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# Purification and characterization of glutamine synthetase from the shoot of Moso Bamboo (*Phyllostachys edulis*)

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**Abstract.** Glutamine synthetase from the shoot of *Phyllostachys edulis* (Moso Bamboo) was purified to apparent homogeneity through molecular sieving, ion exchange and affinity chromatography. The enzyme was purified about 1400-fold with a recovery more than 20%. The enzyme has a molecular weight of 361,000, and consists of eight identical size subunits of 45,000 each. In the synthetase assay, apparent Km values of L-glutamate and ATP are 2.79 mM and 1.76 mM, respectively; however, it exhibited positive cooperativity for $NH_4Cl$ with a Hill coefficient of 1.5 and $S_{0.5}$ value of 0.54 mM. In the transferase assay, apparent Km values of L-glutamine and hydroxylamine were 58.82 mM and 2.99 mM, respectively. The pH optima were 6.8 in the synthetase assay, and 6.3 in the transferase assay. In the presence of $Mg^{2+}$ and 2-mercaptoethanol, the enzyme could be stored at 4°C for two weeks without significant loss of its activity. When the enzyme was incubated at 45°C for 60 minutes, it still retained about 70% of its original activity. Divalent metal ions are important activators for the enzyme activity, with $Mg^{2+}$ being the most effective for biosynthetic activity. At 50 mM concentration, the observed order of effectiveness of different cations is $Mg^{2+} > Co^{2+} > Fe^{2+} = Zn^{2+} > Cu^{2+}$. $Mn^{2+}$ is the most effective for the transferase activity. The $Mg^{2+}$-dependent biosynthetic activity is strongly inhibited by $Mn^{2+}$ and $Fe^{2+}$, whereas the $Mn^{2+}$-dependent transferase activity is strongly inhibited by $Fe^{2+}$, $Ca^{2+}$ and $Zn^{2+}$.

**Key words:** Bamboo shoot; Divalent cation; Glutamine synthetase; Nitrogen assimilation; *Phyllostachys edulis*.

## Introduction

In plants, the primary input of nitrogen occurs through the assimilation of ammonia into an organic form. Glutamine synthetase (L-glutamate:ammonia ligase, ADP forming, EC.6.3.1.2) jointly with the glutamate synthase (L-glutamine:2-oxoglutarate aminotransferase, EC.1.4.7.1) is the major pathway for the assimilation of ammonia into organic compounds, thus glutamine synthetase plays a key role in nitrogen metabolism (Miflin and Lea, 1980). An increasing number of studies have been made on the identification, characterization and subcellular localization of the multiple forms of glutamine synthetase in different tissues of higher plants (Hirel and Gadal, 1980; Ahmad *et al.*, 1982; McCormack *et al.*, 1982; Cullimore *et al.*, 1983; Ericson, 1985; Muhitch, 1989; Vézina and Langlois, 1989; Vézina and Margolis, 1990; Höpfner *et al.*, 1990). In green tissues of most higher plants contain two forms of glutamine synthetase, one located in the cytoplasm and the other located in the chloroplast; however, in non-chlorophyll portions contain only the cytosolic from of glutamine synthetase (Stewart *et al.*, 1980). These two isoform enzymes can be separated by ion exchange chromatography and are distinguished by several kinetic and physical properties (McNally *et al.*, 1983; Beudeker and Tabita, 1985; Yuan and Hou, 1987).

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It was demonstrated that cytosolic glutamine synthetases isolated from green leaves, etiolated leaves, roots, root nodular tissue and seeds of different plant species are all similar proteins (Hirel *et al.*, 1984).

Bamboo plants are one of the major forest vegetations in the oriental countries and are widely planted on many hillsides with many of their shoots serving as

The brei was filtered through a nylon tissue (200 mesh) and centrifuged at 12,000 g for 30 min. Buffer A containing 1% protamine sulfate was added dropwise to the supernatant with stirring in the ratio of 1 : 10 (v/v). After standing for 10 min, the precipitate was removed by centrifugation. The clarified supernatant was brought to 30% saturation with $(NH_4)_2SO_4$ and the

vegetables. *Phyllostachys edulis* (Moso Bamboo) is distributed in the warm-temperate parts of China, and commonly planted throughout Taiwan at altitudes up to 1,600 m. The shoot of Moso Bamboo occurs at the node of the underground rhizome in the winter season and grows rapidly. Hence the shoot represents one type of typical non-green tissue which is undergoing active nitrogen metabolism. During the shoot development, nitrogen and carbon compounds are translocated into the shoot from the maternal bamboo plants through the rhizome, or from the roots directly. Thus it seemed to be of particular interest to study the glutamine synthetase from the bamboo shoots. The present paper describes the purification and characterization of the shoot enzyme, and the effects of divalent metal ions are also presented.

## Materials and Methods

precipitate was removed by centrifugation. The supernatant was brought to 65% saturation with $(NH_4)_2SO_4$, and the precipitated protein was collected by centrifugation and dissolved in a small amount of buffer A. The undissolved portion was removed by centrifugation and the supernatant was loaded on a Sepharose 6B column (2.6 × 80 cm) previously equilibrated with 0.01 M Tris-HCl buffer (pH 7.5) containing 2 mM EDTA, 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol (buffer B) and eluted with the same buffer. Fractions of 5 ml were collected at a flow rate of 40 ml per hour, and those containing glutamine synthetase activity were pooled and loaded on a DEAE-Sephacel column (2.6 × 20 cm) also previously equilibrated with buffer B. The column was washed with buffer B to remove unadsorbed substances (about 300 ml). Poteins were eluted from the column with a linear gradient of 0-0.5 M KCl in 500 ml of buffer B. Fractions of 5 ml were collected at a flow rate of 40 ml per hour, and

*Enzyme Assays*

The biosynthetic activity of glutamine synthetase was assayed based on the release of inorganic phosphate in the presence of $NH_4Cl$ (Shapiro and Stadtman, 1970). The assay mixture contained 50 mM imidazole-HCl buffer (pH 7.0), 7.5 mM ATP, 100 mM L-glutamate, 50 mM $NH_4Cl$, 50 mM $MgCl_2$ and an appropriate amount of enzyme solution in a final volume of 0.4 ml. The reaction was started by adding the enzyme solution, and the enzyme solution was omitted in the blank test. After incubation at 30°C for 15 min, the reaction was terminated by adding 3.6 ml of ferrous sulfate reagent (0.8% $FeSO_4 \cdot 7H_2O$ in 0.015N $H_2SO_4$, prepared freshly), followed by adding 0.3 ml of ammonium molybdate reagent [6.6%$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 7.5 N $H_2SO_4$]. The absorbance at 660 nm was measured after several minutes, using sodium dihydrogen phosphate as the standard. One unit of glutamine synthetase activity was defined as the amount of enzyme catalyzing the release of 1 $\mu$mole of inorganic phosphate per minute at 30°C.

Glutamine synthetase also can be assayed for its transferase activity based on the formation of $\gamma$-glutamyl hydroxamate in the presence of $NH_2OH$ (Shapiro and Stadtman, 1970). The assay mixture contained 40 mM imidazole-HCl buffer (pH 7.0), 30 mM L-glutamine, 3 mM $MnCl_2$, 0.4 mM ADP, 20 mM sodium arsenate, 60 mM $NH_2OH$ and an appropriate amount of enzyme solution in a final volume of 3 ml. The reaction was started by adding $NH_2OH$, and L-glutamine was omitted in the blank test. After incubation at 30°C for 15 min, the $\gamma$-glutamyl hydroxamate formed was determined by adding 1.0 ml of ferric chloride reagent (equal volumes of 10% $FeCl_3 \cdot 6H_2O$ in 0.2 N HCl, 24% TCA and 6 N HCl were mixed together) and the absorbance at 540 nm was measured with the commercial $\gamma$-glutamyl hydroxamate being used as a standard. One unit of transferase activity was defined as the amount of enzyme catalyzing the formation of 1 $\mu$mole of $\gamma$-glutamyl hydroxamate per minute at 30°C.

*Protein Determination*

Proteins in the crude extract and purification steps up to ammonium sulfate precipitation were determined by micro-biuret method (Itzhaki and Gill, 1964). In the purification steps afterwards, protein content was estimated by the dye-binding method of Bradford (1976) using crystalline bovine serum albumin as the standard.

*Polyacrylamide Gel Electrophoresis*

Nondenaturating gel electrophoresis of native protein was performed on 7.5% polyacrylamide slab gels as described by Davis (1964). One-dimensional SDS-polyacrylamide gel electrophoresis was carried out on 12.5% polyacrylamide slab gels according to Laemmli (1970). Protein bands were visualized on slab gels by means of Coomassie blue R staining. The activity of native glutamine synthetase on the slab gel was detected by the transferase assay as described by Barratt (1980).

*Molecular Weight Determination*

The molecular weight of the shoot glutamine synthetase was estimated by a calibrated Sephacryl S-300 column (1.6 × 80 cm) previously equilibrated with 0.05 M Tris-HCl buffer (pH 7.5) containing 2 mM EDTA, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 0.1 M NaCl. Aldolase (158,000), catalase (232,000), ferritin (440,000) and thyroglobulin (669,000) were used as standards. The partition coefficient (Kav) of each protein was calculated. The molecular weight of the subunit of shoot enzyme was estimated by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). $\alpha$-Lactalbumin (14,400), trypsin inhibitor (20,100), carbonic anhydrase (30,000), ovalbumin (43,000), albumin (67,000) and phosphorylase b (94,000) were used as standards. The relative mobility (Rf) of each protein to the tracking dye was calculated.

*Enzyme Kinetics*

Kinetic measurement of the shoot glutamine synthetase was made on the protein purified to the Sephacryl S-300 gel filtration step. Km values for L-glutamate, ATP and $NH_4Cl$ were determined by assaying the biosynthetic activity of the enzyme; and Km values for L-glutamine and hydroxylamine were determined by assaying the transferase activity of the enzyme.

The pH optimum of the biosynthetic activity was established by assaying in cacodylate (pH 5.5 to 7.1), imidazole-HCl (pH 6.0 to 7.6) and Tris-HCl (pH 7.0 to 8.2) buffer solutions. The pH optimum of the transferase activity was established by assaying in citrate (pH 4.7 to 6.9), cacodylate (pH 5.8 to 7.2) and imidazole-HCl (pH 6.5 to 7.6) buffer solutions.

The thermal stability of the enzyme activity was tested. The purified enzyme was dialyzed against 0.01 M Tris-HCl buffer (pH 7.5) to remove all stabilizing ligands. The enzyme was incubated at 45°C in 0.01 M Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$ or 10 mM 2-mercaptoethanol, and the biosynthetic activity of the enzyme was determined at different time intervals.

*Experiments Involving Divalent Metal Ions*

Metal ions were removed from the purified enzyme solution by dialysis for overnight against two changes of 500 ml of 0.01 M Tris-HCl buffer (pH 7.5) containing 2 mM EDTA and 10 mM 2-mercaptoethanol. The requirement of divalent metal ions for biosynthetic and transferase activities of the enzyme were determined, and assay mixtures were described in Table 3. Effects of divalent metal ions on the $Mg^{2+}$-promoted biosynthetic activity and the $Mn^{2+}$-promoted transferase activity of the enzyme were also determined, and assay mixtures were described in Table 4.

## Results

*Emzyme Purification*

The successive purification steps for the glutamine synthetase from the shoot of bamboo *Phyllostachys edulis* are summarized in Table 1. The elution profile of the DEAE-Sephacel is shown in Fig. 1. Only one glutamine synthetase peak was eluted at KCl concentration between 0.2 to 0.3 M. The enzyme was routinely purified to about 1400-fold with a recovery of about 20%. Following purification, a homogeneous enzyme preparation was obtained which was checked by electrophoresis on 7.5% polyacrylamide gel (Fig. 2). Sample lanes of the gel contained only one Coomassie blue-staining band. The sole dye-stained protein band was identified as glutamine synthetase by the activity staining which was performed by incubating the gel in the transferase reaction mixture for one hour at 30°C and then treated with ferric chloride reagent as described in method section to visualize the $\gamma$-glutamyl hydroxamate produced.

*Kinetic Constants for Substrates*

The kinetic properties of the purified enzyme were analyzed by both the synthetase and the transferase assays. The substrate saturation curves were analyzed by the double-reciprocal plots of velocity versus the concentration of one of the variable substrate (Figs. 3 and 4). The purified enzyme followed the Michaelis-Menten kinetics for L-glutamate, ATP, L-glutamine and hydroxylamine, but exhibited nonhyperbolic saturation curve for $NH_4Cl$ (Fig. 3, C), denoting positive cooperativity in this case. Kinetic parameters were estimated and presented in Table 2. In the biosynthetic

**Table 1.** *Purification of glutamine synthetase from the shoot of bamboo* ***Phyllostachys edulis***

| Purification step | Total volume (ml) | Total protein (mg) | Total activity (units)[b] | Recovery (%) | Specific activity (units/mg protein) | Purification (fold) |
|---|---|---|---|---|---|---|
| Crude extract[a] | 1130 | 7293.64 | 364.25 | 100 | 0.050 | 1 |
| Protamine sulfate treatment | 1220 | 5876.79 | 322.81 | 89 | 0.055 | 1.10 |
| 30-60% Ammonium sulfate ppt. | 21 | 1185.17 | 231.01 | 63 | 0.195 | 3.90 |
| Sepharose 6B eluate | 122 | 202.63 | 203.04 | 56 | 1.002 | 20.06 |
| DEAE-Sephacel eluate | 94 | 117.35 | 166.70 | 46 | 1.450 | 28.45 |
| 2'5'ADP-Sepharose 4B eluate | 31 | 1.43 | 96.58 | 27 | 67.536 | 1352.34 |
| Sephacryl S-300 eluate | 28 | 1.06 | 76.49 | 21 | 72.164 | 1445.02 |

[a]Crude extract was prepared from 320 grams of fresh shoots of bamboo.

[b]Glutamine synthetase activity was determined by the transferase assay. One unit of the enzyme was defined as the amount of enzyme catalyzing the formation of 1.0 $\mu$mole of $\gamma$-glutamate hydroxamate per minute at 30°C.

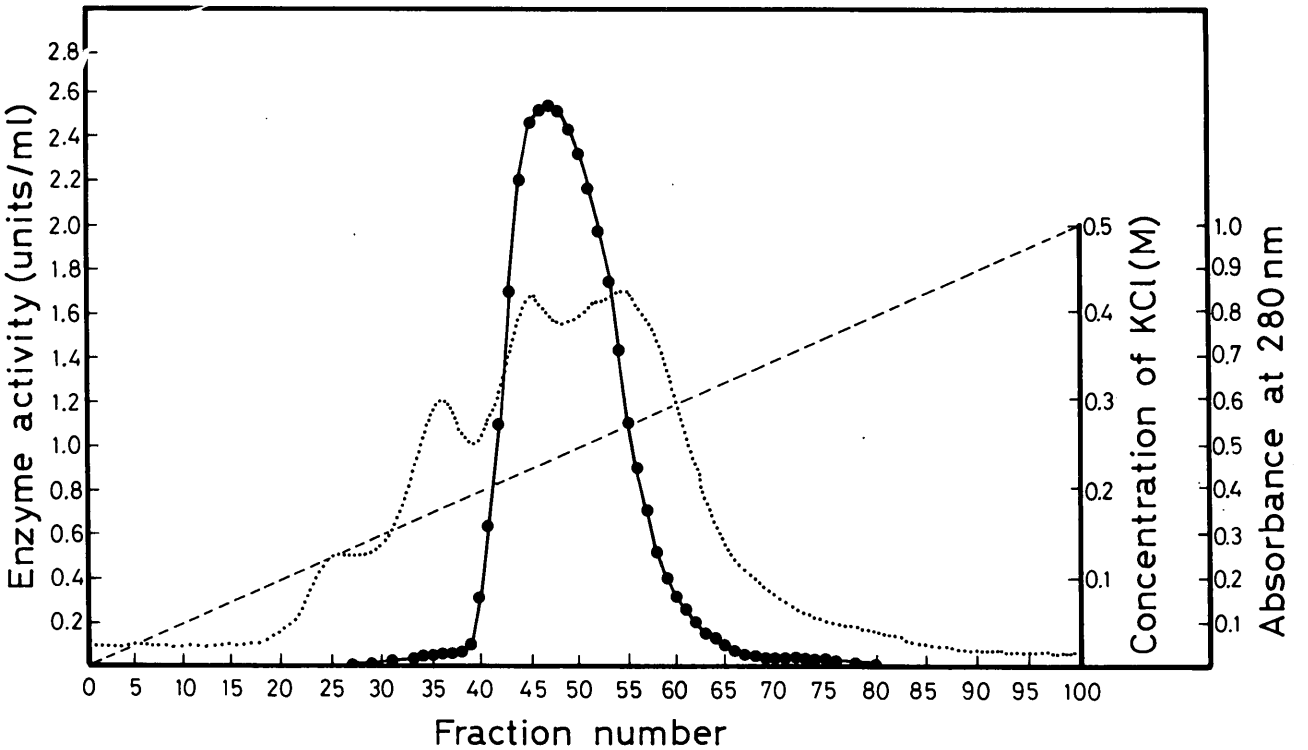


Fig. 1. Elution pattern of glutamine synthetase from the shoot of bamboo *Phyllostachys edulis* using DEAE-Sephacel column (2.0×20 cm). Five ml per fraction was collected with a flow rate of 40 ml per hour, and the enzyme activity was determined by the transferase reaction based on production of γ-glutamyl hydroxamate. The solid line represents the enzyme activity, the dotted line indicats the absorbance at 280 nm, the broken line corresponds to the KCl gradient.

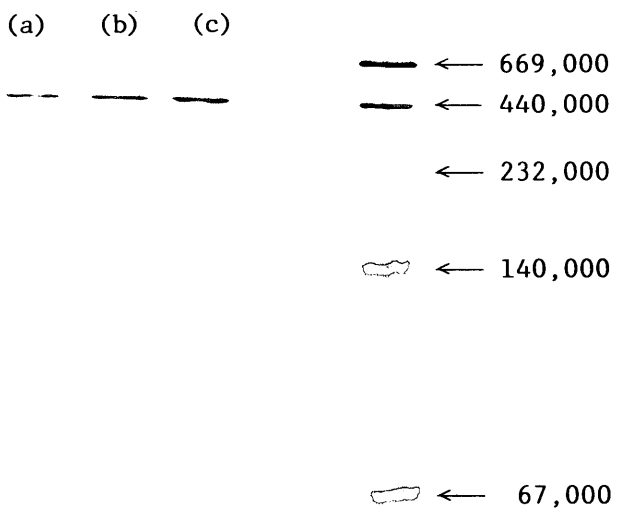


Fig. 2. Polyacrylamide gel electrophoresis of glutamine synthetase from the shoot of bamboo *Phyllostachys edulis* following the purification with Sephacryl S-300 column. Amount of protein loaded in each well: (a) 20 μg, (b) 30 μg and (c) 40 μg.

reaction, apparent Km values for L-glutamate and ATP were 2.79 mM and 1.76 mM, respectively. For the positive cooperative substrate $NH_4Cl$, the estimated Hill coefficient ($n_H$) was 1.5, and the $S_{0.5}$ value estimated at saturating concentrations of L-glutamate and ATP was 0.54 mM. In the transferase reaction, apparent Km values for L-glutamine and hydroxylamine were 58.82 mM and 2.99 mM, respectively; however, the apparent Km value for ADP was so low and no accurate estimation could be made.

*Stability and pH Optimum*

In the presence of stabilizing ligands such as $Mg^{2+}$ and 2-mercaptoethanol, the purified enzyme can be stored at 4°C for more than two weeks without significant loss of its activity. After 30 days at 4°C in 0.01 M Tris-HCl buffer, pH 7.5, containing 2 mM EDTA, 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol, the enzyme still retained more than 85% of its original activity. The thermal stability of the enzyme in the presence or absence of $MgCl_2$ and 2-mercaptoethanol was also examined (Fig. 5). The enzyme was incubated at 45°C in

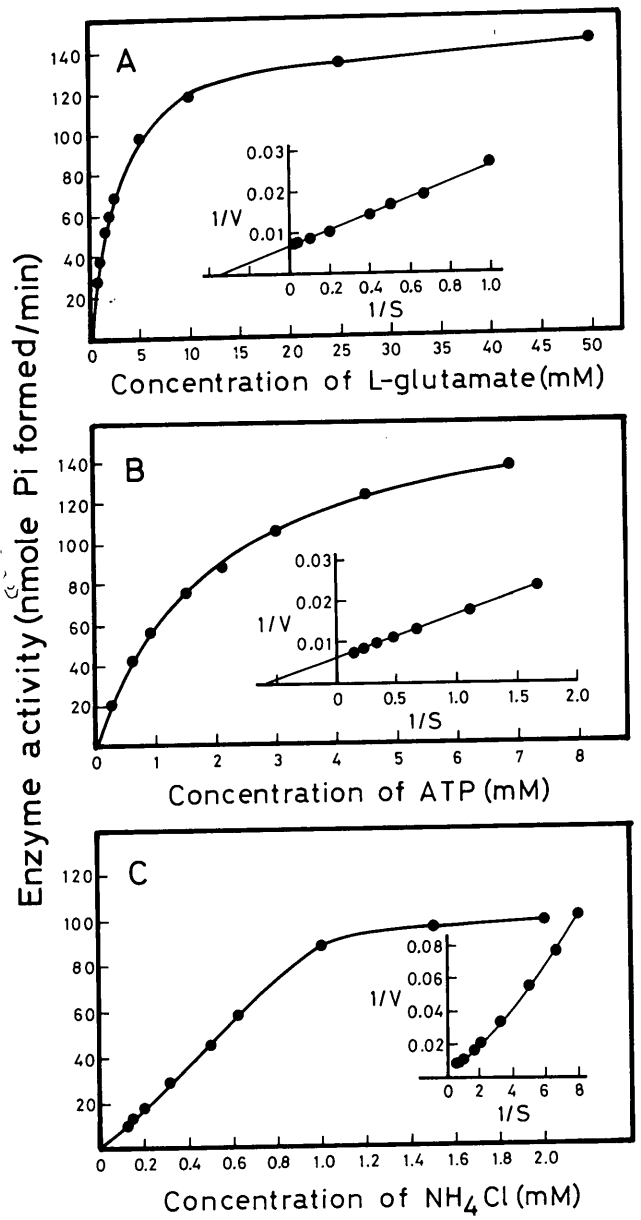


Fig. 3. Substrate saturation curves and double reciprocal plots (insets) with L-glutamate (A), ATP (B) and $NH_4Cl$ (C) as variable substrates for the biosynthetic activity of glutamine synthetase from the shoot of *Phyllostachys edulis*.

0.01 M Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol, the biosynthetic activity was determined at different time intervals, the enzyme activity was found decrease slowly, but still retained about 70% of its original activity after 60 minutes. However, omission of $MgCl_2$ and 2-mercaptoethanol, the thermal stability of this enzyme was strongly

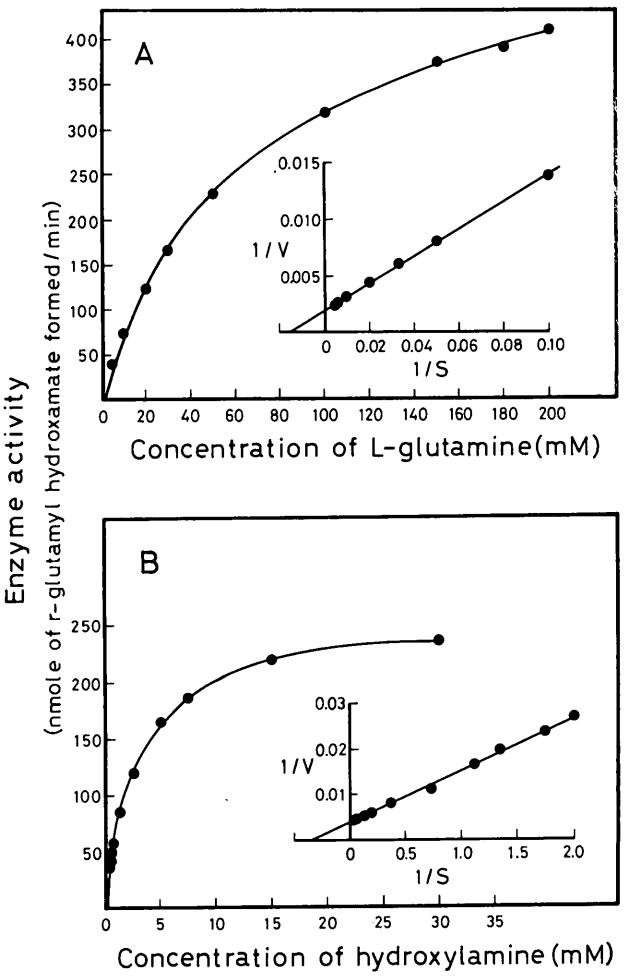


Fig. 4. Substrate saturation curves and double reciprocal plots (insets) with L-glutamine (A) and hydroxylamine (B) as variable substrates for the transferase activity of glutamine synthetase from the shoot of *Phyllostachys edulis*.

**Table 2.** *Affinity for substrates of glutamine synthetase from the shoot of bamboo* ***Phyllostachys edulis***

| Substrates | Apparent Km values (mM) |
|---|---|
| L-Glutamate[a] | 2.79 |
| ATP[a] | 1.76 |
| $NH_4Cl$[a] ($n_H = 1.5$) | 0.54 ($S_{0.5}$) |
| L-Glutamine[b] | 58.82 |
| Hydroxylamine[b] | 2.99 |

[a]Enzyme activity was evaluated by the biosynthetic reaction based on release of inorganic phosphate.
[b]Enzyme activity was evaluated by the transferase reaction based on formation of $\gamma$-glutamyl hydroxamate.

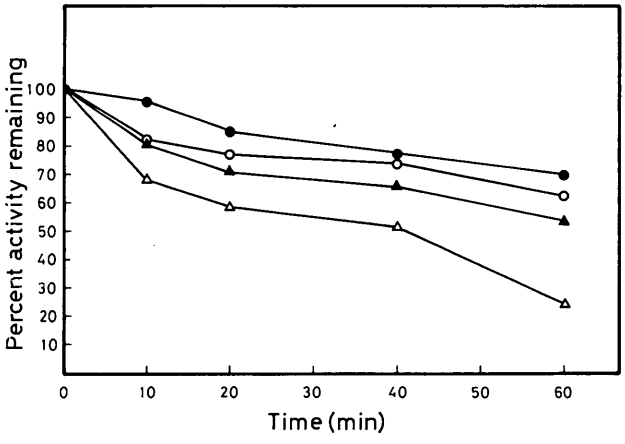


Fig. 5. Thermal inactivation of glutamine synthetase from the shoot of bamboo *Phyllostachys edulis*. Glutamine synthetase was incubated for different time at 45°C in 0.01 M Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol (●); or in 0.01 M Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$ (○); or in 0.01 M Tris-HCl buffer, pH 7.5, containing 10 mM 2-mercaptoethanol (▲); or in 0.01 M Tris-HCl buffer, pH 7.5, only (△). After incubation, the solution was kept in an ice bath and the activity of enzyme was measured by the biosynthetic reaction based on release of inorganic phosphate.

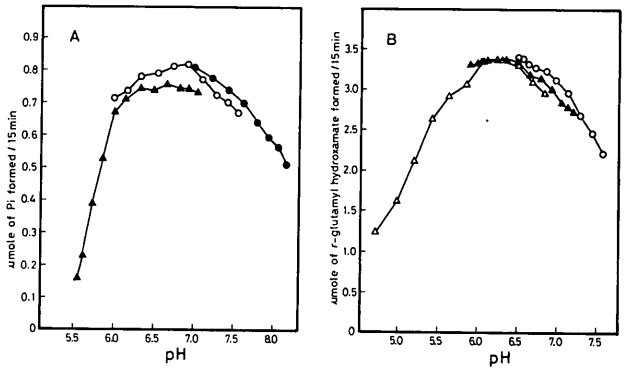


Fig. 6. Effect of pH on the activity of glutamine synthetase from the shoot of *Phyllostachys edulis*. A, The biosynthetic activity was determined from the rate of Pi production and the activity was expressed as μmole of Pi formed in 15 minutes. Assay was performed in 50 mM cacodylate buffer (▲) between pH 5.5 to 7.1, in 50 mM imidazole-HCl buffer (○) between pH 6.0 to 7.6 and in 50 mM Tris-HCl buffer (●) between pH 7.0 to 8.2. B, The transferase activity was determined from the rate of γ-glutamyl hydroxamate formed and the activity was expressed as μmole of γ-glutamyl hydroxamate formed in 15 minutes. Assay was performed in 40 mM citrate buffer (△) between pH 4.7 to 6.9, in 40 mM cacodylate buffer (▲) between pH 5.8 to 7.2 and in 40 mM imidazole-HCl buffer (○) between pH 6.5 to 7.6.

affected. When the enzyme was incubated in the buffer containing 5 mM $MgCl_2$ or 10 mM 2-mercaptoethanol only, the enzyme activity decreased to 62% or 54% of its original activity, respectively, after 60 minutes. Furthermore, as the enzyme was incubated in the buffer without $MgCl_2$ and 2-mercaptoethanol, the enzyme activity decreased to 24% of its original activity after 60 minutes.

The effect of pH on the biosynthetic and transferase activities of the enzyme were shown in Fig. 6. The pH profiles for the biosynthetic activity of the enzyme showed a broad range between pH 6.3 to 7.2 with an optimum pH being around 6.8; and the transferase activity showed a range between pH 6.1 to 6.5 with an optimum pH being around 6.3.

*Molecular Weight*

The molecular weight of the enzyme was estimated by molecular sieve chromatography on Sephacryl S-300 column, and the value obtained was about 361,000 (Fig. 7). When the purified enzyme was electrophoresed in 12.5% SDS-polyacrylamine gel, a single band of protein with molecular weight of 45,000 (Fig. 8). This result, together with the relative molecular weight of the native enzyme, suggests that the enzyme consists of eight identical size subunits.

*Effects of Divalent Metal Ions on the Enzyme Activity*

In the test of assay requirements for the shoot glutamine synthetase showed that neither the synthetase nor the transferase activity could be detected due to omission of the divalent metal ion in both reaction mixtures (data not shown). Therefore, like the glutamine synthetase from other sources, the enzyme from the shoot of *Phyllostachys edulis* requires a divalent metal ion as an activator. The relative rates of biosynthetic and transferase activities in the presence of different divalent cations are given in Table 3. For the biosynthetic activity, $Mg^{2+}$ was the most effective cation. At 50 mM concentration, the observed order of effectiveness of different divalent cations was $Mg^{2+} > Co^{2+} > Fe^{2+} = Zn^{2+} > Cu^{2+}$. At lower concentrations, $Mn^{2+}$ was also an activator of the biosynthetic activity. For the transferase activity, $Mn^{2+}$ was the most effec-

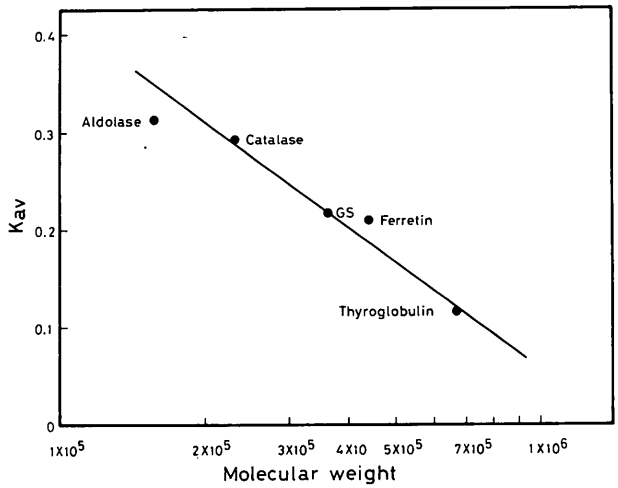


Fig. 7. Estimation of molecular weight of the glutamine synthetase from the shoot of bamboo *Phyllostachys edulis* by gel filtration on Sephacryl S-300 column. Column size: 1.6×80 cm. Elution buffer: 0.05 M Tris-HCl buffer (pH 7.5) containing 2 mM EDTA, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 0.1 M NaCl. Flow rate: 20 ml/h, 2 ml per fraction was collected.

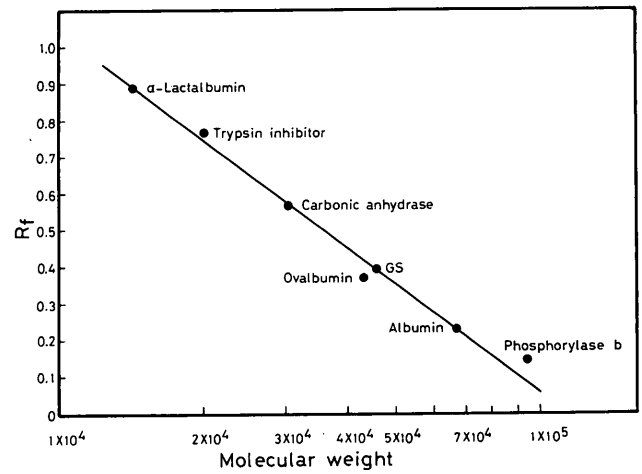


Fig. 8. Estimation of molecular weight of subunit of the glutamine synthetase from the shoot of bamboo *Phyllostachys edulis* by SDS-polyacrylamied gel electrophoresis on a 12.5 % polyacrylamied gel containing 0.1 % SDS.

tive cation followed by $Co^{2+}$ and $Mg^{2+}$. A relatively small enhancement of the transferase activity was obtained with $Fe^{2+}$ and other cations were almost ineffective.

The effect of different divalent metal ions on the $Mg^{2+}$-dependent biosynthetic activity and the $Mn^{2+}$-dependent transferase activity of the shoot glutamine synthetase are shown in Table 4. The biosynthetic activity was strongly inhibited by $Mn^{2+}$ and $Fe^{2+}$. At a lower concentration of 1 mM, $Mn^{2+}$ and $Fe^{2+}$ inhibited the biosynthetic activity about 84% and 71%, respectively. At concentrations higher than 20 mM, $Cu^{2+}$, $Ca^{2+}$, $Zn^{2+}$ and $Ni^{2+}$ also showed strong inhibitory effect on the biosynthetic activity. $Co^{2+}$ was a relatively poor inhibitor at all of the concentrations tested. The transferase activity was strongly inhibited by $Fe^{2+}$, $Ca^{2+}$ and $Zn^{2+}$ when the concentration of cations were higher than 1 mM. However, $Mg^{2+}$, $Co^{2+}$, $Cu^{2+}$ and $Ni^{2+}$ showed relatively less inhibitory effect on the transferase activity.

## Discussion

The presence of two forms of glutamine synthetase in higher plants has been widely reported. These have been placed in four groups depending on the content and intracellular location of isoenzymes (McNally *et al.*, 1983). Only one form of glutamine synthetase was found in the shoot of *Phyllostachys edulis*, and the factors that may regulate its activity were examined. Of the purification methods, the critical step is the treatment of the crude extract with protamine sulfate. Without this step, an extraneous milky substance will precipitate together with proteins in the ammonium sulfate precipitation step and it will seriously interferes following purification steps. From the data that overall purification of 1,400-fold was needed to obtain a homogeneous preparation, it was estimated that the enzyme represented about 0.07% of the total soluble protein. The DEAE-Sephacel column chromatographic pattern (Fig. 1) showed that the only one glutamine synthetase peak was eluted at an ionic strength between 0.2 to 0.3 M KCl. It is quite similar to the chloroplastic glutamine synthetase (McNally *et al.*, 1983). However, the bamboo shoot is a kind of non-green tissue when it was still under the soil surface; thus we would like to consider that the shoot enzyme is more similar to the glutamine synthetase from etiolated leaves (Mann *et al.*, 1980; Nishimura *et al.*, 1982; Höpfner *et al.*, 1990). The enzyme is probably located in plastids of the shoot (Vézina and Langlois, 1989).

The molecular weights of both the native shoot

**Table 3.** *Biosynthetic and transferase activities of glutamine synthetase with various cations*

Biosyntheitc and transferase activities were determined from the rate of formation of Pi and γ-glutamyl hydroxamate, respectively. For the biosynthetic activity, the reaction mixture in a final volume of 0.4 ml contained 50 mM imidazole-HCl buffer (pH 7.0), 7.5 mM ATP, 100 mM L-glutamate, 50 mM $NH_4Cl$, 0.1 ml enzyme preparation (3.5 μg protein) and specified concentrations of various cations. For the transferase activity, the reaction mixture in a volume of 3 ml contained 40 mM imidazole-HCl buffer (pH 7.0), 30 mM L-glutamine, 0.4 mM ADP, 20 mM sodium arsenate, 60 mM $NH_2OH$, 0.1 ml enzyme preparation (3.5 μg protein) and specified concentrations of various cations.

| Divalent cation | Biosynthetic activity[1] | | | | Transferase activity[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration of cations (mM) | | | | Concentration of cations (mM) | | | |
| | 1 | 5 | 20 | 50 | 0.1 | 1 | 5 | 10 |
| $Mg^{2+}$ | 38 | 358 | 764 | 800 | 28 | 388 | 1008 | 1465 |
| $Mn^{2+}$ | 107 | 124 | 12 | 14 | 2233 | 2882 | 2936 | 3456 |
| $Co^{2+}$ | 117 | 297 | 512 | 505 | 54 | 1027 | 1553 | 1493 |
| $Cu^{2+}$ | 20 | 33 | 20 | 28 | 25 | 35 | 50 | 28 |
| $Fe^{2+}$ | 48 | 66 | 102 | 112 | 35 | 91 | 359 | 158 |
| $Ca^{2+}$ | 36 | 13 | 2 | 2 | 38 | 10 | 19 | 3 |
| $Zn^{2+}$ | 40 | 50 | 104 | 111 | 27 | 25 | 35 | 10 |
| $Ni^{2+}$ | 41 | 26 | 13 | 13 | 35 | 25 | 25 | 6 |

[1]nmole Pi produced in 15 minutes at 30°C.
[2]nmole γ-glutamylhydroxamate produced in 15 minutes at 30°C.

enzyme (361,000) and its subunits (45,000) are in the

**Table 4.** *Effects of divalent cations on biosynthetic and transferase activities of glutamine synthetase*

A series of specified concentrations of various cations were added to both reaction mixtures for assay of biosynthetic and transferase activities. The enzyme was preincubated with these reaction mixtures for 10 minutes and assays were performed as described in materials and methods except that the biosynthetic reaction was started by adding the L-glutamate. Biosynthetic and transferase activities were determined from the rate of formation of Pi and γ-glutamyl hydroxamate, respectively.

| Divalent cation added | % Inhibition of biosynthetic activity | | | | % Inhibition of transferase activity | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration of cations (mM) | | | | Concentration of cations (mM) | | | |
| | 1 | 5 | 20 | 50 | 0.1 | 1 | 5 | 10 |
| None | 0 | | | | 0 | | | |
| $Mg^{2+}$ | — | — | — | — | 23 | 25 | 32 | 33 |
| $Mn^{2+}$ | 84 | 93 | 97 | 98 | — | — | — | — |
| $Co^{2+}$ | 20 | 20 | 21 | 25 | 24 | 33 | 60 | 61 |
| $Cu^{2+}$ | 11 | 38 | 94 | 95 | 24 | 41 | 65 | 94 |
| $Fe^{2+}$ | 71 | 78 | 88 | 90 | 28 | 79 | 86 | 92 |
| $Ca^{2+}$ | 25 | 61 | 89 | 92 | 29 | 69 | 97 | 99 |
| $Zn^{2+}$ | 22 | 52 | 81 | 91 | 26 | 67 | 99 | 99 |
| $Ni^{2+}$ | 10 | 27 | 80 | 97 | 24 | 27 | 35 | 70 |

5.) also indicated that these ligands effectively protect the enzyme activity, and $Mg^{2+}$ is more effective than 2-mercaptoethanol. The most striking difference between the bamboo shoot enzyme and enzymes from other plants is its pH optimum for the $Mg^{2+}$-dependent

concentrations of divalent cations required for both the biosynthetic and the transferase activities. It has also been found the glutamine synthetase isolated from pea leaves (O'Neal and Joy, 1974), *Anabaena cylindrica* (Sawhney and Nicholas, 1978) and *Phormidium laminosum* (Blanco *et al.*, 1989). It is possible that these cations can stabilize different conformational states of the enzyme.

The presence of more than one divalent metal ions in reaction mixtures can greatly affect the enzyme activity. The effect of different divalent cations on the $Mg^{2+}$-dependent biosynthetic activity and the $Mn^{2+}$-dependent transferase activity of the bamboo shoot enzyme (Table 4) indicated that all divalent cations tested exhibited inhibitory effects. $Mn^{2+}$ and $Fe^{2+}$ strongly inhibited the $Mg^{2+}$-dependent activity of the enzyme. O'Neal and Joy (1974) also reported that $Mn^{2+}$, $Ca^{2+}$ and $Zn^{2+}$ sharply inhibited the $Mg^{2+}$-dependent activity of the pea leaf glutamine synthetase. It is probable that these divalent cations are competing with $Mg^{2+}$, and that they have a much higher binding constant; however, it is also possible that these cations may have binding sites distinct or partially distinct from the $Mg^{2+}$ binding site. An alternative possibility is that all cations compete for the same $Mg^{2+}$ binding site on the enzyme molecule, but that the initial binding is followed by secondary reactions that vary with the kind of cation bound, thereby yielding a different conformational state for each different cation which

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# 孟宗竹筍中麩醯胺合成酶之純化及特性之探測

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孟宗竹(*Phyllostachys edulis*)筍中之麩醯胺合成酶，經分子篩濾，離子交換和親和力層析等純化步驟後，可純化至視均一之程度。其比活性約增加 1400 倍，回收率可達 20%以上。此酶之分子量爲 361,000，具有 8 個同型子單元，分子量皆爲 45,000。於合成酶活性之基質中，L-Glutamate 及 ATP 之視 Km 值分別爲 2.79 mM 及 1.76 mM；但 $NH_4Cl$ 則呈現正協同性作用，其 Hill 係數爲 1.5，$S_{0.5}$ 爲 0.54 mM。轉移酶活性之基質中，L-Glutamine 及 Hydroxylamine 之視 Km 值分