THE ROLE OF THE CALVIN CYCLE FOR ANOXYGENIC CO_2 PHOTOASSIMILATION IN *ANACYSTIS NIDULANS*

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1. Introduction

Several blue–green algae (cyanobacteria) are capable of utilizing sulfide [1–4], hydrogen [4–7], or other exogenous reductants [4] for the photoassimilation of CO_2 when the water-splitting activity is blocked by DCMU [1–7], by NH_2OH [1,4], or in far-red light [2–4]. Such anoxygenic photosynthesis may involve both photosystems [4] or only photosystem I [2–4,6] depending on the electron donor. During oxygenic photosynthesis the Calvin cycle is the main CO_2 fixing pathway in blue–green algae [8,9] but certain growth and/or assay conditions may lead to a significant initial contribution of PEP carboxylation to the entry of carbon into C_4 acids [10–13]. Nothing is known so far about the path of CO_2 fixation during anoxygenic photosynthesis of blue–green algae. Also *Anacystis nidulans* was recently shown to photoassimilate CO_2 anaerobically by use of electron donors other than water [4,6]. In the present study the distribution of label among the early products of $^{14}CO_2$ photoassimilation by *Anacystis* was determined. Hydrogen and thiosulfate (donors to PSI) or hydrazine and cysteine (donors to PSII) were employed as exogenous photoreductants in the strict absence of oxygen. In addition, activities of several Calvin cycle enzymes and of PEP carboxylase, and the intracellular pool sizes of pyridine nucleotides were measured after pre-incubation of the cells under various conditions. The results suggest an operative Calvin cycle as the main CO_2 fixing reaction sequence during anoxygenic as well as oxygenic photosynthesis in *Anacystis*.

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl) 1,1-dimethylurea; DTT, dithiothreitol; 3-PGA, 3-phosphoglycerate; Tris, *N*-tris-(hydroxymethyl)-aminomethane; tricine, *N*-tris-(hydroxymethyl)-methylglycine

2. Materials and methods

Anacystis nidulans (strain L-1402-1, Göttingen) was grown photoautotrophically under N_2 or H_2 in presence of thiosulfate (replacing sulfate) and ammonium chloride (replacing nitrate) as in [4]. CO_2 assimilation experiments were performed either in a specially constructed assay chamber equipped with a Clark-type oxygen electrode or in a Photo-Warburg apparatus as in [4]. For measurements, the algae were suspended in a buffer solution containing 30 mM Tris/tricine, 1 mM K_2HPO_4 and 1 mM $MgCl_2$ (standard medium, final pH 8.0). Labeled bicarbonate (spec. act. 60 mCi/mmol; final act. 50 μCi/ml algal suspension, final bicarbonate 3 mM) was introduced (zero time) after 5 min pre-incubation under assay conditions including 1 mM unlabeled $NaHCO_3$ to attain metabolic steady state conditions. All incubations were done at 40°C. Light intensity was 18 or 20 W/m^2 (see [4]). Accessory electron donors were employed at the following concentrations (mM): Na-thiosulfate (0.5), hydrazine hydrate (1.5), cysteine (2.5). Experiments with H_2 were performed under an atmosphere of pure H_2 [4]. Oxygen evolution by illuminated algae was inhibited with 10 μM DCMU in case of H_2 and thiosulfate, and with 2 mM NH_2OH in case of hydrazine and cysteine [4]. Photosynthetic O_2 evolution during experiments on anoxygenic

photosynthesis was $<5 \times 10^{-5}$ μmol O_2 $.h^{-1}$ .mg chl^{-1} [4].

Separation of CO_2 fixation products was achieved by thin-layer electrophoresis and conventional chromatography according to [14] as modified [15]. Aspartate, citrulline, 3-PGA and glucose-6-phosphate were identified by co-chromatography with authentic ^{14}C-labeled compounds. Enzyme activities were determined according to [16,17] at 30°C and pH 8.0 in the supernatant (40 000 × *g*, 20 min, 4°C) of crude extracts prepared by sonication of freshly harvested cells suspended in standard medium containing 1 mM dithiothreitol and kept under N_2. Pyridine nucleotides were measured by a combined enzymatic and fluorimetric method [9,18]. Chlorophyll (chl) and protein were determined as in [4].

3. Results and discussion

Figure 1 shows that under conditions of both anoxygenic (A) and oxygenic (B) photosynthesis label from $NaH^{14}CO_3$ initially appeared in 3-PGA in accordance with an operative Calvin cycle [8]. Similar results were obtained when thiosulfate, hydrazine or cysteine served as photosynthetic electron donors (not shown). Controls with DCMU-poisoned cells in the light in absence of exogenous electron donors (fig.2A), as well as dark controls (fig.2B) revealed aspartate as the major initial product of CO_2 fixation. Clearly, availability of sufficient reducing power was a prerequisite for efficient CO_2 assimilation through the Calvin cycle. Measurements on intracellular pool sizes of NADPH supported this conclusion (table 1). The magnitude of the NADPH levels roughly corresponded to the linear rates of total CO_2 incorporation which were found to be in the ratio of about 2200 : 1300 : 470 : 110 : 95 : 14 : 1 with H_2O, hydrazine, cysteine, H_2 and thiosulfate as photosynthetic electron donors, and without donor in the light (+ DCMU) and in the dark, respectively [4].

A remarkable feature was the early appearance of label from $NaH^{14}CO_3$ in citrulline under all experimental conditions tested (fig.1,2) suggesting significant contribution of the carbamoyl phosphate pathway to CO_2 fixation in blue–green algae [13]; yet citrulline cannot be regarded as a rapid initial fixation product as it was hardly detectable before some 20 s incuba-

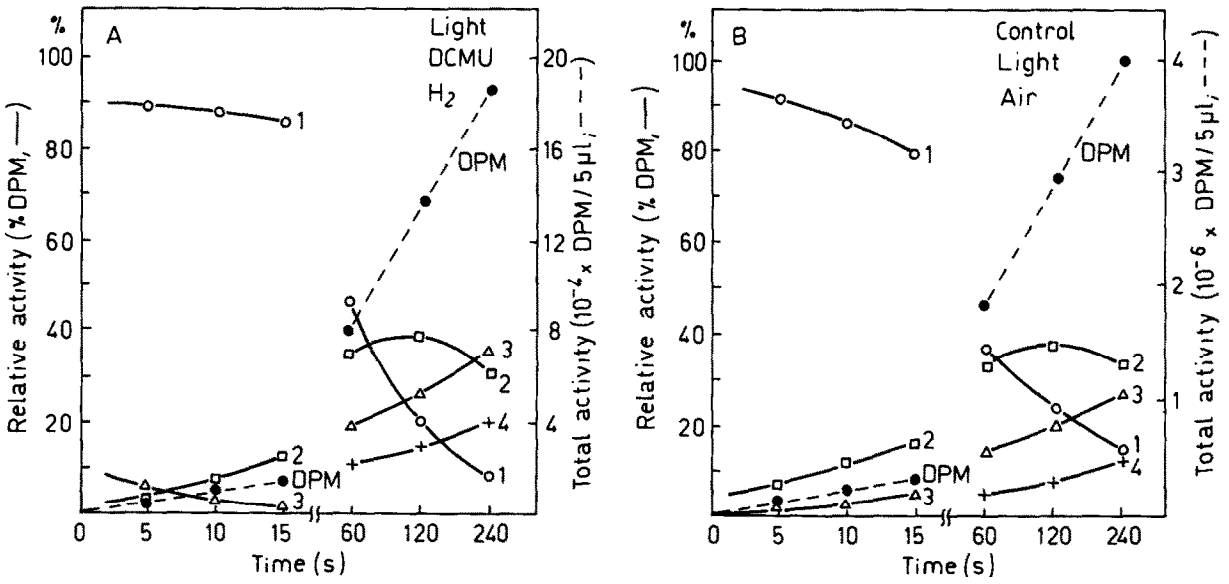

Fig.1. Early time course of radioactivity present in 3–PGA (1), hexose monophosphates (2), aspartate (3) and citrulline (4) under steady state conditions of anoxygenic (A) and oxygenic (B) photosynthesis. For details see section 2. Dotted line gives the increase in total ^{14}C assimilated (right ordinate scale). Apart from differences in absolute rates of ^{14}C incorporation (see text) the labeling pattern was essentially similar when thiosulfate, hydrazine or cysteine served as the photosynthetic electron donors.

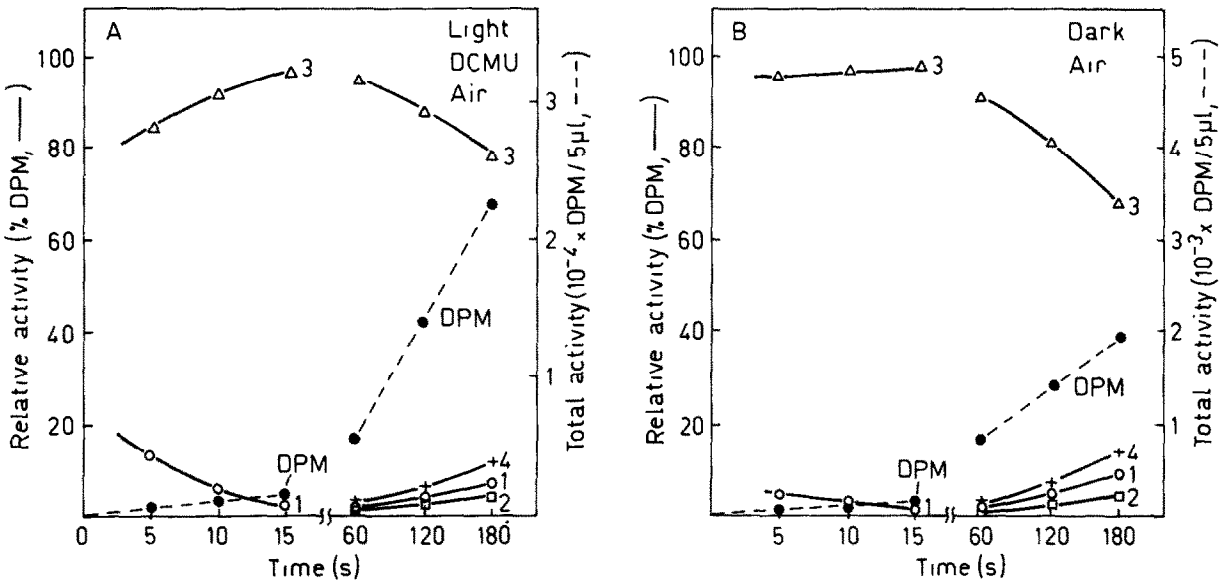

Fig.2. Early products of $^{14}CO_2$ fixation in aerobic conditions in the light in presence of 10 µM DCMU or 2 mM NH_2OH (A) and in the dark (B). Specifications as given in fig.1. Note the greatly diminished absolute rates of CO_2 fixation (dotted line).

Table 1
Intracellular levels of pyridine nucleotides (nmol/mg protein) in extracts obtained by rapid quenching [9] of cells pre-incubated for 30 min under different conditions

	Conditions of pre-incubation			
	Control[a]	Air[b]	Hydrogen[b]	Thiosulfate[b]
NADPH	1.73 ± 0.12	0.10 ± 0.01	0.63 ± 0.05	0.57 ± 0.04
NADP	0.46 ± 0.05	2.12 ± 0.18	1.59 ± 0.10	1.55 ± 0.13
NADH	0.51 ± 0.05	0.49 ± 0.05	0.53 ± 0.06	0.49 ± 0.04
NAD	1.12 ± 0.09	1.12 ± 0.10	1.00 ± 0.09	1.10 ± 0.10
	Hydrazine[c]	Cysteine[c]	Dark, N_2	Dark, Air
NADPH	1.36 ± 0.10	0.98 ± 0.08	2.20 ± 0.18	0.08 ± 0.01
NADP	0.83 ± 0.06	1.19 ± 0.09	0.05 ± 0.01	2.14 ± 0.20
NADH	0.56 ± 0.04	0.52 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
NAD	0.95 ± 0.08	1.00 ± 0.07	1.12 ± 0.09	1.09 ± 0.10

[a] Algae were incubated under conditions of oxygenic photosynthesis
[b] Photosynthetic O_2 evolution inhibited by 10 µM DCMU
[c] Photosynthetic O_2 evolution inhibited by 2 mM NH_2OH

Values given are averages based on 5 expts

Unless otherwise stated pre-incubation was in the light under O_2-free N_2. For comparison the steady state pool sizes under dark anaerobic and dark aerobic conditions are also given (see [20])

Table 2
Activities of several Calvin cycle enzymes and of PEP carboxylase after different conditions of pre-incubation

Enzyme	Conditions of pre-incubation						
	Control[d] 5 min	DCMU, N_2		DCMU, H_2[e]		Dark (1 h)	
		5 min	1 h	5 min	1 h	N_2	Air
RuDP carboxylase[a]							
RuDP	160	163	161	158	160	157	160
R5P	92	92	90	90	93	26	60
Ru5P kinase	95	95	94	95	96	29	64
FDP phosphatase[b]	75	73	76	75	77	70	63
SuDP phosphatase[b]	105	109	106	102	105	102	88
GAP dehydrogenase[b,c]	115	100	85	101	110	95	72
PEP carboxylase	25	45	42	25	25	48	27

[a] Either RuDP or R5P (under conditions of Ru5P kinase assay) were employed as carboxylation substrate [21]
[b] Activities of these enzymes were considerably lower when the extracts were prepared in absence of DTT (cf. [16])
[c] Reaction tested with 3-PGA as substrate and $NADP^+$ as coenzyme [9]
[d] Algae pre-incubated aerobically under conditions of oxygenic photosynthesis
[e] Essentially similar results were obtained when the algae were pre-incubated under conditions of anoxygenic photosynthesis with thiosulfate, hydrazine or cysteine as electron donors (see section 2)

Enzyme activities in the 40 000 × *g* supernatant of algal extracts are given as nmol substrate consumed or product formed .min^{-1} .mg $protein^{-1}$. Unless specified otherwise illuminated samples were pre-incubated under H_2 or N_2 for 5 min or 1 h as indicated.
Abbreviations: FDP, fructose-1,6-diphosphate; GAP, glyceraldehyde-3-phosphate; PEP phosphoenolpyruvate; RuDP, ribulose-1,5-diphosphate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; SuDP, sedoheptulose-1,7-diphosphate

tion with $NaH^{14}CO_3$. Rapid initial incorporation of label into aspartate under conditions of reduced electron supply (fig.2) together with a low rate of overall CO_2 incorporation under these conditions [4], indicated PEP carboxylation to be the prevailing anaplerotic nonreductive CO_2 fixing mechanism in *Anacystis* [10–13,15,17]; however, the significant contribution of the Calvin cycle should not be overlooked (curve 1, fig.2). Low residual activity of the Calvin cycle in darkness was suggested from results of $^{14}CO_2$ fixation studies with two other blue–green algae [19].

The possibility whether certain enzymes involved in CO_2 fixing pathways might be inactivated during prolonged dark incubation or under the conditions of anoxygenic photosyntheses was tested by measuring the activity of the enzymes (table 2). None of the treatments was found to exert a specific influence on any one of the enzymes tested which could explain the greatly varying rates, or patterns, of CO_2 fixation under the respective conditions.

These experiments suggest the Calvin cycle to be the major CO_2 fixing pathway in both anoxygenic and oxygenic photosynthesis of *Anacystis nidulans*, and its function to be controlled basically by the reducing power derived from exogenous electron donors utilizable by the cells. Important anaplerotic routes of CO_2 entry appear to be carbamoyl phosphate synthesis and PEP carboxylation which, under certain growth and/or assay conditions, might perhaps even determine the distribution pattern of primary CO_2 fixation products; this could partly explain the diverging results on CO_2 fixation in blue–green algae (see, e.g., [8–13,15,17,19]). Finally, the results presented here make it likely that other facultative blue–green algae might also employ the Calvin cycle

during anoxygenic CO_2 photoassimilation; clearly, it would be particularly promising to test this possibility by use of species known to display high rates of sulfide-dependent anoxygenic photosynthesis as reported [2,3].

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