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CHAPTER 13

PHOTOSYNTHETIC PRODUCTS OF ZOOXANTHELLAE OF THE REEF-BUILDING CORALS *STYLOPHORA PISTILLATA* AND *SERIATOPORA COLIENDRUM* FROM DIFFERENT DEPTHS OF THE SEYCHELLES ISLANDS

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K. Y. BIL', P. V. KOLMAKOV AND L. MUSCATINE

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K.Y. Bil'[*], P.V. Kolmakov[**] and L. Muscatine[***]

INTRODUCTION

In the world ocean, there is a large group of symbiotic organisms substantially contributing to the total primary productivity of tropical shelf ecosystems (Davies 1977, Porter 1980, Falkowski et al. 1984, Sorokin 1986), in conjunction with macrophytic algae, seagrasses and phytoplankton. This group is the reef-building corals - symbiotic organisms including the polyps of colonial Cnidaria with their endosymbiotic microalgal zooxanthellae. It is known that zooxanthellae provide photosynthetic products not only to themselves but also for the host polyps (Land et al. 1975, Muscatine et al. 1981, Muscatine et al. 1984, Falkowski et al. 1984, Sorokin 1986). However, there are few data in the literature as to the type of photosynthetic carbon metabolism in zooxanthellae and possible changes under the effects of various environmental factors. Only several reports characterizing the type of photosynthesis in endosymbionts are available. In particular, Benson et al. (1978) and Hofmann and Kremer (1981) argue that zooxanthellae fix CO_2 through the C_3 pathway, i.e., carbon photoassimilation occurs with the help of ribulose-1, 5-biphosphate carboxylase and the first stable assimilate is 3-phosphoglycerate. Other works (Ting 1976, Beardall et al. 1976, Trench and Fisher 1983, Tyler and Trench 1986), on the contrary, showed that free-living dinoflagellates and coral zooxanthellae have high levels of phosphoenol-pyruvate carboxylase and malate dehydrogenase activity, and express the opinion that C_4 photosynthesis or mixed C_3-C_4 pathways of photosynthesis are possibly present.

In the present work, we report the results of investigations of CO_2 assimilation rates and the composition of primary and final assimilates in coral zooxanthellae from various depths. The experiments were carried out during the Soviet-American expedition aboard the R/V Akademik A. Nesmeyanov.

METHODS AND MATERIALS

The symbiotic reef-building corals *Stylophora pistillata* (Ester) and *Seriatopora coliendrum*, inhabiting 2-3 m and 36 m depths near Desroches and Praslin Islands (Amirantes and Seychelles Groups), were chosen as the subjects of investigation. Depending on the experiment, fragments of coral colonies or isolated zooxanthellae were used. Zooxanthellae were isolated by the water-pick .TB.6"

* Institute of Soil and Photosynthesis, USSR Academy of Sciences, Puschino, Moscow, USSR
** Institute of Marine Biology, Far East Branch, USSR Academy of Sciences, Vladivostok, 690032, USSR
*** Department of Biology, University of California, Los Angeles, California, USA, 90024-1606

method (Johannes and Wiebe 1970). Polyp tissue was washed by seawater for 1-3 min to separate zooxanthellae from the polyps and fragments of coral skeleton. Zooxanthellae were isolated from the supernatant by precipitation at the repeated centrifugation force of 1500-2000 g), then washed in seawater from the polyp tissue residues. Cleaned zooxanthellae were resuspended in seawater at a concentration of 7.7×10^6 cells per ml. We controlled the purity of fractions and counted cells using a standard compound microscope. ^{14}C was used in the form of bicarbonate dissolved in seawater or as gaseous $^{14}CO_2$ for measuring photosynthetic rates and determining the composition of carbon metabolism products. Zooxanthellae cells were precipitated on 2.4 cm GF/A (USA) glass microfiber filters which were incubated for a fixed time in the exposure chamber with radioactive carbonic acid of bicarbonate. Carbon accumulation rates during photosynthesis were measured at light energies of 50-80 $W \cdot m^{-2}$, at air and water temperatures of 28°C after 10-30 sec pre-illumination of samples. After the exposure to light with radioactive bicarbonate, the samples were fixed by 1 ml of boiling 80% ethanol (EtOH) with formic acid added. The fixed zooxanthellae, together with the glass filters, were ground to homogeneous conditions and analyzed as well as another sample from the coral fragments. The analysis of the assimilate composition from the EtOH-soluble fraction was made using the method of two-dimensional ascending paper chromatography (Benson et al. 1950, Bil' et al. 1981, Bil' 1988). The mixture of butanol, formic acid and distilled water (75:13:12) and water-saturated phenol were used as solvents. Radioactivity of identified assimilates was measured by scintillation counter (Intertechnique, SL-30-300 type) and expressed in percentage of the total radioactivity of all assimilates eluted from the chromatograms.

RESULTS

Photosynthetic rates of isolated zooxanthellae from *Stylophora pistillata* and *Seriatopora coliendrum* obtained at saturating light and optimum temperatures are presented in Table 1. When CO_2 was used as the carbon source, the photosynthetic intensity was 1.5 to 2 times lower than with bicarbonate as the carbon source. However, the increase of CO_2 concentration in the exposure chamber leads to a considerable increase of the rate of ^{14}C assimilation (see experiment 2 in Table 1).

The analysis of primary assimilate composition produced by isolated zooxanthellae and zooxanthellae in polyps shows that these photoautotrophic organisms possess the typical C_3-pathway of photosynthesis. In particular, during a short-term photosynthetic duration (5-15 sec), up to 90-94% of the radioactive carbon from the EtOH-soluble fraction of zooxanthellae is incorporated into 3-phosphoglycerate and other phosphatesters (Table 2). At longer exposures of cells in the radioactive medium, ^{14}C appears in the free sugars, fructose, glucose and saccharose. Intermediate assimilates typical of the C_4 pathway, such as malic and aspartic acids, appear in traces during short-term exposures to light. Such kinetics of carbon transformation during photosynthesis can be observed at various intensities of light flux, environmental temperatures and habitat depths.

It is known that one of the products of photosynthesis in zooxanthellae is glycerol (Muscatine 1980, Schlichter et al. 1983, Schlichter et al. 1984, Battey and Patton 1984). Glycerol was observed in the present study, not only after long-term exposures of zooxanthellae (see Table 3), but even at 5-15 sec where up to 7-8% of the ^{14}C from the EtOH-soluble fraction was incorporated into this assimilate (Table 2). The results presented in Table 2 also suggest that ^{14}C is consecutively transformed from 3-phosphoglycerate into free sugars and glycerol. However, after 15-sec long photosynthetic exposures to $^{14}CO_2$, where zooxanthellae were kept under a normal atmosphere in the dark, we observed an increase in the concentration of radioactive label in alanine, aspartate and glutamate. Radioactive carbon appears also in malate. In these experimental variants, free sugars were nearly absent but the concentration of ^{14}C-glycerol increased (Table 2). The results obtained are consistent with the data from Table 3 which shows that coral zooxanthellae (both isolated and in polyps) from the 36 m depth synthesize 2 to 2.5 times fewer free sugars than corals from depths of 2-3 m. In deep water coral

zooxanthellae, glyerol concentration also is greater (Table 3). As in the case of dark specimens (see Table 2), radioactive alanine, aspartate, glutamate and malate also increase. The form of carbon substrate (HCO^-_3 or CO_2) does not substantially affect the ^{14}C-distribution among photosynthetic products, both in shallow - and deep-water samples (Table 3).

DISCUSSION

It is known that the photosynthetic products of zooxanthellae are quite diverse and in addition to carbohydrates they include such low molecular intermediates as 3-phosphoglycerate, phosphate esters, alanine, aspartate, glutamate, glycine and serine, fatty acids and many lipids (Muscatine and Cernichiari 1969, Schmitz and Kremer 1977, Patton et al. 1977, Blanquet et al. 1979). The main insoluble carbohydrates of zooxanthellae are glucose and glycerol (Muscatine and Cernichiari 1969, Muscatine 1980, Tables 2 and 3). Recently, it was reported that in the intact coral *Acropora scandens*, mannose may be a dominant carbohydrate. But since this polysaccharide had not been found earlier as a photosynthetic product of zooxanthellae, then its discovery by Schmitz and Kremer (1977) may be the result of interactions between the algae and polyps.

Our experiments on the dynamics of carbon accumulation and redistribution of ^{14}C among primary assimilates produced by zooxanthallae of *Stylophora pistillata* and *Seriatopora coliendrum* support the point of view of Benson et al. (1977) and Hofmann and Kremer (1981) that symbiotic dinoflagellates have the typical C_3-pathway of photosynthesis. One can surmise that despite the presence of high concentrations of inorganic carbon in the form of bicarbonate, zooxanthellae prefer to photoassimilate carbon in the form of CO_2 (Table 1). However, it is not excluded that zooxanthellae, as well as *Chlorella* (Aizawa and Miyachi 1979), are capable of fixing carbon either in the form of CO_2 or bicarbonation depending on the long-term effects of certain factors.

As was mentioned above, the products of zooxanthellae photosynthetic carbon metabolism are hexoses and glycerol (Tables 2 and 3). After photoassimilation of ^{14}C-carbon dioxide directly in zooxanthellae, radioactive label is also quickly incorporated into fatty acids (Latyshev, unpublished data) or into esterified lipid drops (Muscatine and Cernichiari 1969, Patton et al. 1977, Blanquet et al. 1979). ^{14}C-distribution between assimilate fractions in zooxanthellae, both in vivo and in vitro, depends to a large extent on conditions of light incubation and/or coral habitat. For example, Schmitz and Kremer (1977) found a high ^{14}C fixation in vivo in EtOH-soluble assimilates. However, pulse-chase experiments showed the rapid transformation of soluble compounds into insoluble ones. In a number of experiments (Schmitz and Kremer 1977), about 50% of the ^{14}C was found in the lipid fraction. In other experiments (Patton et al. 1977, Blanquet et al. 1979, Trench 1979), a considerable labeling of lipids also was obtained using isolated zooxanthellae following a long incubation in host tissue. This is to be expected, since corals and their symbiotic associations are rich in reserve and structural lipids. Some coral tissues, for example, can contain more than 34% lipids per unit dry weight (Patton et al. 1977), and the coral *Goniastrea retiformis* contains up to 3 mg of wax ester (cetyl palmitate) per cm^2 of tissue surface (Benson and Muscatine 1974). The ratio of hexose and glycerol, to our experience, depends considerably upon the light history; zooxanthellae from deep-water corals (Table 3) synthesize chiefly glycerol. In such low-light conditions, a substantial portion of ^{14}C also is shunted away from the Calvin-Benson cycle to synthesize alanine, aspartate, malate and glutmate. Constrastingly, in corals from 2-3 m in depth, the amount of radioactive carbon in C_4-dicarboxylic acids and glycerol decreases, while it increases in free sugars (Table 3).

Since ^{14}C-glycerol can be found even after only 5-15 sec of photosynthesis (Table 2), its formation is most likely conditioned by reduction of a part of dihydrooxiacetonphosphate into glycerol-3-phosphate by glycerol phosphate dehydrogenase ferment, with its further phosphatase hydrolysis to glycerol (Fig 2). One can suppose that if a coral dwells at a considerable depth, glycerol on the one hand promotes

osmotic regulation of cells, and on the other hand it is a main product of energetic transport from zooxanthellae to the polyp body. Glycerol not only provides for glycolysis and gluconeogenesis reactions but it also may be one of the precursors of membrane lipids, such as phosphoacylglycerol and 3-acylglycerol. It should be noted that for symbiotic organisms such as corals, we can only generalize about probable distributions of photosynthetic products between the producer and host, which depends to a great extent on specific habitat conditions.

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Table 1. Photosynthetic rates of zooxanthellae isolated from *Stylophora pistillata* and *Seriatopora coliendrum* depending on carbon substrate and habitat depth. Concentration of various carbon forms was: 1 - *Stylophora pistillata* - HCO_3^- - 6.5 mM, CO_2 - 0.105 mM; 2 - *Seriatopora coliendrum* - HCO_3^- - 18.0 mM, CO_2 - 0.194 mM. • value in parentheses = values of photosynthetic intensity at CO_2 - concentration of 0.56 mM.

Coral species and habitat	Depth m	Photosynthetic rate, $\mu g\ CO_2 \cdot 10^{-6}$ cells$\cdot h^{-1}$	
		Substrate	
		HCO_3^-	CO_2
Stylophora pistillata (Desroches)	2	28.3±3.0	14.9±1.5
Seriatopora coliendrum (Praslin)	3	13.5±1.5	9.7±1.0 (25.0±2.6•)
	36	11.3±1.2	7.4±0.8 (17.6±1.8)

Table 2. Composition of photosynthetic products of zooxanthellae and colony fragments of *Stylophora pistillata* from 2 - 3 m depth (Desroches). "Glycerol" includes glycerol and glycerol-3-phosphate, "Sugars" includes saccharose, glucose and fructose; (l) = light, (d) = dark; (-) = absence or traces of elements; → - transition of a sample from $^{14}CO_2$ $^{12}CO_2$. Relative deviations: for values higher than 10 - 5-10%, lower than 10 - 15-20%.

Sample	Duration (sec) of light incubation $^{14}CO_2$	$^{12}CO_2$	Radioactivity of elements of EtOH-fraction in % of total radioactivity of eluates: Phosphate esters	Sugars	Alanine	Aspartate	Glutamate	Malate	Glycerol
Isolated zooxanthellae	5		93.2	-	-	-	-	-	6.8
	15		91.1	-	-	-	-	-	8.1
	60		25.3	47.9	-	1.4	0.8	-	24.6
	180		15.5	68.0	0.6	1.0	0.6	-	14.3
	15 →	60(l)	27.0	56.1	-	1.1	1.1	-	14.8
	15 →	180(l)	7.8	67.6	-	1.0	2.0	-	21.6
	15 →	60(d)	42.8	0.2	4.5	7.8	10.3	2.3	32.7
	15 →	180(d)	32.7	-	3.7	13.3	25.0	2.5	22.8
Corals	60		32.9	57.6	-	-	-	-	9.5
	180		17.3	72.1	-	1.5	1.6	-	7.5

Table 3. Composition of products of 300 s photosynthesis in isolated zooxanthellae and colony fragments of *Seriatopora coliendrum* with reference to habitat depth (Praslin). Experiments were performed at saturating light at water and air temperature of 28°and concentration of CO_2=0.48mM, HCO_3^-=18.0 mM. Relative deviation: for more than 10 - 5-10%, less than 10 - 15-20%.

Sample	Carbon substrate	Depth m	Radioactivity of elements of EtOH-fraction in % of total radioactivity of eluates							
			Phosphate esters	Sugars	Serine+ Glycine	Alanine	Aspartate	Glutamate	Malate	Glycerol
Isolated zooxanthellae	CO_2	3	4.3	60.9	1.1	0.8	1.2	14.6	0.9	16.2
		36	7.2	22.1	1.0	3.3	10.5	12.4	4.0	39.5
	HCO_3^-	3	20.6	5702	1.3	0.4	1.0	11.0	0.3	8.2
		36	11.3	28.8	0.5	3.7	10.4	19.4	5.9	20.0
Corals	CO_2	3	6.3	51.0	4.5	2.7	3.8	7.8	1.4	22.5
		36	6.8	18.1	0.4	5.1	5.6	10.8	3.0	50.2

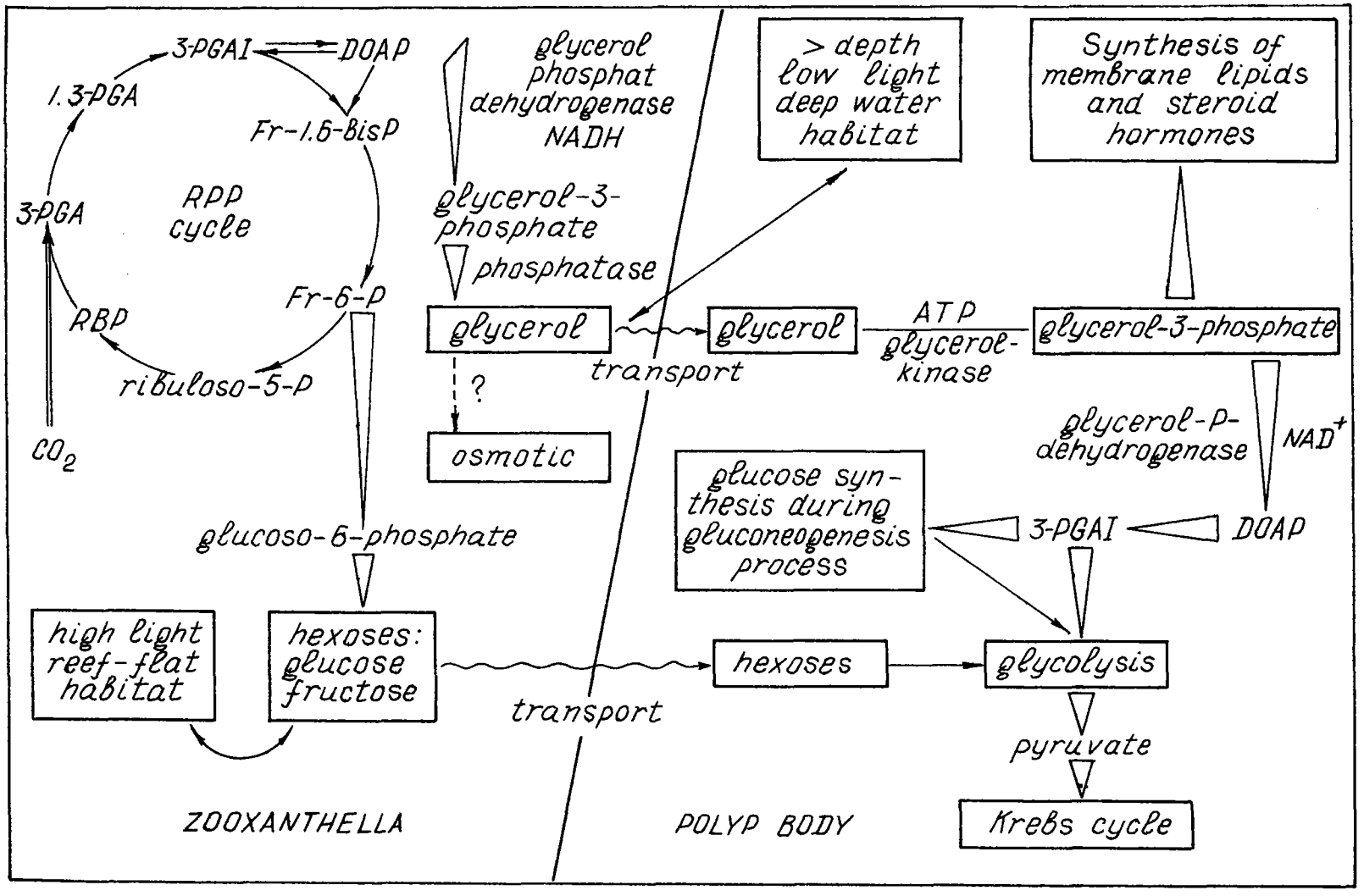

Figure 1. Photosynthetic products of zooxanthellae with reference to habitat depth and possible ways of their utilization by polyps.