Jpn. J. Phycol. (Sôrui) **36**: 202–211, September 20, 1988

The characteristics of photosynthesis and carbon metabolism in *Heterosigma akashiwo* (Raphidophyceae)

Kyoko TAKAHASHI and Tomoyoshi IKAWA

Institute of Biological Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki 305, Japan

TAKAHASHI, K. and IKAWA, T. 1988. The characteristics of photosynthesis and carbon metabolism in *Heterosigma akashiwo* (Raphidophyceae). Jpn. J. Phycol. **36**: 202–211.

The characteristics of photosynthetic CO_2 fixation were studied in the marine raphidophycean flagellate *Heterosigma akashiwo*. The rate of photosynthetic CO_2 fixation was saturated about 150 $W \cdot m^{-2}$ and was not inhibited by higher light intensities at least up to 500 $W \cdot m^{-2}$. Maximum rate of photosynthetic CO_2 fixation was about 300 μmol CO_2 mg Chl.a^{-1}. hr^{-1}. The rate was saturated at about 1 mM $NaHCO_3$ and half-saturation for $NaHCO_3$ was about 0.1 mM. Time course of ^{14}C-incorporation into photosynthetic products showed that 3-phosphoglycerate was the initial product, and 80% methanol-soluble β-1,3-glucans were the main reserve products of photosynthetic CO_2 fixation. Pattern of dark $^{14}CO_2$ fixation after preillumination also suggests that photosynthetic CO_2 fixation in this alga may be carried out by the reductive pentose phosphate cycle (C_3 cycle).

The effect of oxygen on the rate of photosynthetic $^{14}CO_2$ fixation was also studied. The highest rate was obtained under 2% O_2. The rate under 100% O_2 was 30% lower than that under 2% O_2. Under 100% O_2 relatively low levels of intermediates of photorespiratory pathway such as glycolate, serine and glycine were accumulated.

These results indicate that *H. akashiwo* has high photosynthetic activity even under the conditions of the high light, low CO_2, and high O_2 concentrations.

Key Index Words: Dark CO_2 fixation—Heterosigma akashiwo (*Raphidophyceae*)—*Light-enhanced dark CO_2 fixation*—Olisthodiscus luteus—*Photosynthesis*—*Photosynthetic CO_2 fixation*—*Storage product.*

The marine raphidophycean flagellate *Heterosigma akashiwo* (Hada) Hada is one of the most abundunt phytoplankton species in the temperate coastal waters of Japan. Previously, this alga was usually referred to as *Olisthodiscus luteus*, but it was recently pointed out that this species should be treated under the name of *H. akashiwo* (HARA *et al.* 1985). In recent years, the number of investigations of the ecology and physiology of this alga has increased considerably since the recognition of its importance as a principal organism in "red tide" blooms (FUKAZAWA *et al.* 1980, HATANO *et al.* 1983, TAKAHASHI and FUKAZAWA 1982, TOMAS 1979, 1980, WADA *et al.* 1985, WATANABE *et al.* 1982).

However, relatively little is known about the photosynthetic process in this alga. TOMAS (1980) has reported the effects of light intensity and temperature on the rate of photosynthesis and the cellular concentrations of nitrogen and carbon in an axenic clone of *O. luteus* following incubation in both indoor and out-door growth chambers. The major photosynthate of *O. luteus* was reported by BIDWELL (1957) to be mannitol, and HELLEBUST (1965) also found it to be the major carbon compound excreted from this alga. However, no investigation has been carried out on the

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (59390002, 60040062, 62304006) and grant from the Nissan Science Foundation.

carbon pathway during photosynthesis, nor the effect of oxygen on photosynthetic carbon metabolism in the raphidophycean algae including *H. akashiwo*.

The purpose of the present study is to characterize photosynthesis and photosynthetic carbon metabolism and to elucidate carbon fixation in the dark in *H. akashiwo*.

Materials and Methods

Algal culture

Heterosigma akashiwo (Hada) Hada was obtained from M. TAKAHASHI, Department of Botany, University of Tokyo. The alga was originally isolated by S. YAMOCHI, Osaka Prefecture Fisheries Experimental Station, from Tanigawa Fishing Port, Osaka Bay in 1979. Cells were grown axenically in 2-liter Erlenmeyer flasks containing 1 liter of PES medium (PROVASOLI, 1968), together with Jamarine S artificial seawater (Jamarine Laboratory, Osaka, Japan) at 18‰ salinity and enriched with 200 mg $NaNO_3$ and 40 mg Na_2HPO_4 per liter of medium. The medium was adjusted to pH 8.0 with KOH. Illumination was provided by cool-white fluorescent tubes at an intensity of about $12\ W \cdot m^{-2}$ at flask level under continuous bubbling with ordinary air.

Cells in a late exponential phase of growth (6–7 days old) were harvested by gentle filtration through Millipore filter SM (5 μm pore size), washed three times with reaction medium containing 25 mM HEPES and enriched PES medium (pH 8.0), and resuspended in the medium at a concentration of 5–10 μg chlorophyll *a* per m*l*. A small amount of silicon (Toshiba Silicon) was added to the reaction medium to prevent foaming.

Photosynthetic $^{14}CO_2$ fixation

Photosynthetic $^{14}CO_2$ fixation was carried out using 1 m*l* or 6 m*l* of algal suspension placed in spitz-type test tube (15×145 mm or 30×164 mm) at 23°C, and bubbled with CO_2-free air from a long hypodermic needle at a flow rate of $120\ ml \cdot min^{-1}$ throughout preillumination and subsequent photosynthetic $^{14}CO_2$ fixation. The tube was illuminated from one side with a halogen lamp. After 10-min preillumination, photosynthetic $^{14}CO_2$ fixation was started by injecting 240 μCi ($56.1\ mCi \cdot mmol^{-1}$) $NaH^{14}CO_3$ per m*l* of algal suspension, and stopped by treating with methanol as described below. After a scheduled photosynthetic period, suspending algal cells were collected quickly with suction through a glass-filter disc (Whatman GF/A, 25 mm diameter) and the cells were dipped into 80% hot methanol together with the disc. Illumination was continued throughout these processes. The algal suspension was heated in a water bath at 65°C for 5 min. After removal of glass-fiber disc, the suspension was acidified by the addition of acetic acid. A part of the algal suspension was then analysed for ^{14}C fixation products.

Analysis of $^{14}CO_2$-fixation products

The algal cells suspended in methanol were filtered through a Millipore filter (HA type, 0.45 μm pore size, 25 mm diameter). The cells on the membrane filter were extracted several times with a small amount of 80% hot methanol. Extracts were combined (80% methanol-soluble fraction) and a portion of the mixture was removed to determine the radioactivity. The radioactivity of the residue on the membrane filter (80% methanol-insoluble fraction) was determined with a liquid scintillation spectrometer. The rest of methanol extract was dried *in vacuo* at 35°C and dissolved in a small amount of 80% methanol to be chromatograph two-dimensionally on Whatman No. 3MM filter paper. The individual compounds were identified as described by SUZUKI and IKAWA (1985). Free sugars used as standards were also co-chromatographed on Toyo filter paper No. 50 with solvent systems, *n*-butanol-acetic acid-water (5:4:2 v/v), *n*-butanol-pyridine-water (6:4:3 v/v) (FRENCH and WILD, 1953), and ethyl acetate-pyridine-water (6:4:3) (WHISTLER

and HICKSON, 1954).

The three spots of ^{14}C-compounds left in the lower Rf regions after two-dimensional chromatography were eluted with water for treatment with β-1,3-glucanase, which was prepared from *Trichoderma viride* by the method of HORITSU *et al.* (1973). The resulting product giving one radioactive spot on re-chromatography was co-chromatographed with authentic glucose.

Determination of chlorophyll a

Chlorophyll *a* was measured spectrophotometrically in methanol extracts by the procedure of IWAMURA *et al.* (1970).

Results

Effects of light intensity and $NaHCO_3$ concentration on photosynthetic CO_2 fixation

Fig. 1 shows the rate of photosynthesis under ambient air condition as a function of light intensity. The rate of photosynthetic CO_2 fixation was saturated at about 150 $W \cdot m^{-2}$ and was not inhibited by higher light intensities at least up to 500 $W \cdot m^{-2}$. Maximum rate of photosynthetic CO_2 fixation was about 300 μmol CO_2 mg Chl.$a^{-1} \cdot hr^{-1}$.

Fig. 2 shows the effect of $NaHCO_3$ concentrations on the rate of photosynthetic $^{14}CO_2$ fixation at pH 8.0. The rate was saturated at about 1.0 mM and half-saturation for $NaHCO_3$ was 116 μM. These data suggested that characteristics of photosynthetic CO_2 fixation in this alga adapted to a higher light intensisty and a lower concentration of inorganic carbons.

Time course of photosynthetic CO_2 fixation

The total amount of photosynthetic CO_2 fixation increased linearly for 10 min at 0.7 mM $NaHCO_3$ and 250 $W \cdot m^{-2}$ (Fig. 3). The percent of ^{14}C incorporated into the 80% methanol-soluble fraction attained about 84% while those into the insoluble fraction was only 16% after 10-min $^{14}CO_2$ fixation. In addition, about a half of ^{14}C of the latter was localized in the β-1,3-glucans during this period (data not shown). Time course of ^{14}C incorporation into individual compounds are shown in Fig. 4. More than 80% of ^{14}C in the methanol-soluble fraction after 15-sec photosynthesis was incorporated into 3-phosphoglycerate (PGA) and a small portion of other sugar phosphates, but it decreased quickly thereafter. In contrast, the radioactivities of

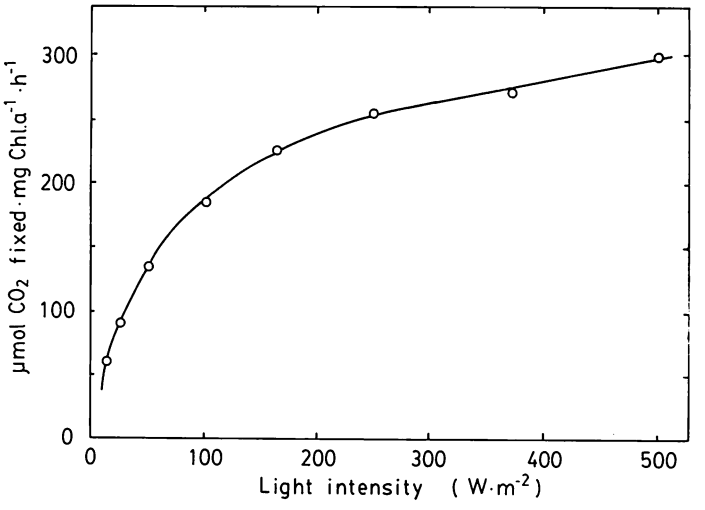

Fig. 1. Effect of light intensity on the rate of photosynthetic $^{14}CO_2$ fixation in *Heterosigma akashiwo* cells under ambient air condition. The rate was calculated from the amount of ^{14}C fixed for 5 min after 10-min preillumination. Incubation temperature and $NaHCO_3$ concentration were 23°C and 0.7 mM, respectively. Other experimental conditions are described in the text.

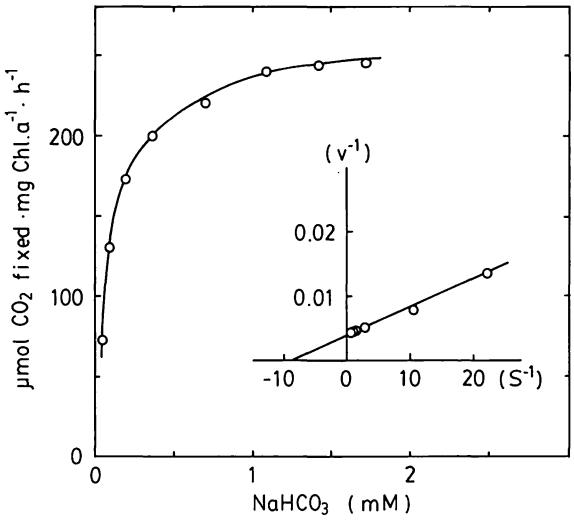

Fig. 2. Effect of $NaHCO_3$ concentration on the rate of photosynthetic $^{14}CO_2$ fixation in *Heterosigma akashiwo* cells under ambient air condition. Chlorophyll *a* content and the light intensity were 5.2 $\mu g \cdot ml^{-1}$ and 250 $W \cdot m^{-2}$. Other experimental conditions are described in the text.

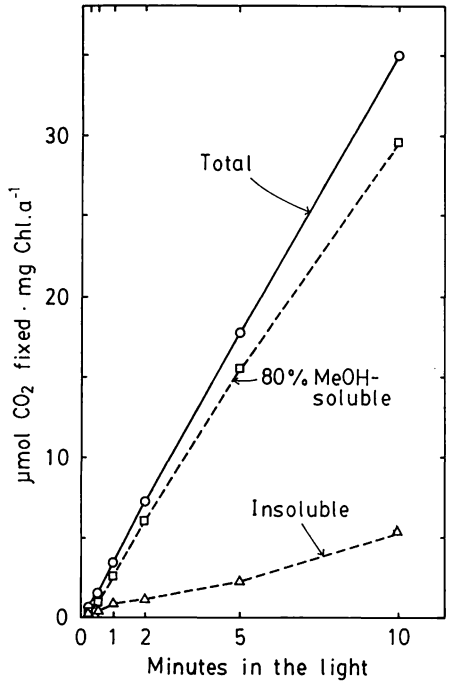

Fig. 3. Time course of ^{14}C-incorporation into 80% methanol-soluble and -insoluble fraction during photosynthetic $^{14}CO_2$ fixation in *Heterosigma akashiwo* cells under ambient air condition. Light intensity and $NaHCO_3$ concentration were 250 $W \cdot m^{-2}$ and 0.7 mM, respectively. Other experimental conditions are described in the text. ○, total activity; □, 80% methanol-soluble fraction; △, 80% methanol-insoluble fraction.

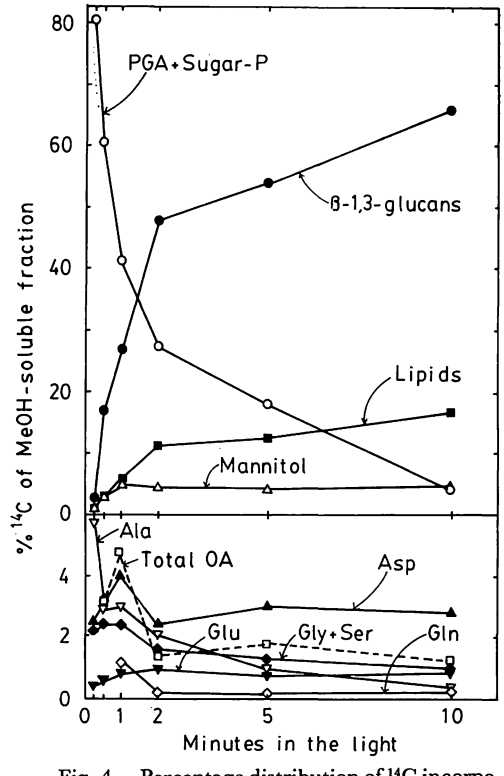

Fig. 4. Percentage distribution of ^{14}C incorporated into individual products *versus* time of photosynthetic $^{14}CO_2$ fixation in *Heterosigma akashiwo* cells. Data are from the experiment described in Fig. 3. Symbols: Ala, alanine; Asp, aspartate; Gln, glutamine, Glu, glutamate; Gly, glycine; Ser, serine; Total OA, total organic acids such as malate, succinate and glycolate.

amino acids as well as β-1,3-glucan are remarkably increased for the first few minutes. The results clearly indicate that PGA was the first product of CO_2 fixation, and that radioactivity was transferred to β-1,3-glucans and amino acids. It may well be that, therefore, the pohtosynthetic CO_2 fixation in *H. akashiwo* is mainly carried out through the reductive pentose phosphate cycle. It should be pointed out here that the ^{14}C-incorporation into β-1,3-glucans of 80% methanol-soluble fraction rapidly increased with time to occupy about 60% of the total activity after 10-min photosynthesis, whereas a very small amount of the activity, i.e. about 4% of the total, were incorporated into mannitol.

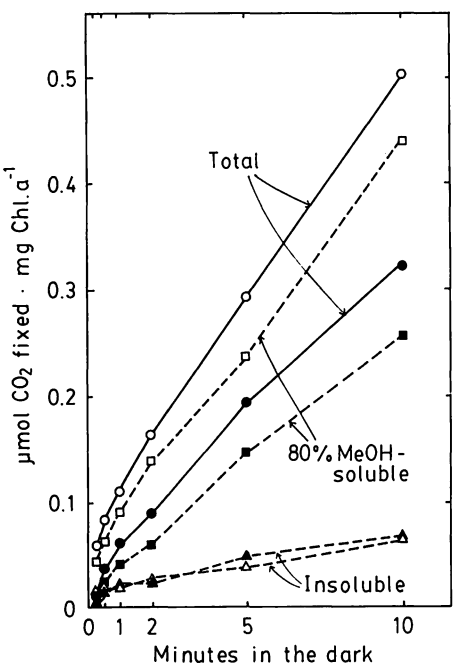

Fig. 5. Time course of ^{14}C-incorporation into 80% methanol-soluble and -insoluble fractions during dark $^{14}CO_2$ fixation by preilluminated and non-preilluminated cells of *Heteosigma akashiwo*. Open symbols: dark $^{14}CO_2$ fixation after 10-min preillumination (250 $W \cdot m^{-2}$) under CO_2-free air condition; closed symbols: dark $^{14}CO_2$ fixation without preillumination. $NaH^{14}CO_3$ solution (0.7 mM) was added in the dark immediately after turning off the light or after 20 min of continuous darkness.
○, ●, total activity; □, ■, 80% methanol-soluble fraction; △, ▲, 80% methanol-insoluble fraction.

Time courses of dark $^{14}CO_2$ fixation by preilluminated and non-preilluminated cells

The incorporation of ^{14}C in the non-preilluminated cells proceeded almost linearly with time and the rate of dark CO_2 fixation was only 1% of that of photosynthesis (Fig. 5). Incorporation of ^{14}C during 10-min dark CO_2 fixation was 40% larger in amount in the preilluminated cells than that in non-preilluminated ones (Fig. 5).

Time courses of ^{14}C incorporation in the individual products during dark $^{14}CO_2$ fixation with and without preillumination are shown in Figs 6 and 7. Aspartate and glutamate were the major products of dark $^{14}CO_2$ fixation with or without preillumination. On the other hand, most of ^{14}C was incorporated into PGA immediately after the addition of $^{14}CO_2$ to the preilluminated cells, but it decreased rapidly during the rest of the time periods. The percentages of ^{14}C incorporations into aspartate and glutamate increased initially with time in either

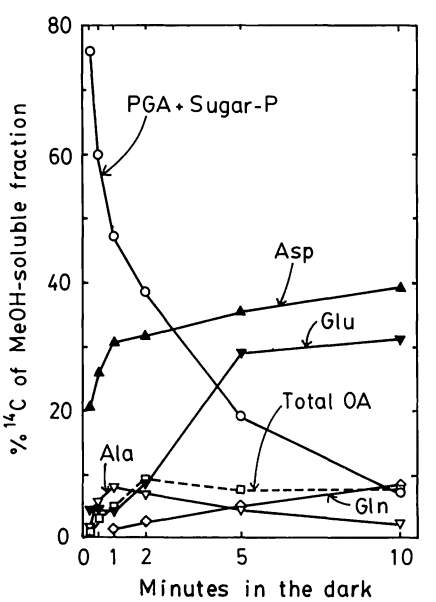

Fig. 6. Percentage distribution of ^{14}C incorporated into individual products *versus* time of dark $^{14}CO_2$-fixation after 10-min preillumination. Data are from the experiment described in Fig. 5. ○, PGA+sugar phosphates; ▽, alanine; ▲, aspartate; ▼, glutamate; ◇, glutamine; □, total organic acids.

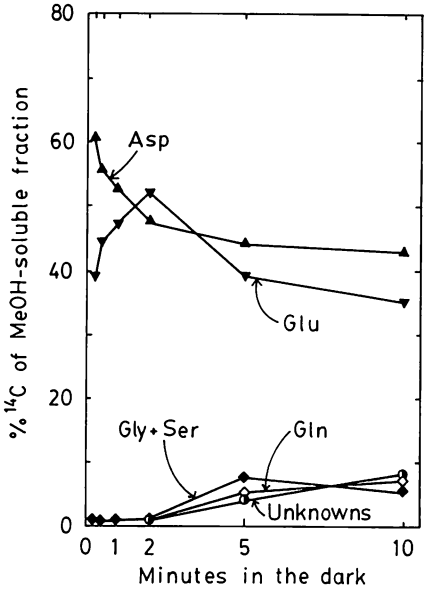

Fig. 7. Percentage distribution of ^{14}C incorporated in individual products *versus* time of dark $^{14}CO_2$-fixation without preillumination. Data are from the experiment described in Fig. 5. ◆, glycine+serine; ◑, unknown compounds, others see legend for Fig. 6.

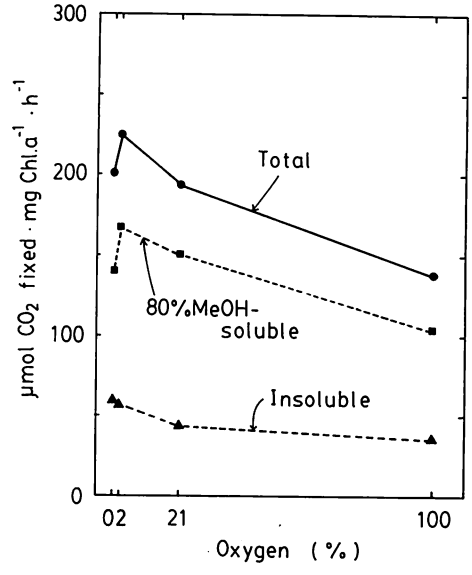

Fig. 8. Effect of oxygen concentration on the distribution of ^{14}C in the 80% methanol-soluble and -insoluble fractions in *Heterosigma akashiwo* cells. The rate was calculated from the amount of ^{14}C fixed for 5 min at 23°C. Light intensity and $NaHCO_3$ concentration were 250 W·m^{-2} and 0.7 mM, respectively. ●, total activity; ■, 80% methanol-soluble fraction; ▲, 80% methanol-insoluble fraction.

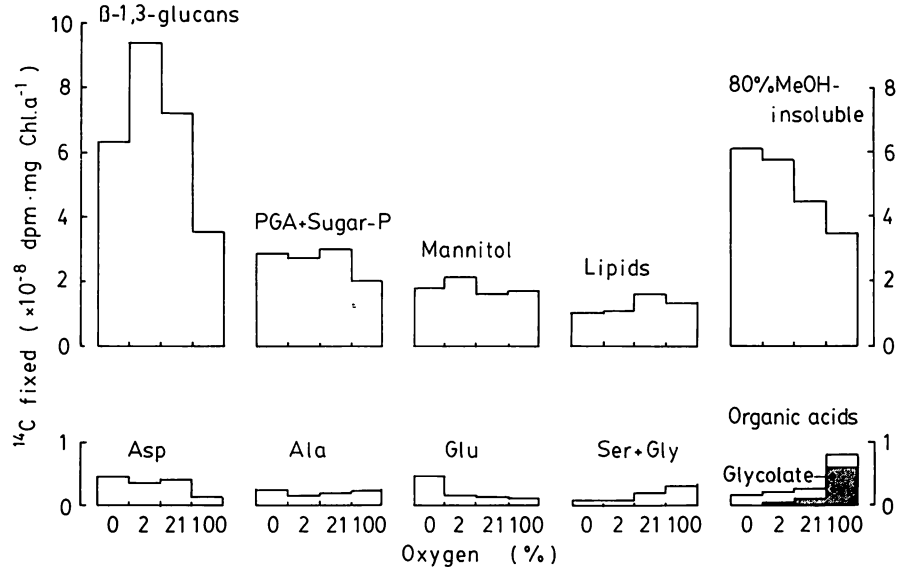

Fig. 9. Effect of oxygen concentration on the distribution of ^{14}C incorporated in individual products during 5-min photosynthetic $^{14}CO_2$ fixation. Data are from the experiment described in Fig. 8. Symbols: Ala, alanine; Asp, aspartate; Glu, glutamate; PGA+Sugar-P, PGA+sugar phosphates; Ser+Gly, serine+glycine.

preillumination or non-preillumination, but it decreased gradually thereafter, while those into other several amino acids, though they were small in amount, tend to increase slightly (Fig. 7).

Effect of oxygen on the photosynthetic CO_2 fixation

The effect of O_2 concentration on the rate of photosynthetic $^{14}CO_2$ fixation was determined at a high light intensity (250 $W \cdot m^{-2}$). As shown in Fig. 8, the highest rate was obtained under 2% O_2. Increase in the O_2 concentration above 2% caused decrease in the rate of photoysnthesis. The rate under 100% O_2 was 30% lower that under 2% O_2. Under anaerobic condition, the rate of photosynthesis was inhibited to about 10% of that under 2% O_2. Since a considerably large amount of ^{14}C was fixed under this concentration of O_2 in the 80% methanol-soluble fraction, some amount of O_2 seemed absolutely to favor ^{14}C-incorporation into this fraction.

Fig. 9 shows the effect of O_2 concentration on the distribution of ^{14}C in the products of 5-min photosynthetic $^{14}CO_2$ fixation. The amount of ^{14}C in 80% methanol-soluble β-1,3-glucans, was predominantly influenced by the O_2 concentration. Although the amounts of ^{14}C in glycolate, glycine and serine increased with increasing O_2 concentration, they were very small under O_2 concentrations up to 21%, and that in glycolate was only 4% of the total ^{14}C fixed even under 100% O_2. These results suggest that photorespiration occurs during photosynthesis at O_2 concentrations higher than 21% at saturating $NaHCO_3$ concentration (0.7 mM), but its inhibitory contribution to the photosynthesis is not very high.

Discussion

Detailed studies on the metabolic pathways of CO_2 fixation have not been made in raphidophycean algae including *Heterosigma akashiwo*, although numerous contributions have dealt with the ecological and physiological features of this alga and *Olisthodiscus*, a species having been identified later as *Heterosigma*. The data obtained in the present experiments suggest that *H. akashiwo* probably fixed CO_2 via the convensional C_3 pathway because PGA was the main primary product formed photosynthetically, and the label of PGA was subsequently transferred to other compounds as in the manner typical of C_3 plants, while C_4 acids comprised only minor part of the labeled compounds (Fig. 4). The main storage products of this photosynthetic process are 80% methanol-soluble β-1,3-glucans of yet unidentified size.

BIDWELL (1957) reported that *Olisthodiscus* sp. accumulated about 35% of total ^{14}C into mannitol and about 10% into an alcohol-insoluble glucan after 12 hr of photosynthesis in the presence of $H^{14}CO_3^-$, and he concluded that mannitol is the main product of photosynthesis in the alga. On the other hand, HELLEBUST (1965) has shown that *Olisthodiscus* sp. cells excreted about 10% of ^{14}C photoassimilates as mannitol in the log phase of growth during 48 hr of alternate 12-hr light and dark periods and it increased to more than 50% during the stationary growth phase.

In the present experiments, however, the percentage of ^{14}C in mannitol attained a maximum stationary level (4%) after 2 min of photosynthesis (Fig. 4). And the amount of ^{14}C-mannitol excreted was only 1.5% of the total ^{14}C fixed in the cells after 5 min of photosynthesis (data not shown). The percentage of radioactivity incorporated into mannitol differed depending on the culture condition. More than 20% of ^{14}C was incorporated into mannitol when the cells were cultured without aeration. Thus the accumulation of mannitol would seem to depend upon physiological and environmental parameters. These results suggest that mannitol may function in part as an osmotic regulation substance in *H. akashiwo* as reported in prasinophycean algae (ASHINO-FUSE and IKAWA 1981, HELLEBUST 1976, KIRST 1975).

On the other hand, most of the ^{14}C was found in the 80% methanol-soluble β-1,3-glucans, which contained over 56% of the total ^{14}C fixed in the cells after 5 min of photosynthesis (Fig. 4), while the amount of ^{14}C fixed in the 80% methanol-insoluble β-1,3-glucan and lipids, which were considered to be storage products in diatoms and brown algae (CRAIGIE 1974, HANDA 1969, HOLDSWORTH and COLBECK 1976, KREMER and BERKS 1978, YAMAGUCHI *et al.* 1968), was relatively small in amount in comparison with that fixed in the 80% methanol-soluble β-1,3-glucans. Furthermore radioactivity in the 80% methanol-soluble glucans in *H. akashiwo* was markedly decreased during the chase period in the dark (data not shown). It is postulated from these facts that the 80% methanol-soluble β-1,3-glucans are the major storage product of photosynthesis in *H. akashiwo*.

Enhancement of dark CO_2 fixation after preillumination in the absence of CO_2 has been observed both in higher plants and in algae (MIYACHI 1979). Analysis of $^{14}CO_2$ fixation products revealed that the main initial product was PGA in C_3 plants whereas it was malate and aspartate in C_4 plants, and the percentage of radioactivity incorparated in the initial $^{14}CO_2$ fixation products continued to decrease rapidly during the rest of time periods (MIYACHI 1979). Light-enhanced dark CO_2 fixation was also observed in *H. akashiwo* (Fig. 5), although the extent of the enhancement in this alga was smaller than those in *Chlorella* and *Anacystis* (HOGETSU and MIYACHI 1970, MIYACHI 1979). Distribution of radioactivity incorporated in the initial ^{14}C fixation product during light-enhanced dark $^{14}CO_2$ fixation (Fig. 6) was considerably different from those during dark $^{14}CO_2$ fixation without preillumination (Fig. 7). About 76% of the total ^{14}C incorporated was found in PGA after 15 sec of light-enhanced dark $^{14}CO_2$ fixation, but the radioactivity decreased rapidly during the rest of the time periods. The pattern of ^{14}C fixation products during light-enhanced dark $^{14}CO_2$ fixation is consistent with those in C_3 plants (MIYACHI 1979). These result also suggest that *H. akashiwo* is a C_3 plant.

Photosynthesis in terrestrial C_3 plants is inhibited considerably by oxygen even under ambient air conditions (21% O_2, 0.03% CO_2). The inhibition is mainly associated with photorespiration derived from oxygenase activity of RuBP carboxylase/oxygenase (BECK 1979).

Oxygen inhibition of photosynthesis has been also observed in many species of various algal divisions (WARBURG 1920, GAFFRON 1940, TAMIYA and HUZISIGE 1949, BEARDALL and MORRIS 1975, COLEMAN and COLMAN 1980, KREMER 1980, SHELP and CANVIN 1980, BIRMINGHAM *et al.* 1982). Other algae, on the other hand, seem to exhibit a different photosynthetic response to O_2. Little or no effect of O_2 on photosynthesis was reported in several algae (LLOYD *et al.* 1977, COLEMAN and COLMAN 1980, BEER and ISRAEL 1986). Furthermore, oxygen enhancement of photosynthetic $^{14}CO_2$ fixation has been observed in the blue-green alga *Anacystis nidulans* (MIYACHI and OKABE 1976), and in the cryptophycean alga *Chroomonas* sp. (SUZUKI and IKAWA 1984a, b and 1985).

In *H. akashiwo* cells, the pattern of photosynthetic $^{14}CO_2$ fixation closely resemble those of C_3 plants (Fig. 4). However, the effect of O_2 on photosynthesis in *H. akashiwo* cells seems to differ from that in terrestrial C_3 plants. Photosynthetic $^{14}CO_2$ fixation was inhibited by anaerobiosis as well as high concentrations of O_2, and the highest rate of CO_2 fixation was obtained under 2% O_2 (Fig. 7). The percent radioactivity incorporated into the intermediates of the photorespiratory pathway, such as glycolate, glycine and serine, was almost negligible at concentrations up to 21%, and very small even under 100% O_2 (Fig. 8). These results were consistent with those in *Chroomonas* (SUZUKI and IKAWA 1985).

The lack of inhibition of photosynthesis at high light intensity (Fig. 1), low sensitivity to O_2 (Fig. 7), and relatively high af-

finity to inorganic carbon (Fig. 2) may be important features to cause a bloom in natural waters.

References

ASHINO-FUSE, H. and IKAWA, T. 1981. Photosynthesis and carbon metabolism in *Tetraselmis* sp. (Prasinophyceae). Jap. J. Phycol. **29**: 189–196.

BEARDALL, J. and MORRIS, I. 1975. Effects of environmental factors on photosynthesis patterns in *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. **11**: 430–434.

BECK, E. 1979. Glycolate synthesis. p. 325–337. *In* M. GIBBS and E. LATZKO [ed.] Encyclopedia of Plant Physiology. New series Vol. 6, Photosynthesis II. Springer-Verlag, Berlin.

BEER, S. and ISRAEL, A. 1986. Photosynthesis of *Ulva* sp. III. O_2 effects, carboxylase activities, and the CO_2 incorporation pattern. Plant Physiol. **81**: 937–938.

BIDWELL, R.G.S. 1957. Photosynthesis and metabolism of marine algae. I. Photosynthesis of two marine flagellates compared with *Chlorella*. Can. J. Bot. **35**: 945–950.

BIRMINGHAM, B.C., COLEMAN, J.R. and COLMAN, B. 1982. Measurement of photorespiration in algae. Plant Physiol. **69**: 259–262.

COLEMAN, J.R. and COLMAN, B. 1980. Effect of oxygen and temperature on the efficiency of photosynthetic carbon assimilation in two microscopic algae. Plant Physiol. **65**: 980–983.

CRAIGIE, J.S. 1974. Storage products. p. 206–235. *In* W.D.P. STEWART [ed.] Algal Physiology and Biochemistry. Blackwell Sci. Pub., Oxford.

FRENCH, D. and WILD, G.M. 1953. Correlation of carbohydrate structure with papergram mobility. J. Am. Soc. **75**: 2612–2616.

FUKAZAWA, N., ISHIMURA, T., TAKAHASHI, M. and FUJITA, Y. 1980. A mechanism of 'red tide' formation. I. Growth rate estimation by DCMU-induced fluorescence increase. Mar. Ecol. Prog. Ser. **3**: 217–222.

GAFFRON, H. 1940. Studies on the induction period of photosynthesis and light respiration in green algae. Amer. J. Bot. **27**: 204–216.

HANDA, N. 1969. Carbohydrate metabolism in the marine diatom *Skeletonema costatum*. Mar. Biol. **38**: 189–199.

HARA, Y., INOUYE, I. and CHIHARA, M. 1985. Morphology and ultrastructure of *Olisthodiscus luteus* (Raphidophyceae) with special reference to the taxonomy. Bot. Mag. Tokyo **98**: 251–262.

HATANO, T., HARA, Y. and TAKAHASHI, M. 1983. Preliminary study on the effects of photoperiod and nutrients on the vertical migratory behavior of a red tide flagellate *Heterosigma akashiwo*. Jap. J. Phycol. **31**: 263–269.

HELLEBUST, J.A. 1965. Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanog. **10**: 192–206.

HELLEBUST, J.A. 1976. Effect of salinity on photosynthesis and mannitol synthesis in the green flagellate *Platymonas suecica* Can. J. Bot. **54**: 1735–1741.

HOGETSU, D. and MIYACHI, S. 1970. Effect of oxygen on the light-enhanced dark carbon fixation in *Chlorella* cells. Plant Cell Physiol. **45**: 178–182.

HOLDSWORTH, E.S. and COLBECK, J. 1976. The pattern of carbon fixation in the marine unicellular alga *Phaeodactylulm tricornutum*. Mar. Biol. **38**: 189–199.

HORITSU, H., SATAKE, T. and TOMOYEDA, M. 1973. Purification of an β-D-(1,3)-glucanase from *Rhizopus niveus*. Agr. Biol. Chem. **37**: 1007–1012.

IWAMURA, T., NAGAI, H. and ICHIMURA, S. 1970. Improved methods for determining contents of chlorophyll, protein, ribonucleic acid, and deoxyribonucleic acid in planktonic populations. Int. Revue ges Hydobiol. **55**: 131–147.

KIRST, J. 1975. Beziehungen zwischen Mannitkonzentration und osmotischer Belastung bei der Brackwasseralge *Platymonas subcordiformis* Hanzen. Z. Pflanzenphysiol. **76s**: 316–325.

KREMER, B.P. and BERKS, R. 1978. Photosynthesis and carbon metabolism in marine and freshwater diatoms. Z. Pflanzenphysiol. **87**: 146–165.

LLOYD, N.D.H., CANVIN, D.T. and CULVER, D.A. 1977. Photosynthesis and photorespiration in algae. Plant Physiol. **59**: 936–940.

MIYACHI, S. 1979. Light-enhanced dark CO_2 fixation. p. 68–76. *In* M. GIBBS and E. LATZKO [ed.] Encyclopedia of Plant Physiology. New series Vol. 6 Photosynthesis II. Springer-Verlag, Berlin.

MIYACHI, S. and OKABE, K. 1976. Oxygen enhancement of photosynthesis in *Anacystis nidulans* cells. Plant Cell Physiol. **17**: 973–986.

PROVASOLI, L. 1968. Media and prospects for the cultivation of marine algae. p. 63–75. *In* A. WATANABE and A. HATTORI [ed] Culture and Collections of Algae. Proc. U.S.-Japan Conf. Hakone, 1966., Jap. Soc. Plant Physiol., Tokyo.

SHELP, B.J. and CANVIN, DT. 1980. Photorespiration and oxygen inhibition of photosynthesis in *Chlorella pyrenoidosa*. Plant Physiol. **65**: 780–784.

SUZUKI, K. and IKAWA, T. 1984a. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas*

sp. (Cryptophya) I. Some characteristics of the oxygen effect. Plant Cell Physiol. **25**: 367–375 (1984).

SUZUKI, K. and IKAWA, T. 1984b. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta) II. Effect of inhibitors, uncouplers and an artificial electron mediator on the inhibition of $^{14}CO_2$ fixation by anaerobiosis. Plant Cell Physiol. **25**: 377–384.

SUZUKI, K. and IKAWA, T. 1985. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta). III. Effect of oxygen on photosynthetic carbon metabolism. Plant Cell Physiol. **26**: 1003–1010.

TAKAHASHI, M. and FUKAZAWA, N. 1982. A mechanism of "red-tide" formation II. Effect of selective nutrient stimulation on the growth of different phytoplankton species in natural water. Mar. Biol. **70**: 267–273.

TAMIYA, H. and HUZISIGE, H. 1949. Effect of oxygen on the dark reaction of photosynthesis. Stud. Tokugawa Inst. **6**: 83–104.

TOMAS, C.R. 1979. *Olisthodiscus luteus* (Chrysophyceae). III. Uptake and utilization of nitrogen and phosphorus. J. Phycol. **15**: 5–12.

TOMAS, C.R. 1980. *Olisthodiscus luteus* (Chrysophyceae). IV. Effects of light intensity and temperature on photosynthesis, and cellular composition. J. Phycol. **16**: 149–156.

WADA, M., MIYAZAKI, A. and FUJII, T. 1985. On the mechanisms of diurnal vertical migration behavior of *Heterosigma akashiwo* (Raphidophyceae). Plant Cell Physiol. **26**: 431–436.

WARBURG, O. 1920. Uber die Geschwindigkeit der photochemischen Kohlensauerzersetzung in lebenden Zellen. 2. Biochem. Z. **103**: 188–217.

WATANABE, M.M., NAKAMURA, Y., MORI, S. and YAMOCHI, S. 1982. Effects of physicochemical factors and nutrients on the growth of *Heterosigma akashiwo* Hada from Osaka Bay, Japan. Jap. J. Phycol. **30**: 279–288.

WHISTLER, R.L. and HICKSON, J.L. 1954. Maltotetraose and crystalline pentadecaacetylmaltotetraitol. J. Am. Chem. Soc. **76**: 1671–1673.

WILLENBRINK, J., KREMER, B.P., SCHMITZ, K. and STIVASTAVA, L.M. 1979. Photosynthetic and light independent carbon fixation in *Macrocystis*, *Nerocystis*, and some selected pacific Laminariales. Can. J. Bot. **57**: 890–897.

YAMAGUCHI, T., IKAWA, T. and NISIZAWA, K. 1966. Incorporation of radiocarbon from H $^{14}CO_3^-$ into sugar constituents by a brown alga, *Eisenia bicyclis*, during photosynthesis and its fate in the dark. Plant Cell Physiol. **7**: 217–229.

高橋京子・猪川倫好：ラフィド藻 *Heterosigma akashiwo* における光合成炭素代謝特性

海産ラフィド藻 *H. akashiwo* の光合成炭酸固定活性は，最大約 300μ mol CO_2·mg Chl.a^{-1}·hr^{-1} で他の近縁の藻類に比べ非常に高い活性を持つことが示された。またこの活性は 500 W·m^{-2} 以上の強光下でも阻害されず，無機炭素に対し比較的高い親和性を持つこと（$Km_{app(HCO_3^-)}$=0.1 mM）が明らかにされた。光合成 $^{14}CO_2$ 固定産物の経時的変化の解析から，光合成炭酸固定は炭素還元回路（C_3 回路）によって行われ，主要な貯蔵産物として80%メタノール可溶性のβ-1,3-グルカンを生成することが示された。また光合成炭酸固定に対する酸素の影響を調べたところ，本藻は C_3 植物であるにもかかわらず，高い酸素耐性を有することが明らかになった。これらの特性は，本藻が昼間，海表面付近で活発な光合成を行うことによりもたらされると推定される低炭酸，高酸素環境下でも，十分高い光合成活性を保つ上で，非常に有利であると考えられる。（305 つくば市天王台1-1-1 筑波大学生物科学系）