Jpn. J. Phycol. (Sôrui) 39: 385–387, December 10, 1991

Yasushi Fujimori, Katsuhito Nakamura, Yuichi Maruoka, Tsuneo Matsubayashi and Goro Tamura: Immunological comparison of algal cysteine synthases

Key Index Words: Chlorella—*cysteine synthase*—*immunological comparison*—Nannochloropsis—Porphyra—Scenedesmus—Spirulina.

Yasushi Fujimori, Goro Tamura, Department of Agricultural Chemistry, Faculty of Horticulture, Chiba University, Matsudo, Chiba, 271 Japan
Katsuhito Nakamura, (Present Adress) Department of Biology, Faculty of General Education, Gifu University, Gifu, 501-11 Japan
Yuichi Maruoka, (Present Adress) The Calpis Food Industry Co., LTD, Fuchinobe 5-11-10, Sagamihara, Kanagawa, 229 Japan
Tsuneo Matsubayashi, Chlorella Industry Co., LTD, Hisatomi 1343, Chikugo, Fukuoka, 833 Japan

Cysteine synthase (CSase, EC 4.2.99.8) catalyzes the synthesis of L-cysteine from *O*-acetyl-L-serine and sulfide. CSases from a variety of higher plants have been extensively purified and characterized (Masada *et al.* 1975, Tamura *et al.* 1976, Bertagnolli and Wedding 1977, Murakoshi *et al.* 1985, Ikegami *et al.* 1988, Nakamura and Tamura 1989), however relatively little is known about the properties of algal CSases (Schmidt 1977, Leon *et al.* 1987, Diessner and Schmidt 1981, Fujimori *et al.* 1991). In the present experiment, the antiserum raised against *Chlorella* and *Porphyra* CSases have been prepared and, using these antiserum, the immunological comparison of CSases from five species of algae [*Chlorella* and *Scenedesmus* (Chlorophyceae); *Nannochloropsis* (Eustigmatophyceae); *Porphyra* (Rodophyceae); *Spirulina* (Cyanophyceae)] was examined.

For the development of anti-*Chlorella* CSase serum, the purification of the enzyme was performed by the procedures described in the previous paper (Nakamura and Tamura 1989). The purified *Chlorella* CSase had a specific activity of 270 μmol L-cysteine formed per min per mg of protein and also showed a single band in PAGE. The purified enzyme was emulsified with Freund's complete adjuvant and injected subcutaneously into a previously unimmunized rabbit. Each rabbit was injected 4 times in the same manner. After 8 weeks, whole blood was collected by cardiac puncture and the serum was obtained after centrifugation. As for *Porphyra* CSase, the methods for purification and preparation of the antiserum were described in the previous paper (Fujimori *et al.* 1991). The *Porphyra* CSase had a specific activity of 408 μmol cysteine formed per min per mg protein and showed a single band in PAGE.

Table 1 shows the presence of CSase in several algae of different families. Each alga in the table was macerated in 30 mM Tris-HCl buffer (pH 8.0) containing 10 mM 2-mercaptoethanol and 0.5 mM EDTA and homogenized by sonication except *Porphyra* that was extracted by autolysis, and the homogenate was subjected to enzyme assay.

Specific activity is expressed as cysteine formation in 1 μmol per min per mg protein of crude extract. On a wet weight basis, 16 to 30-fold amount of activities compared with that from *Porphyra* and *Spirulina* were detected in *Chlorella* and *Nannochloropsis*, respectively. When expressed on a protein basis, however, algal preparations exhibited approximately the same value except for *Spirulina*.

As shown in Fig. 1, the anti-*Chlorella* CSase thoroughly inactivated the CSase from *Chlorella, Scenedesmus* and *Nannochloropsis*. However, this anti CSase serum was almost ineffective in inactivating the *Porphyra* and *Spirulina* enzymes (Fig. 1A). The amount of anti-*Chlorella* serum, which caused 50% inhibition of *Chlorella, Scenedesmus* and *Nannochloropsis*

Table 1. Cysteine synthase activity in several algae.

Species	CSase Activity (units/g wet weight)	Specific Activity (units/mg protein)
Chlorella[a]	24.0	1.09
Scenedesmus[a]	7.1	0.76
Nannochloropsis[b]	39.0	0.84
Porphyra[c]	1.3	0.34
Spirulina[d]	1.5	0.08

[a] *Chlorella* (*Chlorella* sp.) and *Scenedesmus* (*Scenedesmus* sp.) were grown in the medium which contained, per liter: KNO_3, 2.5 g; KH_2PO_4, 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; Fe-EDTA, 15 mg; H_3BO_3, 2.86 mg; $MnCl_2 \cdot 4H_2O$, 1.81 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg and Na_2MoO_4, 0.021 mg (pH 7.0).

[b] *Nannochloropsis* (*Nannochloropsis oculata*) were grown in the medium which contained, per liter: $(NH_4)_2SO_4 \cdot 5H_2O$, 100–300 mg; calcium superphosphate, 15–20 mg; urea, 5 mg; CLEWAT-32 (purchased from Teikoku Kagaku Sangyou Co.) (pH 8.0). After the cultivation in this medium for few days, following supplemental elements were added, per liter of medium: KNO_3, 100 mg; Na_2HPO_4, 20 mg; Fe-EDTA, 3 mg.

[c] *Porphyra* (*Porphyra yezoensis*) was grown in Tokyo bay in winter.

[d] *Spirulina* (*Spirulina platensis*) was cultured by the method of Ogawa and Terui (1970). It was done in the SOT medium (pH 9.0–9.2) at 33°C, under continuous light (7–10 klux).

[a,b] Light (5–10 klux) and air containing CO_2 (1–2%) were supplied.

CSase activities were 10, 7 and 16 μl, respectively.

On the other hand, anti-*Porphyra* CSase reacted most with *Porphyra* CSase only slightly with the *Chlorella, Nannochloropsis* and *Spirulina* CSase, and to an intermediate degree with the *Scenedesmus* CSase (Fig. 1B). Then, the 50% inhibition values for the anti-*Porphyra* CSase to *Porphyra, Scenedesmus, Nannochloropsis, Chlorella* and *Spirulina* CSase were calculated to be 9, 35, >140, >250 and >250 μl, respectively.

Based on the immuno-inactivation results it appears that *Chlorella* and *Nannochloropsis* CSases are immunologically closely related, although not identical, while both CSases differ substantially from the *Scenedesmus* CSase. Moreover, the total lack in cross-reactivity between these serum and the CSase from *Spirulina* (Fig. 1A, B) may indicate that the surface protein structure of Cyanophyceae CSase is widely different from that of Chlorophyceae, Eustigmatophyceae and Rodophyceae.

The magnitude of the reactivities of antiserum were as follows: anti-*Chlorella* CSase (*Scenedesmus* > *Chlorella* and *Nannochloropsis* > > *Porphyra* and *Spirulina*); anti-*Porphyra* CSase (*Porphyra* > *Scenedesmus* > > *Spirulina, Chlorella* and *Nannochlorosis*). Then we can speculate that the order of the antigenic

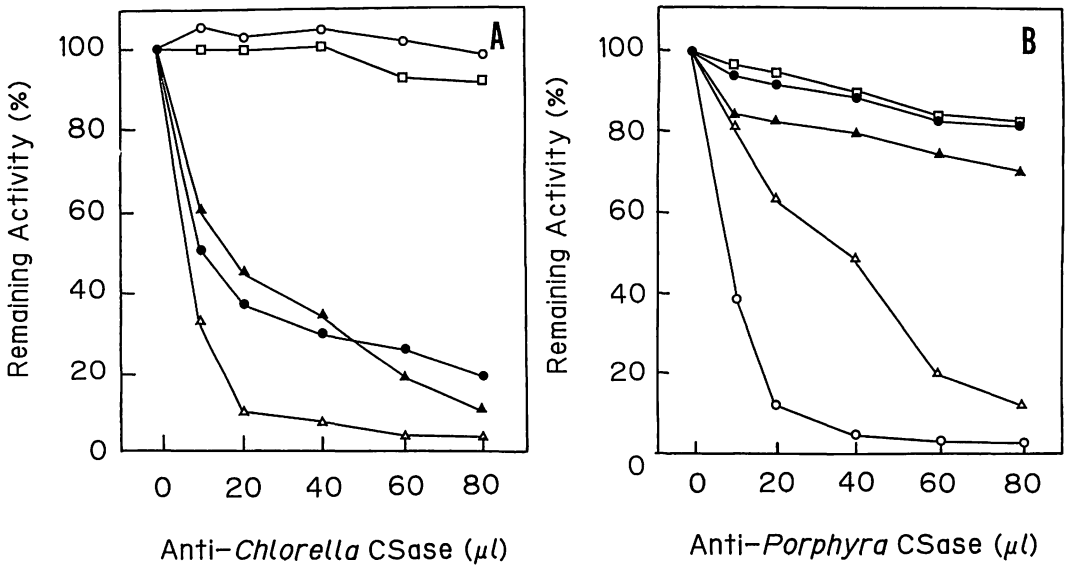

Fig. 1. Immuno-inactivation of the CSase from *Chlorella* (●), *Scenedesmus* (△), *Nannochloropsis* (▲), *Porphyra* (○) and *Spirulina* (□). Titrations were performed with anti-*Chlorella* CSase (A) and anti-*Porphyra* CSase (B). Extraction of CSases from the thalli was done by sonication except *Porphyra* enzyme which was extracted by autolysis.

reactivity between *Chlorella* and other four species are; *Chlorella-Nannochloropsis-Scenedesmus---Porphyra---Spirulina.*

Nannochloropsis had been belonging to Chlorophyceae group (Droop 1955). However, it was reclassified to Eustigmatophyceae (a kind of Xanthophyceae) for the particular difference of the chloroplast pigment composition and cell structures (Antia *et al.* 1975, Hibberd 1981). It is so curious that the *Chlorella* CSase is immunologically more closely related to the enzyme from *Nannochloropsis* than that from *Scenedesmus*. On the other hand, *Scenedesmus* CSase had some common antigenic determinants of the enzyme not only in *Chlorella* but also in *Porphyra.*

Acknowledgements

We are grateful to Mr. Shigeru Araki of Yamamoto Nori Research Laboratory for providing us with *Porphyra* thalli, and Dr. Keishiro Wada of Kanazawa University for donation of *Spirulina platensis* OU-1 strain. We also thank Dr. Kazuo Fukushima of Nihon University School of Dentistry at Matsudo, for his guidence in preparation of the antiserum.

References

Antia, N. J., Bisalputra, T., Cheng, J. Y. and Kalley, J. P. 1975. Pigment and cytological evidence for reclassification of *Nannochloris oculata* and *Monallantas salina* in the Eustigmatophyceae. J. Phycol. **11:** 339–343.

Bertagnolli, B. L. and Wedding, R. T. 1977. Purification and initial kinetic characterization of different forms *O*-Acetylserine sulfhydrylase from seedlings of two species of *Phaseolus.* Plant Physiol. **60:** 115–121.

Diessener, W. and Schmidt, A. 1981. Isoenzymes of cysteine synthase in the cyanobacterium *Synechococcus* 6301. Z. Pflanzenphysiol. **102:** 57–68.

Droop, M. R. 1955. Some new supra-littoral *Protista.* J. Marine Biol. Asc. of the U. K. **34:** 233–245.

Fujimori, Y., Nakamura, K. and Tamura, G. 1991. Cysteine synthase from a red alga *Porphyra yezoensis*: purification and properties. Jpn. J. Phycol. **39:** 7–14.

Hibberd, D. J. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Bot. J. of the Linnean Soci. **82:** 93–119.

Ikegami, F., Kaneko, M., Kamiyama, H. and Murakoshi, I. 1988. Purification and characterization of cysteine synthases from *Citrullus vulgaris.* Phytochemistry **27:** 697–701.

Leon, J., Romero, F., Galvan, F. and Vega, J. M. 1987. Purification and physicochemical characterization of *O*-acetyl-L-serine sulfhydrylase from *Chlamydomonas reinhardtii.* Plant Science **53:** 93–99.

Masada, M., Fukushima, K. and Tamura, G. 1975. Cysteine synthase from rape leaves. J. Biochem. **77:** 1107–1115.

Murakoshi, I., Ikegami, F. and Kaneko, M. 1985. Purification and properties of cysteine synthase from *Spinacia oleracea.* Phytochemistry **24:** 1907–1911.

Nakamura, K. and Tamura, G. 1989. Five isoforms of cysteine synthase in rape leaves. Agric. Biol. Chem. **53:** 2537–2538.

Ogawa, T. and Terui, G. 1970. Studies on the growth of *Spirulina platensis* (I) On the pure culture of *Spirulina platensis.* J. Ferment. Technol. **48:** 361–367.

Schmidt, A. 1977. Exchange of cysteine-bound sulfide with free sulfide and cysteine synthase activity in *Chlorella pyrenoidosa.* Z. Pflanzenphysiol. **84:** 435–449.

Tamura, G., Iwasawa, T., Masada, M. and Fukushima, K. 1976. Some properties of cysteine synthase from radish roots. Agric. Biol. Chem. **40:** 637–638.

藤森　泰・中村勝人・丸岡裕一・松林恒夫*・田村五郎：藻類システイン合成酵素の免疫学的比較

緑藻 *Chlorella* 及び紅藻 *Porphyra* からそれぞれ精製したシステイン合成酵素 (CSase) を抗原として用いて，それぞれの酵素に対する抗 CSase 血清を作製し，5 種類の藻類 (*Chlorella, Scenedesmus, Nannochloropsis, Porphyra, Spirulina*) の CSase について，免疫滴定を行ない比較を試みた。その結果，*Chlorella* と真正眼点藻 *Nannochloropsis* の CSase は共に 2 種の抗 CSase 血清に対し類似した反応を示し，免疫学的に近い関係が見られた。緑藻 *Scenedesmus* の CSase は，上記のものとは異なる滴定曲線を示し，*Chlorella* 及び *Nannochloropsis* の CSase と免疫学的にかなり異なることが示唆された。また，藍藻 *Spirulina* の CSase は，両抗 CSase 血清にほとんど反応を示さなかった。(271 千葉県松戸市松戸648　千葉大学園芸学部生物化学研究室，*833福岡県筑後市久富1343　クロレラ工業株式会社)