Exogenous hexoses cause quantitative changes of pigment and glycerolipid composition in filamentous cyanobacteria

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Abstract

Cyanobacteria *Spirulina platensis* and *Nostoc linckia* were grown in the presence of 5 mM and 50 mM glucose or 5 mM mannose, non-metabolisable glucose analogue that effectively triggers the repression of photosynthesis. Glucose evoked active cyanobacterial growth but chlorophyll (Chl) content decreased to some extent and porphyrins were excreted. The content of monogalactosyldiacylglycerol decreased in glucose-grown cyanobacteria and that of phosphatidylglycerol increased substantially. Mannose inhibited cyanobacteria growth as well as Chl synthesis, however, phosphatidylglycerol contents were higher than in respective control samples. In cyanobacterial cells glucose may not only inhibit photosynthetic processes, but also cause structural transformations of membranes which may be necessary for the activity of respiratory electron transport chain components under heterotrophic conditions.

Additional key words: chlorophyll; glucose; mannose; monogalactosyldiacylglycerol; phosphatidylglycerol; *Nostoc linckia*; *Spirulina platensis*; sugars.

Introduction

Much attention is paid now to the regulatory role of sugars, particularly glucose, in photosynthetic processes. Semenenko's workgroup pioneered the dissection of glucose control mechanisms in algal photosynthesis (Semenenko 1978, Semenenko *et al.* 1984). Later it was shown that sugars are not only the substrates in carbon and energy metabolism and in polymer biosynthesis but they can also act as primary messengers in the signal transduction to the genome (Rolland *et al.* 2002). In contrast to special-purpose hormone molecules, sugars are present in the cells in the millimolar range, not nano- to micromolar, and take part in intermediary metabolism (Smeekens 2000). The metabolic and signalling functions of sugars are not readily separated, but different approaches have been elaborated to overcome this hindrance, *e.g.* the use of non-metabolizable analogues. For example, hexokinase, the first enzyme in the hexose assimilation pathway, is one of the key sensors and signal transmitters of sugar repression (Jang *et al.* 1997, Smeekens 2000). Mannose, a glucose epimer at the second carbon atom, can be phosphorylated by hexokinase but does not undergo further metabolism and is capable of repressing promoters of genes for photosynthetic components at considerably lower concentrations than glucose itself (Jang and Sheen 1994, Pego *et al.* 2000).

While the bulk of information concerning sugar-induced signal transduction in bacterial, yeast, mammalian, and higher plant systems is accumulated to date, much less is known about glucose regulation of metabolism, especially photosynthesis, in cyanobacteria. In this group of oxygen-evolving prokaryotes both photosynthetic and respiratory electron transport chains are present in the same thylakoid membrane and share common components (Schmetterer 1994). The lipid composition of most cyanobacteria resembles that of inner envelope and thylakoid membranes of higher plant chloroplasts (Wada and Murata 1998). Cyanobacteria contain four main glycerolipid classes: uncharged galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) along with two anionic lipids, sulfonated glycolipid sulfoquinovosyldiacylglycerol (SQDG) and the phospholipid phosphatidylglycerol (PG). On the contrary, bacterial and extra-chloroplast plant membranes contain mainly phospholipids. So the complexes involved in cyanobacterial respiration operate in the lipid environment that differs drastically

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Abbreviations: Chl, chlorophyll; DGDG, digalactosyldiacylglycerol; GlcDG, monoglucosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; 16:0, hexadecanoic acid (palmitic acid); 16:1, Δ9-hexadecenoic acid (palmitoleic acid); 18:0, octadecanoic acid (stearic acid); 18:1, Δ9-octadecenoic acid (oleic acid); 18:2, Δ9,12-octadecadienoic acid (linoleic acid); α-18:3, Δ9,12,15-octadecatrienoic acid (α-linolenic acid); γ-18:3, Δ6,9,12-octadecatrienoic acid (γ-linolenic acid).

from that in mitochondria and bacteria.

The purpose of the present study was to dissect the effects of glucose, a key metabolite of a photosynthetic cell, on the chlorophyll (Chl), glycerolipid, and fatty acid composition of filamentous cyanobacteria *Spirulina platensis* and *Nostoc linckia*. Mannose was also employed in the experiment to distinguish the possible signalling role of glucose.

Materials and methods

Organisms and culture conditions: Algologically pure cultures of filamentous cyanobacteria from culture collection IBASU-B (Membranology and Phytochemistry Department, M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine) were examined. Soil cyanobacterium *Nostoc linckia* (Roth.) Born *et* Flah. gathered on the north-facing slope of 'Evolution Canyon' (Lower Nahal Oren, Mt. Carmel, Israel) (Vinogradova *et al.* 1995) was studied in comparison with freshwater cyanobacterium *Spirulina platensis* (Nordst.) Geitl. Cyanobacteria were grown in sterile conditions in 750 cm^3 Erlenmeyer flasks each filled with 330 cm^3 of liquid medium described in Zender and Gorham (1960) for *N. linckia* and in Pinevich *et al.* (1970) for *S. platensis*. Growth temperature was 27–28 °C and the cultures were stirred up twice a day. Cyanobacterial cultures were irradiated 12 h per day with "white" fluorescent lamps supplying 70 μmol(photon) m^{-2} s^{-1}. On the 15th day of growth the cultivation conditions were changed by adding 5 mM glucose, 50 mM glucose, or 5 mM mannose. All biochemical parameters were determined after 4 d of growth with sugars.

Chl and lipid analyses: Cyanobacterial cells were harvested by centrifugation at 1 500×*g* for 20 min and washed thrice with distilled water. Chl *a* concentration was measured in acetone extracts by the Wettstein procedure (cited from Gavrilenko *et al.* 1975).

Lipids were extracted and purified according to Bligh and Dyer (1959). Lipid extracts were fractionated by thin-layer chromatography on glass plates with silica gel. Solvent systems chloroform/methanol/water 65 : 25 : 4, by vol. (Nichols 1963) and acetone/benzene/water 91 : 30 : 8, by vol. (Sato and Murata 1982) were used. Spots of individual lipid classes were visualized by exposing the plates to iodine vapour. Phosphatidylglycerol was determined as inorganic phosphate after mineralization of dried lipid residue with perchloric acid (Rodionov and Kholoptseva 1974). Glycolipid content was measured with the procedure proposed by Svennerholm (1956). Total lipid extracts were subjected to methanolysis with 2 % H_2SO_4 in methanol (by vol.) at 80 °C for 1 h (Christie 1982). The resulting fatty acid methyl esters were analysed with a gas-liquid chromatograph *Chrom-4* (*Laboratorní přístroje*, Czech Republic) equipped with a flame-ionization detector. The column (2 m × 3 mm internal diameter) was packed with *Chromaton N-AW* (*Chemapol*, Czech Republic) with 30 % polyethylene glycol adipinate. The column temperature was 180 °C. Retention times of individual fatty acid methyl esters were compared with those of standard compounds (*Sigma*).

Fluorescence measurements were performed with a spectrofluorometer *Hitachi-850* (Japan).

Results were the averages from three independent experiments, each analysed three times. Standard deviations were always less than 5 %.

Results

S. platensis was characterized by slower photoautotrophic growth, but the addition of 5 mM glucose almost immediately resulted in appreciable increase of its biomass (Fig. 1). In *N. linckia* the effect of the same glucose concentration was prominent only on the fourth day of cultivation. The intensive growth of both cyanobacterial strains was observed in the presence of 50 mM glucose; however, growth rates of *S. platensis* were somewhat higher. The retardation of growth in response to adding of mannose in *S. platensis*, in contrast to *N. linckia*, was rather moderate and lowering with time.

The decrease of Chl *a* content in *N. linckia* cells in the presence of sugars was always detectable though not great (Fig. 2). On the contrary, in *S. platensis* 5 mM glucose did not affect this parameter and the addition of 50 mM glucose or 5 mM mannose declined the cellular content of that main photosynthetic pigment substantially.

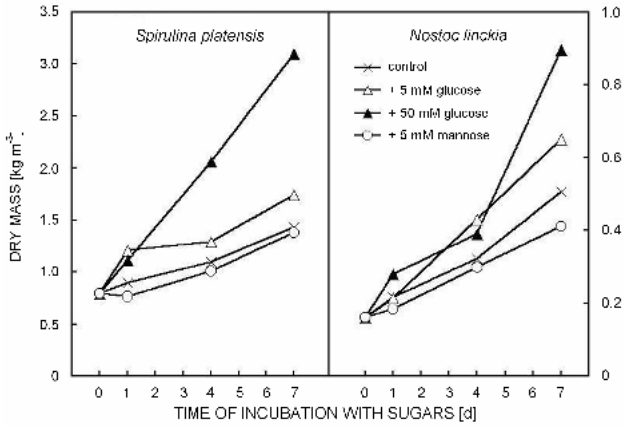

Fig. 1. Growth rates of *Spirulina platensis* and *Nostoc linckia* in the presence of sugars.

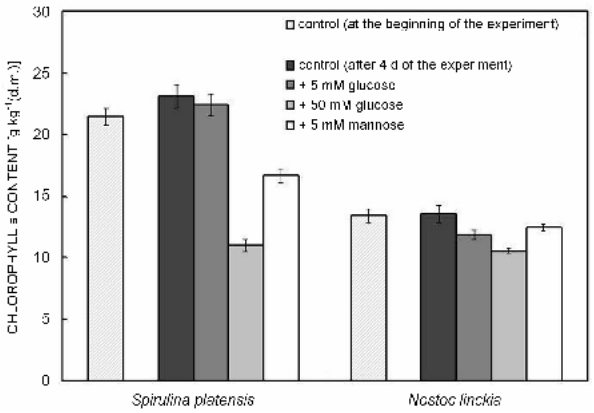

Fig. 2. Chlorophyll *a* content of *Spirulina platensis* and *Nostoc linckia* after 4 d of growth with sugars.

For further elucidation of the routes of cyanobacterial photosynthetic apparatus reorganization in response to glucose impact, pigment excretion was also examined. When glucose was added to *N. linckia and S. platensis* (regardless of concentration), tetrapyrrol compounds with reddish-orange fluorescence (λ_{max} ~ 615–618 nm) appeared in the cultural media shortly after. The position of Soret band (λ_{max} ~ 400 nm) and maxima in the visible region (porphyrin Q-band) in the excitation spectrum (Fig. 3) allowed identify the pigment as porphyrin, most probably coproporphyrin. Little amounts of uroporphyrin III and/or protoporphyrin IX may also be present because their spectral properties are similar.

Lipid compositions of *S. platensis* and *N. linckia* are compared in Table 1. Four major glycerolipid classes, MGDG, DGDG, SQDG, and PG, were always detected.

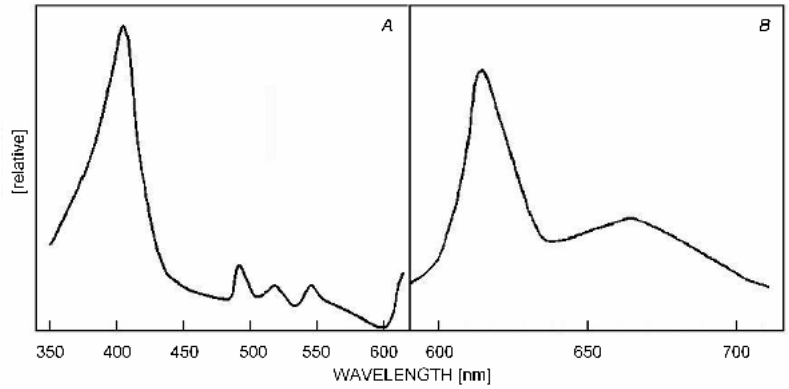

Fig. 3. Typical fluorescence emission (*A*) and excitation (*B*) spectrum of cyanobacterial growth medium after glucose supplementation.

Higher contents of all lipids, especially PG, were peculiar to *S. platensis* cells. *N. linckia* in comparison with *S. platensis* was characterised by somewhat higher parts of galactolipids but strikingly lower PG proportion. Both absolute and relative contents of MGDG tended to diminish when sugars were added to cyanobacterial cultural media (the only exception was the unaltered relative proportion of MGDG in *N. linckia* cells cultivated with 50 mM glucose). DGDG content decreased in *S. platensis* cultivated on 50 mM glucose and 5 mM mannose but it rose in mannose-grown *N. linckia* cells. SQDG parts of total glycerolipid content were similar in both strains and did not vary significantly under experimental conditions. However, the content of that lipid was lowered in all sugar-grown *N. linckia* cells and also in *S. platensis* supplied with 50 mM glucose. The increase of PG content

Table 1. Glycerolipid content of *Spirulina platensis* and *Nostoc linckia* after 4 d of growth with sugars. Ratio means molar ratio of phospholipids and glycolipids.

		Glycerolipid [g kg^{-1}(d.m.)]				[mol %]				Ratio	PG/SQDG
		MGDG	DGDG	SQDG	PG	MGDG	DGDG	SQDG	PG		
S. platensis	Control	28.01±0.80	9.11±0.29	15.41±0.72	11.44±0.51	45.8	12.3	23.1	18.8	0.23	0.81
	+ 5 mM glucose	24.57±1.12	9.52±0.34	16.40±0.65	15.95±0.74	38.7	12.3	23.7	25.3	0.34	1.07
	+ 50 mM glucose	20.04±1.04	5.31±0.27	11.35±0.53	13.85±0.67	41.1	8.9	21.4	28.6	0.40	1.34
	+ 5 mM mannose	22.17±0.83	6.72±0.26	15.49±0.68	16.93±0.63	37.6	9.4	24.1	28.9	0.41	1.20
N. linckia	Control	17.36±0.86	6.02±0.24	10.33±0.36	2.56±0.10	50.5	14.4	27.6	7.5	0.08	0.27
	+ 5 mM glucose	14.50±0.70	5.60±0.26	9.87±0.48	2.67±0.11	47.0	14.9	29.4	8.7	0.10	0.30
	+ 50 mM glucose	15.38±0.61	5.38±0.23	8.67±0.40	2.75±0.09	50.3	14.5	26.1	9.1	0.10	0.35
	+ 5 mM mannose	14.30±0.65	7.32±0.37	9.40±0.33	4.28±0.02	43.0	18.1	25.9	13.0	0.15	0.50

Table 2. Fatty acid composition of total lipids from *Spirulina platensis* and *Nostoc linckia* after 4 d of growth with sugars.

		Fatty acid [mol %]						
		16:0	16:1	18:0	18:1	18:2	γ-18:3	α-18:3
S. platensis	Control	47.1	5.0	0.8	3.6	17.0	26.5	
	+ 5 mM glucose	45.6	4.6	1.0	4.3	18.4	26.1	
	+ 50 mM glucose	43.8	4.8	1.7	8.9	15.6	25.2	
	+ 5 mM mannose	43.3	4.7	1.1	5.4	17.1	28.4	
N. linckia	Control	41.4	11.3	2.5	11.3	18.3		15.2
	+ 5 mM glucose	39.5	10.1	2.5	19.4	15.3		13.2
	+ 50 mM glucose	40.9	9.5	2.4	14.2	14.5		18.5
	+ 5 mM mannose	40.5	9.7	2.4	17.1	14.8		15.5

in response to the presence of hexoses was the general tendency and that effect was most prominent in the case of 5 mM mannose. Molar ratio of PG to glycolipids and that of PG to SQDG always rose under sugar supplementation.

S. platensis and *N. linckia* belong to different groups in respect to the fatty acid composition of their lipids (Wada and Murata 1998): the former strain contains γ-linolenic acid and the latter contains α-linolenic acid. The main quantitative differences of *N. linckia* in comparison with *S. platensis* were: (*a*) increased de-saturation in C_{16} acids, (*b*) higher percent of 18:0 and especially of 18:1, and (*c*) lower proportion of 18:3 (Table 2). The most remarkable effect of sugar presence on fatty acid composition of both cyanobacterial strains was the significant increment of 18:1 proportion while the percentage of 16:0 was slightly reduced.

Discussion

Cyanobacteria are phototrophic prokaryotes uniquely capable of oxygenic (plant type) photosynthesis and aerobic (cytochrome oxidase based) respiration (Schmetterer 1994). Glucose, the main substrate of respiration, can act as an effective inhibitor of photosynthetic processes (Semenenko 1978, Jang and Sheen 1994), and the details of membrane structure rearrangements in response to the switching between different types of nutrition are still the matter of thorough research.

The examination of growth rates reveals that the freshwater cyanobacterium *S. platensis* responds to the adding of hexoses more rapidly than the soil cyanobacterium *N. linckia*. Moreover, the former strain may possess higher adaptive potential that allows maximal advantage from additional source of carbon and energy and effective coping with the inhibitory influence of mannose.

The reduction of Chl content together with porphyrin excretion in the presence of glucose may be due to the blocking of photosynthetic pigment biosynthesis at the stage of coproporphyrinogen III formation. This assumption is supported by the fact that the excretion of tetrapyrrol compounds occurs also during the dark growth of the cultures, however at much lower rates than in the case of mixotrophic growth.

Troxler and Bogorad (1966) observed the excretion of porphobilinogen, porphyrins (uroporphyrin III, coproporphyrin III, protoporphyrin IX), and blue phycobilin pigment into the cultural medium of *Cyanidium caldarium* under 5-aminolevulinic acid induction both in the light and in the darkness. The ability for porphyrin excretion was detected in photosynthetic bacteria, organotrophic bacteria, and some fungi (Bykhovskij and Zaytseva 1989) and it was used extensively for the microbiological synthesis of those compounds. Stadnichuk *et al.* (1998) showed that in the mixotrophic culture of the unicellular red alga *Galdieria partita* light induction of photosynthetic pigment biosynthesis occurs simultaneously with the excretion of coproporphyrin(ogen) III. They supposed that light affects earlier stages of the biosynthetic pathways of Chl and phycobilins than glucose does. These data correspond well with our present results.

Monoglucosyldiacylglycerol (GlcDG), the immediate precursor of MGDG in its biosynthetic pathway, was not detected in our study. Normally it comprises less than 1 % of the total cyanobacterial glycerolipids (Sato and Murata 1982). Sato (1994) revealed that in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 GlcDG content rose up to 12 % when the cells were grown in the presence of glucose under photoheterotrophic conditions. On the contrary, filamentous cyanobacteria studied in the present experiment did not accumulate distinguishable amounts of that lipid. Hence under diminished MGDG requirement, the regulation of MGDG biosynthesis might occur at the stage before GlcDG formation and all newly synthesized GlcD might be almost immediately converted to MGDG by epimerization of glucose to galactose unit.

Phosphatidylglycerol, the sole cyanobacterial phospholipid, has a net negative charge at physiological pH, just as SQDG does. Benning *et al.* (1993), Guler *et al.* (1996), and Yu *et al.* (2002) have proposed that the possible physiological role of sulfolipids in photosynthetic membranes is the partial substitution of anionic

phospholipids for the purpose of phosphate saving. However, the increased PG content in cyanobacterial cells grown under heterotrophic conditions infers that this lipid may be indispensable for proper functioning of some respiratory complexes. This suggestion is consistent with the reports that PG is required for normal functioning of not only photosystem 2 (Gounaris *et al.* 1983, Kruse and Schmid 1995, Hagio *et al.* 2000), but cyanobacterial cytochrome oxidase (Peschek and Schmetterer 1981) and higher plant cytochrome b_6f complex (Doyle and Yu 1985) as well. PG accumulation was triggered by non-metabolizable mannose even more intensely than by glucose, implying that the mechanisms of hexokinase-mediated regulation of gene expression might be involved.

Making a generalization, we suggest that glucose has a regulatory effect on cyanobacterial cell metabolism (apparently including hexokinase-mediated pathway) that results not only in the inhibition of photosynthetic processes but also in the quantitative redistribution of membrane glycerolipids in favour of phosphatidylglycerol, the only cyanobacterial phospholipid.

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