Cellular Differentiation of Sucrose Metabolism in *Anabaena variabilis*

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Dedicated to Professor Dr. Otto Kandler *on the Occasion of His 65th Birthday*

Anabaena variabilis, Vegetative Cells, Sucrose Synthase, Heterocysts, Alkaline Invertase

Upon treatment of whole filaments of *Anabaena variabilis* with a French pressure cell, the activity of sucrose synthase (UDP glucose: D-fructose 2-α-D-glucosyl transferase, EC 2.4.1.13) was found to be associated almost exclusively with the vegetative cells. The sucrose-cleaving enzyme, an alkaline invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26), appears to be localized solely within the heterocysts. The implications of these findings, in the light of relevant data from the literature, are summarized in a model representing the physiological differentiation and molecular exchange between vegetative cells and heterocysts.

Introduction

It has been shown that the heterocysts and vegetative cells in filamentous blue-green algae interact biochemically: NH_4^+ formed by the reduction of N_2 is incorporated by GS, and the glutamine thus synthesized in the heterocysts is transported to the vegetative cells [1, 2], whereas carbon fixed by the action of ribulose-1,5-bisphosphate carboxylase in vegetative cells is translocated to the heterocysts [3]. Since the major pathway of carbon degradation in the heterocysts is represented by the oxidative pentose phosphate pathway [4–6], it has been suggested that the carrier of carbon from the vegetative cells to the heterocysts may be a sugar. Experiments carried out by Jüttner and Carr appeared to demonstrate that maltose is the carbon compound in question [7]. However, pulse-chase experiments performed in our laboratory with whole filaments of *Anabaena variabilis* revealed sucrose to be the dominant soluble carbohydrate in the alga, and that this sugar is rapidly synthesized and exhibits a high turnover rate [8]. In addition to sucrose, trehalose, trehalose glucosides and trehalose fructosides were present in small amounts, whereas labeled maltose could not be detected on radioautograms [8].

The present study reports on sucrose and trehalose synthesizing and cleaving enzymes in extracts of vegetative cells and heterocysts of *Anabaena variabilis*.

Abbreviations: Fru, fructose; F6P, fructose-6-phosphate; α-G1P, α-D-glucose-1-phosphate; G6P, D-glucose-6-phosphate; GS, glutamine synthase; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid.

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Materials and Methods

Organism

Anabaena variabilis Kütz. (ATCC 29413) was grown aerobically in continous light at 30 °C in an 8-fold dilution of the medium of Allen and Arnon [9] in 10-liter flasks.

Preparation of cell-free extracts of vegetative cells and heterocysts

5 liters of filament suspension (5–8 μg Chl × ml^{-1}) were harvested by filtration and the filaments washed and resuspended in ice cold 50 mM HEPES buffer, pH 7.5, containing 10 mM $MgCl_2$ and 5 mM DTE. The suspension was passed through a chilled French pressure cell at 3000 lb × in^{-2} (vegetative cells were broken by this treatment whereas heterocysts were not) and centrifuged at 1000 × *g* for 5 min. The supernatant was centrifuged at 30000 × *g* for 10 min (the resulting supernatant corresponding to a cell-free extract of vegetative cells) and assayed for enzyme activities as described below. The pellet

obtained from the first centrifugation step was twice more subjected to the pressure cell and subsequent centrifugation procedure described above and the corresponding high-speed centrifugation supernatants were also tested for enzyme activities. In a final procedure the resulting low-speed centrifugation pellet was subjected to a French cell pressure of 20000 lb × in^{-2}. The supernatant obtained following centrifugation of this homogenate at 30000 × *g* corresponds almost exclusively to a cell-free extract of heterocysts.

Enzyme assays

To assaying sucrose synthase (EC 2.4.1.13) and sucrose phosphate synthase (EC 2.4.1.14) activity, the cell-free extracts were incubated with 1 μmol [^{14}C]UdP-glc (1.85 MBq $mmol^{-1}$) and 1 μmol Fru, respectively, as well as with 1 μmol [^{14}C]UdP-glc (1.85 MBq $mmol^{-1}$), 1 μmol F6P and 4 μmol sodium-fluoride as an inhibitor of phosphatase activity. The test solution for assaying sucrose phosphorylase (EC 2.4.1.7) contained 1 μmol [^{14}C-α]G1P (1.85 MBq $mmol^{-1}$) and 1 μmol Fru. Trehalose phosphate synthase (EC 2.4.1.15) was determined by incubation with 1 μmol [^{14}C]UdP-glc (1.85 MBq $mmol^{-1}$) and 1 μmol G6P. Incubation time for all assays described above was 30 min; incubation took place at 32 °C and was terminated by adding 0.5 ml 96% ethanol and boiling for 1 min. The reaction products were analyzed after removal of the heat-denaturated proteins by centrifugation. The carbohydrates in the supernatant were separated by one dimensional descending paper chromatography (Whatman Nr. 1) using ethylacetate : pyridine : water (100:40:30 by vol). Invertase (EC 3.2.1.26) and trehalase (EC 3.2.1.28) were assayed as described by Bergmeyer [10] and Keller *et al.* [11]. Glucose-6-phosphate dehydrogenase activity was tested according to Pearce and Carr [12].

Enzyme activities are quoted as nmol substrate used or product formed × mg $protein^{-1}$ × min^{-1} (= mU). Protein was determined by the method of Sedmark and Grossberg [13].

Results

In order to obtain information concerning the biosynthesis and degradation of sucrose and trehalose in *Anabaena variabilis*, the well known enzyme activities involved in these processes [14–18] were tested in cell-free extracts from vegetative cells and heterocysts of this alga obtained by differential treatment in a French pressure cell. The data obtained are compiled in Table I and Fig. 1 and compared with the activities of glucose-6-phosphate dehydrogenase, a marker enzyme of heterocysts, having been described in earlier reports [4, 6].

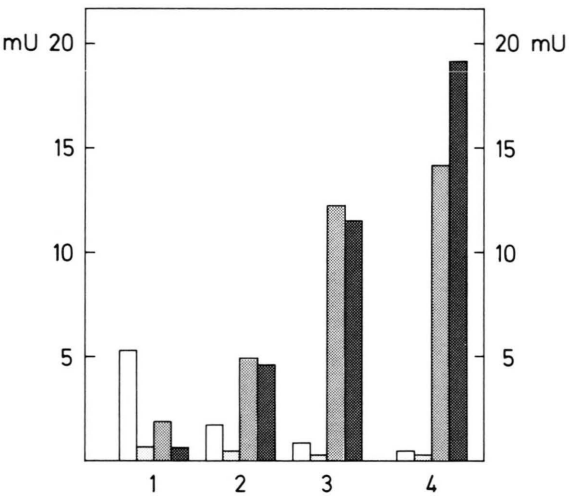

Fig. 1. Time course of solubilization of sucrose synthase-□, trehalase-□, glucose-6-phosphate dehydrogenase-▨ and alkaline invertase ■ activities (mU) during 4 French press cell treatment steps as described in Materials and Methods.

Enzyme	Cell-free extract	
	Vegetative cells	Heterocysts
Sucrose synthase	5.25*	0.41
Sucrose phosphate synthase	0	0
Sucrose phosphorylase	0	0
Alkaline invertase	0.64	19.2
Trehalose phosphate synthase	0	0
Trehalase	0.52	0.28
Glucose-6-phosphate-dehydrogenase	1.9	14.1

* ± 20 μg Cu × l^{-1}.

Table I. Activities (in mU of substrate used or product formed) of enzymes of carbohydrate metabolism in *Anabaena variabilis*.

Of the sucrose synthesizing enzymes only sucrose synthase was found to be active in extracts from *Anabaena variabilis*, this activity being ten times higher in vegetative cells than in heterocysts. A sucrose cleaving activity can be attributed to an alkaline invertase (pH optimum 7.5–7.8; data not shown), the activity of which was associated almost exclusively with the heterocysts (19.2 mU). The time course of the solubilization of alkaline invertase during pressure treatment resembles that of glucose-6-phosphate dehydrogenase.

Trehalose-6-phosphate synthase activity as described for yeast [17] and for the pollen of *Lilium longiflorum* [18] could not be demonstrated in extracts of either vegetative cells or heterocysts. Rates of trehalase, however, were 0.52 mU in vegetative cells extracts and about half of this value in extracts from heterocysts.

Discussion

The results obtained in the present study demonstrate unequivocally the presence of sucrose synthase in vegetative cells and of alkaline invertase in heterocysts. Glucose liberated by the action of the latter enzyme could be phosphorylated by hexokinase [6] and feed the oxidative pentose phosphate cycle of the heterocysts (Fig. 2). Fructose may move back into the vegetative cells or be subject to the same fate as glucose, since phosphoglucoisomerase is described to exhibit a high activity in heterocysts [6].

As detailed in the literature, the donation of electrons to nitrogenase may originate from photosystem I, NADPH, H_2 or pyruvate in heterocysts (Fig. 2). NADPH generated by the oxidative pentose phosphate cycle appears to be the most important source of electrons, since high nitrogenase activity has repeatedly been observed in the presence of NADPH, NADPH:ferredoxin oxireductase and ferredoxin. The data presented herewith may serve to underline this concept, but it is not possible to conclude at present whether NADPH is the sole electron donor *in vivo*. The finding that the transport of electrons from donors to nitrogenase requires a membrane potential may also indicate the presence of a membrane-bound link between NADPH, pyruvate and H_2 [19]. This, however, would not inhibit the role of sucrose as the mediator of electron transfer from vegetative cells to heterocysts. In this context it is of interest that Cu at a concentration of 10 $\mu g \times l^{-1}$ resulted in a complete inhibition of N_2-fixation in blue-green algae, whereas photosynthesis activity was reduced to an extent of only 10% [20]. The employment of this same Cu concentration in pulse-chase experiments with whole filaments of *Anabaena variabilis* resulted in a drastic reduction of sucrose metabolism, whereas trehalose metabolism – being in any event incapable of supplying sufficient reducing power for nitrogenase *in vivo* – was not affected whatsoever (Schilling and Ehrnsperger, in press). Since sucrose synthase activity was not inhibited by even a two-fold higher Cu concentration (Table I) than mentioned above (ref. [20]), a regulatory function of sucrose formation in relation to N_2-fixation may be postulated.

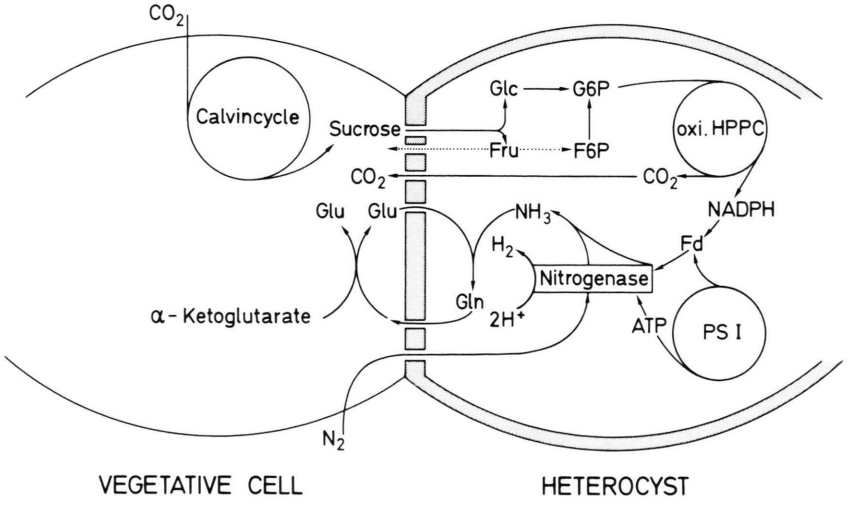

Fig. 2. Model representing the carbon and nitrogen interrelationships between a vegetative cell and a heterocyst in *Anabaena variabilis*.

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