Effect of Salinity on Photosynthetic $^{14}CO_2$ Fixation and Amino Acid Pools of *Bellerochea yucatanensis* (v. Stosch) and *Thalassiosira rotula* (Meunier)

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The marine diatoms *Bellerochea yucatanensis* and *Thalassiosira rotula* were grown at different salinities (20/25, 35, and 40/45‰ salinity (S), respectively) under normal air (0.035 vol.% CO_2). No significant variations in the percentage of gross photosynthetic products (*e.g.* total amino acids, sugar phosphates) were found as a function of salinity during growth. The bulk of the soluble ^{14}C-radioactivity was detected in amino acids. ^{14}C-labelling of glutamine increased markedly with salinity. Low salt – grown algae are characterized by enhanced amino acid pools, mainly of aspartic acid, asparagine and glutamine. It was found that the tested amino acids are not involved in osmoregulation.

Introduction

Marine diatoms adapted to different salinities showed no variations in growth [1]. Photosynthetic activity of several marine diatoms decreased linearly with increasing salinity; optimal photosynthesis was observed at 10 to 24‰ S [2]. It was found that besides the Calvin cycle, a β-carboxylation reaction exists in marine diatoms [3, 4]. This pathway was less sensitive to water and salt stress than the Calvin cycle in higher plants and the cyanobacterium *Synechocystis* [5, 6]. In many organisms osmoregulatory substances have been detected. In diatoms, proline and K^+ might have an osmoregulatory function [7].

This paper presents data on $^{14}CO_2$ fixation and amino acid pools in cells of *Bellerochea yucatanensis* and *Thalassiosira rotula* grown at different salinity levels. The aim was to obtain more information on osmoregulatory substances and on the response of photosynthetic CO_2 fixation by different salinity – adapted marine diatoms.

Materials and Methods

The tropical marine diatom *Bellerochea yucatanensis* (v. Stosch) was isolated by Prof. Dr. Werner, Marburg on the coast of Yucatan, Mexico, whereas the temperate water species *Thalassiosira rotula* (Meunier) was collected in the North Sea by Dr. E. Hagmeier, Helgoland. Both species were grown in practically axenic monocultures at 20 °C under normal air (0.035 vol.% CO_2), irradiated with white fluorescent lamps (Osram, 40 W/25-1) at a 14 : $\overline{10}$ h light : dark regime (intensity: 1 mW cm^{-2}). *B. yucatanensis* was cultivated at 20, 35 and 45‰ S and *T. rotula* at 25, 35 and 40‰ S for more than 4 days. A nutrient solution according to von Stosch *et al.* [8] was used.

Chlorophyll *a* and chlorophyll c_1+c_2 were measured with the method of Jeffrey and Humphrey [9]. Estimation of carotenoids was performed after Myers and Kratz [10] in 90% acetone. Amides and free amino acids were separated and analyzed by reversed-phase high performance liquid chromatography (HPLC) (Beckman Instruments Model 342) after pre-column derivatization with *o*-phthaldialdehyde. A gradient solvent system with (A) sodium acetate, 0.05 N, pH 6.8/methanol/tetrahydrofurane (80/19/1) and (B) methanol/sodium acetate (80/20) as mobile phase (varied after Gibson [11]) was used. Additional details have been published by Döhler and Zink [12].

Algae were harvested during exponential growth, concentrated on paper filters and resuspended in fresh nutrient solution. ^{14}C-kinetic experiments were performed by the procedure described by Döhler [13]. Suspensions were placed into a special assimilation chamber of plexiglass and [^{14}C]-bicarbonate

Abbreviation: S, salinity.

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(1.98 GBq/nmol) added after 20 min of photosynthesis. After different photosynthetic labelling periods samples were removed by a syringe and extracted with ethanol. Separation of the alcohol-soluble metabolites was performed according to the method of Schürmann [14] by thin-layer chromatography and high voltage electrophoresis. The radioactive spots were scraped from the plates and ^{14}C-radioactivity estimated by scintillation spectroscopy.

Results and Discussion

Growth of *Bellerochea* and *Thalassiosira* cells adapted to different salinities was not affected by the salt concentration of the nutrient solution. This is in agreement with findings of other authors [1, 15]. The content of the individual pigments increased linearly with salt concentration, but no significant differences could be measured. McLachlan [16] also found no variation in the chlorophyll *a* content of several diatoms between 2.5 and 35‰ S.

The photosynthetic $^{14}CO_2$ fixation rate was slightly lower in the 20/25‰ S – cultivated *B. yucatanensis* and *T. rotula* cells than of the higher salinity – grown cells. Contrary to this observation Qasim *et al.* [2] described an enhanced $^{14}CO_2$ assimilation by low salinity-grown diatoms. The results of ^{14}C-incorporation into several soluble photosynthetic products of *B. yucatanensis* and *T. rotula* grown under normal salt conditions (35‰ S) are presented in Fig. 1. The bulk of the ^{14}C-radioactivity was found in amino acids of both diatoms, usually in alanine, aspartate and glutamate. These amino acids were ^{14}C-labelled very rapidly (after 30 s photosynthesis). The high proportion of ^{14}C-incorporation into aspartate suggests the presence of a β-carboxylation pathway in marine diatoms. This was suggested by findings of several groups [3, 4, 17].

^{14}C-labelling of 3-phosphoglycerate, sugar monophosphates, and sugar bisphosphates shows clearly that the Calvin cycle is also operative in marine

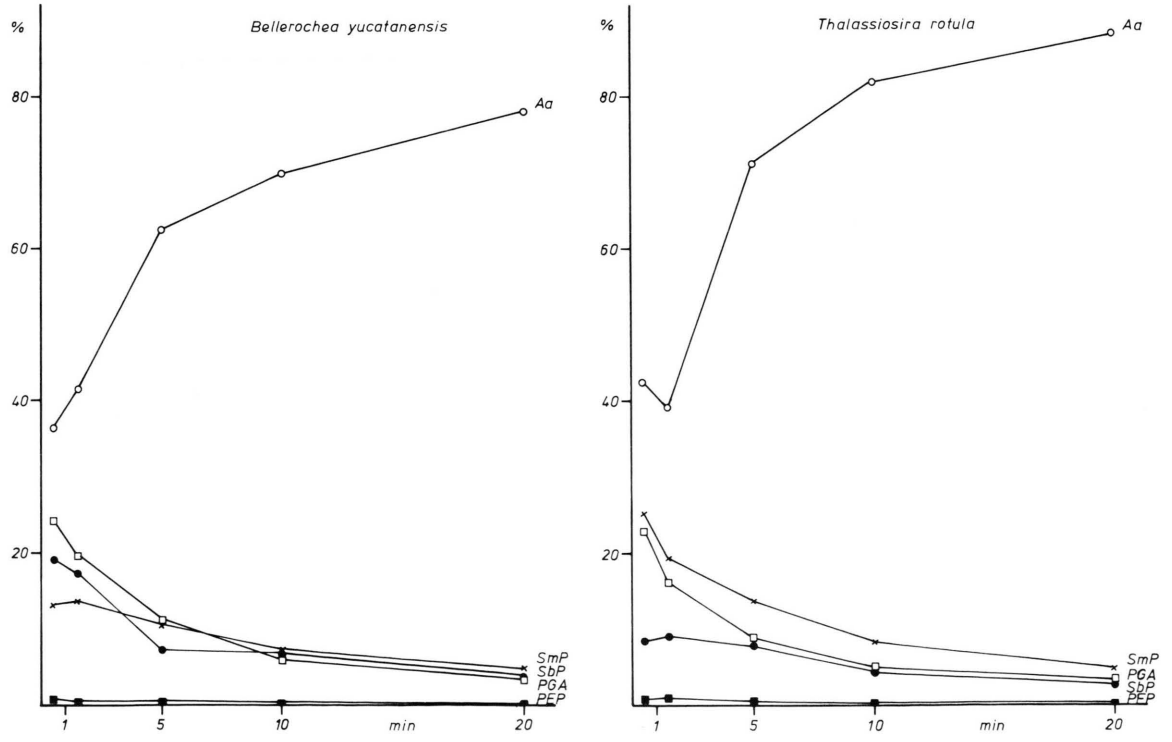

Fig. 1. Kinetics of ^{14}C-incorporation into several photosynthetic products of *Bellerochea yucatanensis* (v. Stosch) and *Thalassiosira rotula* (Meunier) grown at 35‰ S, 20 °C, and normal air (0.035 vol.% CO_2). [^{14}C]-Bicarbonate was added after 15 min of photosynthesis. For further details see Materials and Methods. Aa: total amino acids; PEP: phosphoenolpyruvate; PGA: 3-phosphoglycerate; SbP: sugar bisphosphates; SmP: sugar monophosphates; %: percent of total soluble products.

Table I. ^{14}C-incorporation into several soluble photosynthetic products of *Bellerochea yucatanensis* and *Thalassiosira rotula* cells grown in different salinities after 20 min photosynthesis. Values are presented in dpm·µg chlorophyll a^{-1} and in % of total soluble products. For further details see Materials and Methods.

	B. yucatanensis						*T. rotula*					
	20‰ S	%	35‰ S	%	45‰ S	%	25‰ S	%	35‰ S	%	40‰ S	%
Total amino acids	1132	76.0	1481	84.0	1250	82.0	1577	73.0	1374	79.0	1566	86.0
3-Phosphoglycerate	51	3.4	79	4.5	106	6.9	81	3.7	72	4.2	71	3.9
Fructose 6-phosphate	45	3.0	29	1.6	30	2.0	318	15.0	46	2.6	97	5.3
Glucose 1/Glucose 6-phosphate	115	7.7	59	3.3	78	3.8			99	5.7		
Uridinebisphosphate-glucose	86	5.8	25	1.4	–	–	101	4.7	65	3.7	36	2.0
Fructose/Sedoheptulosebisphosphate	57	3.8	93	5.3	62	4.1	88	4.1	80	4.6	53	2.9
Total soluble products	1486		1766		1526		2165		1736		1823	

diatoms. It was found in *Bellerochea* that NH_4^+ stimulates β-carboxylation [4]. The kinetic patterns of ^{14}C-incorporation into the photosynthetic products of 20/25‰ or 40/45‰ S-grown algae did not change markedly in comparison to those of the 35‰ S-cultivated cells (data not shown). However, differences were observed in ^{14}C-distribution of some specific products (Table I). Total radioactivity in amino acids after 20 min photosynthesis showed a slight diminution of percentage proportion of in low salinity-grown diatoms. Glutamine labelling increased from 3.4% in 20‰ S – to 20.8% of the total soluble products in 45‰ S-cultivated *B. yucatanensis* cells. Similar results were obtained with *T. rotula* (data not shown). ^{14}C-label in sugar monophosphates and uridine-bisphosphate-glucose decreased with increasing salt concentration during growth of the algae. Our results with low salinity-grown algae indicate a slight enhancement of β-carboxylation. Contrary to these findings, salt stress usually results in an increased β-carboxylation [18]. It must be emphasized, however, that we used marine diatoms adapted to the salt concentration of the nutrient solution. The enhanced ^{14}C-labelling of glutamine at high salinity can be attributed to an increased biosynthesis via glutamate dehydrogenase.

Table II. Pool sizes and total amino acid content of *Bellerochea yucatanensis* and *Thalassiosira rotula* grown at different salinities. Values are presented in nmol·µg chlorophyll a^{-1}. For further details see Materials and Methods.

	B. yucatanensis			*T. rotula*		
	20‰ S	35‰ S	45‰ S	25‰ S	35‰ S	40‰ S
Aspartic acid	5.9	2.8	1.8	8.5	1.8	1.7
Glutamic acid	11.1	6.1	6.5	6.3	5.4	5.0
Asparagine	2.3	0.3	0.5	5.5	0.2	0.2
Serine	0.8	0.4	0.2	0.6	0.3	0.3
Glutamine	16.5	0.8	1.2	47.3	0.8	3.0
Histidine	–	–	–	–	–	–
Glycine	1.5	0.1	0.2	1.0	0.3	0.3
Threonine	0.6	0.2	0.1	0.2	0.1	0.1
Arginine	5.7	–	–	2.6	–	–
Alanine	2.0	1.0	1.5	0.9	0.8	1.4
Tyrosine	0.9	0.1	0.1	–	–	–
Tryptophane	0.1	0.1	–	–	–	–
Methionine	0.3	0.1	–	–	0.1	–
Valine	0.5	0.1	0.1	0.1	0.2	0.1
Phenylalanine	0.4	0.1	–	–	–	–
Isoleucine	0.2	0.1	–	–	0.2	–
Leucine	0.3	0.1	–	–	0.1	–
Lysine	0.9	–	0.2	–	1.4	–
Total amino acids	50.0	12.4	12.4	73.0	11.7	12.1

In other series of experiments pool sizes of amides and free amino acids in different salinity-grown diatoms were estimated by HPLC (Table II). Total amino acid content was markedly enhanced in low salinity grown cells compared to both normal and high salt-grown *B. yucatanensis* and *T. rotula* cells. Practically all the amino acids in low salinity-grown algae are significantly higher than those values in 35‰ S and 40‰ S-cultivated cells. These data suggest that free amino acids may have no osmoregulatory function in high salinity-adapted diatoms. Schobert [19] found in *Phaeodactylum tricornutum* that parallel to the salt concentration an increase in proline content was observed. However, Liu and Hellebust [7] could not detected a correlation between salinity and proline content in *Cyclotella cryptica*. Rather, these authors suggest an osmoregulatory function for Na^+, K^+ and Cl^- in diatoms. Pools of aspartate, asparagine and glutamine are markedly increased compared to the other amino acids of *B. yucatanensis* and *T. rotula* cells grown at low salinity.

The enhanced ^{14}C-labelling and pools of glutamine can be possibly explained by an inhibition of glutamate synthase, whereas the activity of glutamine synthetase may be not affected by low salt concentration. Further studies on enzymes of nitrogen metabolism should give more information about the role of glutamine. Investigations on the activity of glutamate synthase and glutamine synthetase are in progress.

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