consisted of 0.2 m*l* of 0.4 M L-glutamate, 0.5 m*l* of 1.13×10^{-3} M NADP (or 1.4×10^{-3} M NAD), 2.0 m*l* of 0.25 M Tris-HC*l* buffer, pH 8.43, and 0.1 m*l* of enzyme solution. These standard mixtures were used through this work if not otherwise stated.

The reaction mixture was placed in a cuvette of 1 cm path in a Shimadzu Model UV-200S double-beam spectrophotometer and changes in absorbance at 340 nm were measured at room temperature within the first 10 min of incubation.

For some incubation mixtures, protein contents were determined by the methods of LOWRY *et al.* (1951) to obtain the specific activities (SA) of GDH, using crystalline bovine serum albumin as standard. SA was expressed by the formula, Absorbance $\times 1/t \times 1/p \times D$, where t is incubation time (minutes), p is mg protein per m*l* of incubation mixture, and D is the dilution rate of ammonium sulfate-fractioned enzyme solution.

Results

Co-enzyme specificity: Two kinds of standard mixtures containing NAD or NADH were incubated at 31°C for 10 min, but non of the absorbance changes were observed in contrast to the NADP or NADPH-containing standard mixtures, even when the reaction time was prolonged twice. Therefore, the enzyme extract seemed to contain practically no NAD(H)-dependent GDH activity.

Optimum pH: The optimum pH of GDH was estimated in both (a) and (b) reactions at 31.5°±0.5°C, respectively. Incubation periods were 5 min and 10 min for (a) and (b) reactions, respectively. As shown in **Fig. 1**, the pH optimum of the GDH for glutamate formation is lower by approximately 1 than that for its reverse reaction.

Time course of the reaction: The time

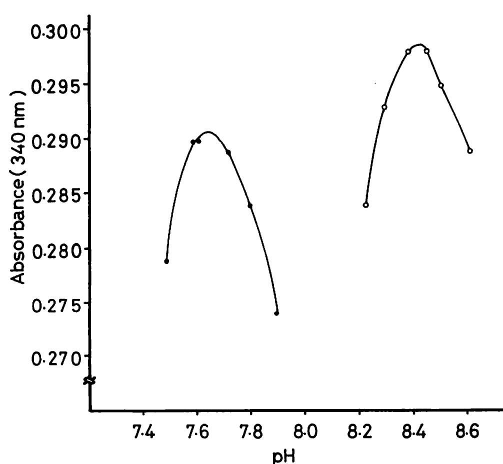

Fig. 1. pH-Activity-Curves of (a) (—●—) and (b) (—○—) reactions. Reaction time of (a): 5 min and (b): 10 min. Reaction temp.: 31.5°±0.5°C.

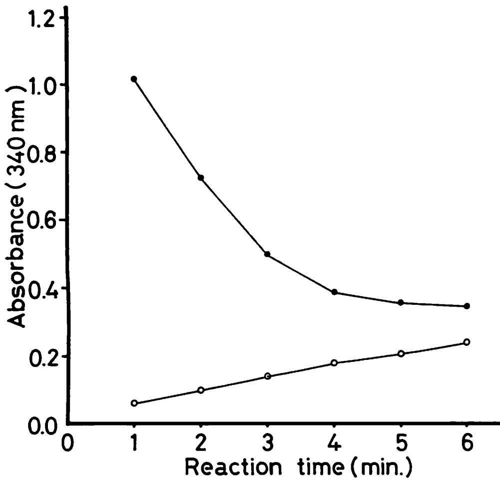

Fig. 2. Time course of (a) (—●—) and (b) (—○—) reactions. pH of (a) reaction: 7.61 and (b) reaction: 8.39. Reaction temp.: 31.4°± 0.4°C.

course of both (a) and (b) reactions was observed at 31.4°±0.4°C, and the results are shown in **Fig. 2.** Under these conditions, the (a) reaction proceeded almost linearly for the first 3 minutes but followed by a rapid slowing down thereafter while the (b) reaction proceeded almost linearly for at least 6 minutes.

Thermostability: A GDH solution was first heated at various temperatures for 10 min, then incubated at 25.5°±0.5°C under the standard conditions and the activities of individual reaction mixtures in the (a) reaction were measured. The results are shown in **Fig. 3.** The GDH was almost completely inactivated by heating at 50°C for 10 min, and the enzyme seemed fairly sensitive for temperature. The similar fact was also found during the storage of fresh fronds in a deep freezer (−40°C). The specific activity, 10.13, for the fresh fronds harvested on July. 19 has decreased to 4.5 after an about one and half

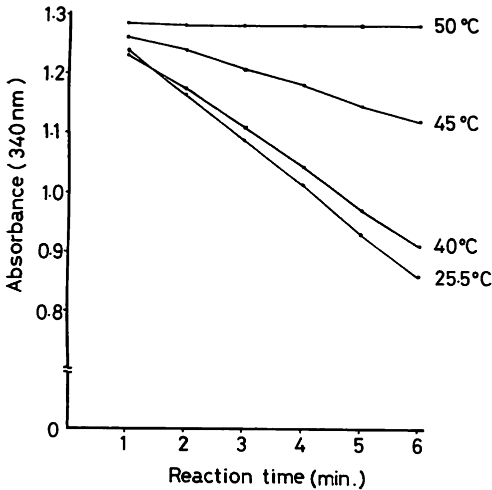

Fig. 3. Thermostability of (a) reaction. Enzyme solutions were heated for 10 min at temperatures indicated. pH 7.65±0.02 and reaction temp.: 25.5°±0.5°C.

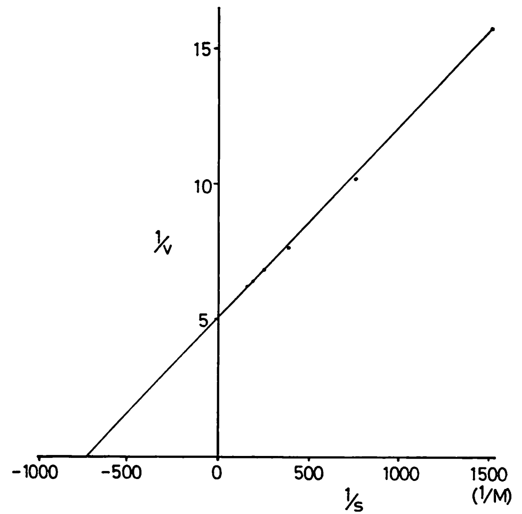

Fig. 4. Lineweaver-Burk plots of α-ketoglutarate in (a) reaction.

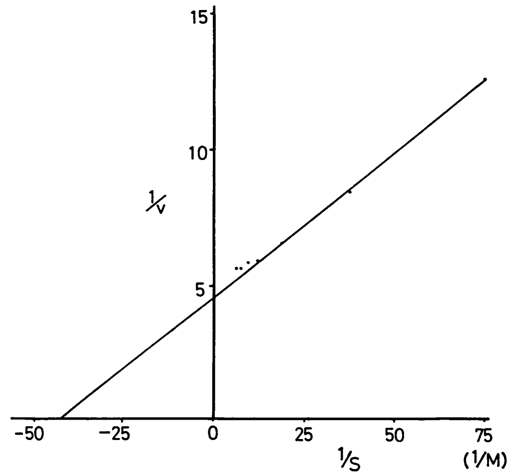

Fig. 5. Lineweaver-Burk plots of ammonia in (a) reaction.

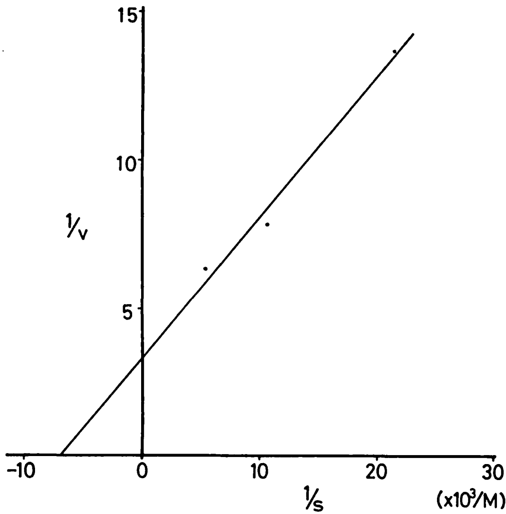

Fig. 6. LINEWEAVER-BURK plots of NADPH in (a) reaction.

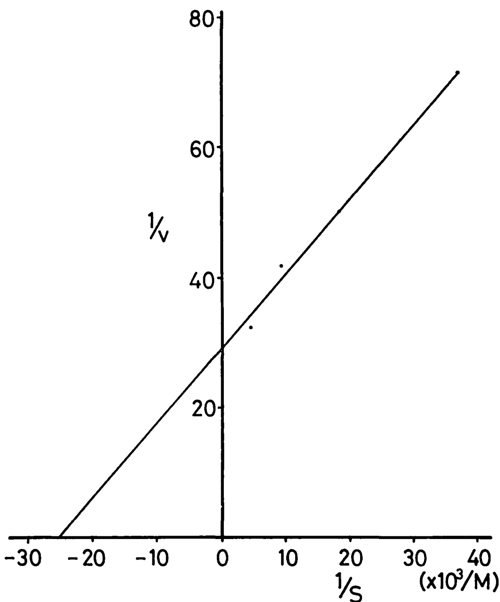

Fig. 7. LINEWEAVER-BURK plots of NADP in (b) reaction.

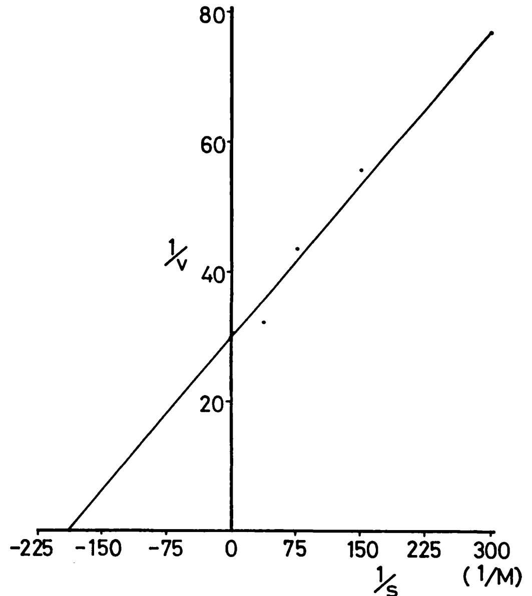

Fig. 8. LINEWEAVER-BURK plots of L-glutamate in (b) reaction.

Table 1. Km and Vmax values of each substrate.

Substrate	Km ($\times 10^{-3}$M)	Vmax (Absorbance)
(a)Reaction,pH 7.65		
α-Ketoglutarate	1.3750	0.260
Ammonia	23.810	0.225
NADPH	0.1423	0.034
(b)Reaction,pH 8.43		
L-Glutamate	5.1981	0.033
NADP	0.0396	0.034

month-storage.

Michaelis constants for substrates and co-enzymes: The initial reaction velocities for substrate and co-enzyme each in (a) and (b) reactions were determined, and their LINEWEAVER-BURK plots were obtained. The results are shown in **Figs. 4 to 8** for α-ketoglutarate, ammonia, NADPH, NADP, and glutamate, respectively. The MICHAELIS constants calculated from the plots are shown in **Table 1** together with their Vmax values.

It is clear in the Table 1, MICHAELIS constants for co-enzymes (NADPH and NADP) are similarly small and the value for NADP is smallest of the two, while the value for ammonia is very large and it is approximately 600 times as large as that for NADP.

Effect of some chemicals: The activity of

GDH was remarkably inhibited by *p*-chloromercuribenzoate (PCMB) and its 3.23×10^{-7} M concentration suppressed nearly 94% of (a) reaction in the standard mixture upon incubation at 33°C for 5 min. In contrast, EDTA at 10^{-6} to 10^{-2} M concentrations showed no inhibition in the standard mixture upon incubation at 25°C for 5 min. Similarly, some metal ions such as Ca^{2+}, Mg^{2+} and Zn^{2+} did not effect this enzyme activity under the same conditions at a concentration of at least 10^{-3} M upon incubation at 26°C for 5 min.

Discussion

As expected, the MICHAELIS constant of *Bryopsis* GDH for ammonia was also found to be largest among the reactants in the reactions catalyzed by this enzyme as with the GDH's of other sources. Although the Km value of this green alga for ammonia was about twice higher than that of *Porphyra* GDH (SATO *et al.*, 1975), these Km values of the algal GDH's were far smaller than those of higher plants already having been reported (BULEN, 1956; PAHLICH and JOY, 1971). However, *Bryopsis* GDH was found to be inhibited with PCMB at a low concentration as with those of mitochondria as well as chloroplasts of bean hypocotyls suggesting its being a SH-enzyme.

The affinity of *Bryopsis* GDH for NADP was much higher than that for NADPH while that for glutamate was much lower than that for α-ketoglutarate. This was the similar trend for the *Porphyra* GDH.

It was found that not only most GDH's of higher plants (JOY, 1969; GIVAN *et al.*, 1970; PAHLICH and JOY, 1971; LEA and THURMAN, 1972; WALLANCE, 1973) but the GDH of *Ulva lactuca* (JACOBI, 1957) can utilize both NAD(H) and NADP(H) as co-enzyme. In contrast, the GDH of corn leaves (BULEN, 1956) did not utilize NADP(H) as co-enzyme while *Porphyra* GDH (SATO *et al.*, 1975) and the present GDH extracted from *Bryopsis maxima* utilized only NADP(H) as co-enzyme. These results may suggest that NAD(H)- and NADP(H)- dependent GDH activities are caused by different enzymes.

According to the study of KRETOVICH *et al.* (1970), NADP(H)-dependent GDH activity of *Chlorella pyrnoidosa* was enhanced by growing on ammonia medium while the activity decreased on nitrate medium, but NAD(H)-dependent GDH activity in the same source received no effect upon this change of N-source. This might also support the above assumption.

References

ARIMA, Y. and KUMAZAWA, K. 1977. Evidence of ammonium assimilation via the glutamine synthetase-glutamate synthase system in rice seedling roots. Plant & Cell Physiol. **18**: 1121-1129.

BARASH, I., SADON, T. and MOR. H. 1973. Induction of a specific isoenzyme of glutamate dehydrogenase by ammonia in oat leaves. Nature New Biology **244**: 150-152.

BARASH, I., SADON, T. and MOR, H. 1974. Relationship of glutamate dehydrogenase levels to free amino acids, amides and ammonia in excised oat leaves. Plant & Cell Physiol. **15**: 563-566.

BASSHAM, J. A. and KIRK, M. 1964. Photosynthesis of amino acids. Biochim. biophys. Acta, **90**: 553-562.

BULEN, W. A. 1956. The isolation and characterization of glutamic dehydrogenase from corn leaves. Arch. Biochem. Biophys. **62**: 173-183.

DHARMAWARDENE, M. W. N., HAYSTEAD, A. and STEWARD, W. D. P. 1973. Glutamine synthetase of the nitrogen-fixing alga, *Anabena cylindrica*. Arch. Mikrobiol. **90**: 281-295.

GIVAN, C. V., GIVAN, A. L. and LEECH, R. M. 1970. Photoreduction of α-ketoglutarate to glutamate by *Vicia fava* chloroplasts. Plant physiol. **45**: 624-630.

HAYSTEAD, A., DHARMAWARDENE, M. W. N. and STEWART, W. D. P. 1973. Ammonia assimilation in a nitrogen-fixing blue-green alga. Plant Sci. Letts. **1**: 439-445.

JACOBI, G. 1957. Enzyme des Aminosäure-Stoffwechsels in *Ulva lactuca*. Transaminasen und Aminosäure-Dehydrogenasen. Planta, **49**: 561-577.

JOY, K. W. 1969. Nitrogen metabolism of *Lemna*

minor. II. Enzymes of nitrate assimilation and some aspects of their regulation. Plant Physiol. **44**: 849-853.

JOY, K.W. 1971. Glutamate dehydrogenase changes in *Lemna* not due to enzyme induction. Plant Physiol. **47**: 455-446.

KRETOVICH, W.L., EVSTIGNEEVA, Z.G. and TOMOVA, N.G. 1970. Effect of nitrogen source on glutamate dehydrogenase and alanine dehydrogenase of *Chlorella*. Can. J. Bot. **48**: 1179-1183.

LEA, P.J. and THURMAN, D.A. 1972. Intracellular locarization and properties of plant L-glutamate dehydrogenases. J. exp. Biol. **23**: 440-449.

LEA, P.J. and MIFLIN, B.J. 1974. Alternative route for nitrogen assimilation in higher plants. Nature, **251**: 614-616.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. 1951. Protein measurement with Folin phenol reagegent. J. Biol. Chem. **193**: 265-273.

MIFLIN, B.J. 1974. The localization of nitrite reductase and other enzymes related to amino acid biosynthesis in the plastids of root and leaves. Plant Physiol. **54**: 550-555.

O'NEAL, D. and JOY, K.W. 1973. Localization of glutamine synthetase in Chloroplasts. Nature New Biology, **246**: 61-62.

PABLICH, E. and JOY, K.W. 1971. Glutamate dehydrogenase from pea roots: Purification and properties of the enzyme. Can. J. Biochem. **49**: 127-138.

SATO, M., SATO, Y. and TSUCHIYA, Y. 1975. Occurrence of glutamate dehydrogenase in purple leaver, *Porphyra yezoensis*. Bull. Jap. Soc. Sci. Fish. **41**: 337-341. (in Japanese)

SMITH, D.C., BASSHAM, J.A. and KIRK, H. 1961. Dynamics of the photosynthesis of carbon compounds. II. Amino acid synthesis. Biochim. biophys. Acta, **48**: 299-313.

WALLANCE, W. 1973. The distribution and characteristics of nitrate reductase and glutamate dehydrogenase in the maize seedling. Plant Physiol. **52**: 191-196.

YAMADA, T., IKAWA, T. and NISIZAWA, K. 1978. Partial purification and some properties of RuDP carboxylase from a green alga, *Bryopsis maxima*. Jap. J. Phycol. **26**: 49-52.

西澤一俊・安斎　寛・奥川雄治：　オオハネモ (*Bryopsis maxima*) のグルタミン酸脱水素酵素の研究

最近までは植物におけるアンモニア同化の主役をなすと考えられていたグルタミン酸脱水素酵素 (GDH) に関する研究は，高等植物や微細藻では詳しく行われているが，多細胞または多核藻における知見は少い。そこで私共が従来 N-代謝や C-固定の研究に使っているオオハネモにおける GDH につき，若干の酵素化学的研究を行った。すなわちその抽出液の硫安分画標品を用い，至適酸度や熱安定性，補酵素特異性，二・三の金属の影響，Michaelis 定数などを観察測定し，既に報告されている結果と比較した。(154 東京都世田谷区下馬 3-34-1，日本大学農獣医学部水産学科)

◎書籍頒布について　会員および，その他ご希望の方に頒布致しますので代金を添えて学会事務局までお申し込み下さい。

(1) **山田幸男先生追悼号**　本学会では昨年 8 月に故山田幸男先生の追悼号を刊行致しました。この事業は，追悼号刊行実行委員会の手で進められ，その経費はすべて各界各位の寄附金によって充当されました。A5 版 i～xxviii，1～418 頁，山田先生の遺影・経歴・業績一覧・追悼文および内外の藻類学者より寄稿された論文50編 (英文26，和文24) が掲載されています。**価格，国内 5500 円，国外 6000 円 (含送料)**。

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