CO_2 Fixation in *Anabaena cylindrica*

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The cyanobacterium *Anabaena cylindrica* grown in a nitrogen – free medium at + 25 °C was used for short-term ^{14}C-kinetics experiments under different conditions. During the dark/light transients the initial fixation products were mainly sugar monophosphates and 3-phosphoglyceric acid (Calvin cycle intermediates), aspartate (10% of total radioactivity) and glycine/serine. Iodoacetamide (0.01 M) caused an inhibition of photosynthetic $^{14}CO_2$ fixation and a ^{14}C-incorporation into aspartate, glutamate and 3-phosphoglyceric acid only. During dark $^{14}CO_2$ assimilation labelling of these products could be measured, too. In a nitrogen atmosphere (N_2 + 0.04 vol. % CO_2) a strong labelling of sugar monophosphates mainly at the beginning of photosynthetic period could be observed. In an oxygen atmosphere (100% O_2) an enhanced label of aspartate and glycerate and a decreased radioactivity in sugar monophosphates were found. Our results were discussed with reference to the operating of a phosphoenolpyruvate carboxylation reaction besides the Calvin cycle and to the glycolate metabolism.

Introduction

Recent studies on photosynthetic CO_2 fixation of several cyanobacteria demonstrate that besides the Calvin cycle there exist a light triggered carboxylation of phosphoenolpyruvate similiar to the C_4 pathway. This conclusion was drawn from investigations of the kinetics of ^{14}C-incorporation into photosynthetic intermediates of *Synechococcus* (= *Anacystis nidulans*) [1, 2] and supported by findings of relatively high activities of PEP carboxylase in the same and other cyanobacteria [3]. On the other hand, the dominant role of the reductive pentosephosphate cycle could be shown operating in several cyanobacteria [4 – 9]. Different conditions during growth and experiments seem to be the reason for the contractionary findings. Changes in intermediates of carbon metabolism result from anoxygenic conditions of *Synechococcus* [1] or from the addition of N_2 or NH_4^+ to N_2-fixing cultures of *Anabaena cylindrica* [10]. In the present study of short-term kinetic experiments the ^{14}C-metabolism by *Anabaena cylindrica* has been followed also under the influence of iodoacetamide.

Materials and Methods

Anabaena cylindrica Lemm. (*strain Cu 1403/2a*) obtained from the Culture Center of Algae and Protozoa, Cambridge was grown axenically at 25 °C in a nitrogen-free medium of Allen and Arnon [11]. For more details see [10].

After a period of nitrogen starvation the cyanobacteria were concentrated by centrifugation and resuspended in a fresh nutrient medium (BG 11). An aliquot of 9.5 ml or 19 ml *Anabaena* suspension was brought into a special plexiglass assimilation chamber and adapted at + 25 °C in the dark for 30 minutes. During adaptation air was bubled through the suspension only and magnetically stirred. Experiments were started by adding 0.5 ml $NaH^{14}CO_3$ (specific activity: 59.1 mCi/mmol; 10 µCi/ml suspension of *Anabaena*) and illuminated at 10 000 lux using a projector. After different photosynthetic or dark periods samples of 1 or 2 ml were removed from the suspension into weighed syringes containing 4 or 8 ml ethanol. Extracts were centrifugated and the residues again extracted with 50% ethanol and distilled water. The extracts of each sample were concentrated to 0.1 ml by evaporation at + 40 °C and 0.3 ml distilled water added to it. Using this procedure we obtained ethanol- and water-soluble ^{14}C-labelled compounds.

The ^{14}C-labelled products were separated according to the method of Schürmann [12] using a Pherograph (type 68, Hormuth & Vetter, Wiesloch). 1 – 20 µl of the extracts were spotted onto a cellulose thinlayer plate (20 × 20 cm; Merck, Darmstadt). High voltage electrophoresis was done at 1000 V, 20 – 25 mA for 50 minutes. Chromatography was

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performed in the second dimension with 2-butanol/formic acid/water (6 : 1 : 2). For total activity a 0.1 ml aliquot of each extract was measured after contact with 0.1 ml trichloracetic acid using a Tracerlab Corumatic scintillation counter. The radioactive spots were scrabbed from the thin-layer plates and put into scintillation vials.

Results and Discussion

All experiments were carried out to follow the route of $^{14}CO_2$ fixation in *Anabaena cylindrica*. The filaments of *Anabaena* exhibit very different cell types: vegetative cells and heterocysts. Jüttner and Carr [13] found no ^{14}C-labelled products of the Calvin cycle in heterocysts. We have carried out our experiments with filaments and not with isolated heterocysts. This species is the main object for studying the nitrogen fixation and assimilation in cyanobacteria also in relationship to the ^{14}C-incorporation into several amino acids [10].

In short term kinetics experiments of $NaH^{14}CO_3$ incorporation the majority of label were seen in sugar phosphates, 3-phosphoglyceric acid and glycerate/citrate. The pattern of the primary photosynthetic products (10 sec light exposure after a 30 min dark period) showed a labelling in phosphoenolpyruvate, glycine, serine and aspartate. Under our experimental conditions ^{14}C appeared most rapidly in phosphorylated compounds of the reductive pentose phosphate cycle and in amino acids.

Fig. 1 (light) demonstrate the dominant role of the Calvin cycle when the data are replotted in percentage distribution of the total radioactivity of soluble compounds. The negative slope of the curves for 3-phosphoglyceric acid and aspartate and the very early labelling of phosphoenolpyruvate (not shown in the figure) can be interpreted that the carboxylation of phosphoenolpyruvate exists in *Anabaena cylindrica*, too. It could not be clearly indicated whether 3-phosphoglyceric acid or aspartate was the first product of ^{14}C-incorporation. For this reason we studied the effect of iodoacetamide (0.01 M) on photosynthetic $^{14}CO_2$ fixation. The result of this experiment seen in Table I showed the majority of ^{14}C-labelling in aspartate and glutamate. High concentrations of iodoacetamide (0.1 M) caused a strong inhibition of photosynthetic $^{14}CO_2$ fixation rate which was under these conditions lower than the dark $^{14}CO_2$ fixation. Döhler [2, 5] had studied the effect of 0.01 M iodoacetamide on the pattern of photosynthetic products in *Anacystis nidulans* (*Synechococcus*), too. In comparison to the results obtained with *Anabaena* a much higher $^{14}CO_2$ fixation rate and labelling of aspartate could be found. Under the influence of 0,001 M iodoacetamide the activity of ribulose-1,5-bisphosphate carboxylase was inhibited by 90 to 95% and that of phosphoenolpyruvate carboxylase 7 to 18% only. We assume that *Anabaena* can fix $NaH^{14}CO_3$ via a light triggered PEP carboxylation reaction but the Calvin cycle is the main fixing pathway. Relatively high labelling of aspartate were observed also in the cyanobacteria *Anacystis* [1, 8, 9] and *Aphanocapsa* [14]. Colman, Cheng and Ingle [3] found in preparations of *Anacystis, Oscillatoria* and *Anabaena flos-aquae* a 5- to 15-fold higher activity of phosphoenolpyruvate carboxylase than that of ribulose-1,5-bisphosphate carboxylase. These findings suggest that these cyanobacteria have the enzymatic capability to fix CO_2 by a phosphoenolpyruvate reaction like C_4 pathway. We conclude from our $^{14}CO_2$ fixation data of *Anabaena cylindrica* experiments that this cyanobacterium is able to fix CO_2 via C_4 pathway to a low part only. The main route under these conditions is

Table I. Kinetics of ^{14}C-incorporation into photosynthetic products of *Anabaena cylindrica* after treatment with 0.01 M iodoacetamide (30 min).

Substance	10 sec		1 min		2 min		5 min		10 min	
	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]
aspartate	42	5.4	110	72.4	523	83.4	1228	81.9	1071	89.5
glutamate			40	26.3	65	10.4	243	16.2	126	10.5
glycerate/citrate	116	14.8								
3-phospho-glyceric acid	171	21.8			39	6.2	29	1.9		
sugar monophosphates	456	58.1								

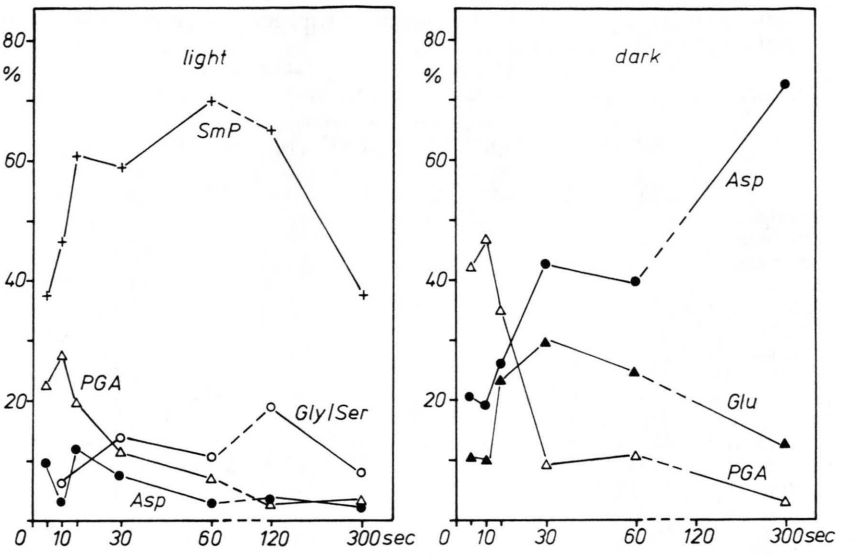

Fig. 1. Kinetics of $^{14}CO_2$ fixation in the light and in the dark of *Anabaena cylindrica* at + 25 °C under normal air conditions. Radioactivity present in aspartate (Asp), glutamate (Glu), glycine/serine (Gly/Ser), 3-phosphoglyceric acid (PGA) and sugar monophosphates (SmP).

the Calvin cycle. More recently Döhler and Roßlenbroich (in press) observed in differently pigmented *Anabaena cylindrica* in strong white light grown cells a most rapidly ^{14}C-incorportion into amino acids, mainly aspartate.

The relative high radioactivity observed in glutamate at the beginning of the photosynthetic period (5 sec) could be attributed to a part of the continous dark $^{14}CO_2$ fixation. The dark $^{14}CO_2$ fixation rate is very low compared to the photosynthetic ^{14}C-incorporation (Fig. 1, dark). The high labelling of 3-phosphoglyceric acid and sugar monophosphates mainly at the beginning of the dark CO_2 fixation can be attributed to the operation of the oxidative pentosephosphate cycle. Radioactivity found in malate, glutamate and citrate demonstrates the operation of

Table II. Kinetics of ^{14}C-incorporation into photosynthetic products of *Anabaena cylindrica* after exposure to a nitrogen atmosphere (N_2 + 0.04% CO_2) for 30 minutes.

Substance	10 sec		30 sec		90 sec		2 min		5 min		10 min	
	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]
aspartate	118	8.3	128	7.1	3722	9.4	5867	6.5	16127	5.8	24960	4.1
asparagine							5360	5.9	2822	1.0	41370	6.9
glutamine							1037	1.1	2823	1.0	10580	1.8
glutamate	40	2.8			402	1.0	260	0.3	8313	3.0	16380	2.7
alanine					378	1.0	2313	2.6	2620	0.9	5255	0.9
glycine/serine					4315	10.9	46273	51.0	40150	14.5	50785	8.4
citrulline							630	0.7	1330	0.5	2250	0.4
threonine							235	0.3	2390	0.9	7990	1.3
isoleucine							777	0.9	276	0.1	920	0.2
malate					157	0.4			803	0.3	1040	0.2
glycerate/citrate	78	5.5			3895	9.9	8410	9.4	16320	5.9	24795	4.1
3-phosphoglyceric acid	237	16.6	138	7.7	1958	5.0	407	0.4	9243	3.3	7190	1.2
sugar monophosphates	952	66.8	1398	77.8	23157	58.6	14387	15.9	95953	34.6	82515	13.7
fructose bisphosphate			133	7.4	735	1.9			1863	0.7	1670	0.3
sucrose							3677	4.1	36220	13.1	75680	12.6
phosphoglycolate									157	0.1	530	0.1
glycolate					112	0.3	190	0.2	397	0.1	820	0.1
uridinbisphosphate					662	1.7	277	0.3	3823	1.4	3525	0.6
glucose												
phosphoenolpyruvate									627	0.2	1530	0.3

Table III. Kinetics of ^{14}C-incorporation into photosynthetic products of *Anabaena cylindrica* after exposure to an oxygen atmosphere (100% O_2) for 30 minutes.

Substance	10 sec		15 sec		30 sec		1 min		2 min		5 min		10 min	
	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]	[dpm]	[%]
aspartate	230	18.2	146	28.7	511	31.8	831	5.0	5076	8.4	29530	14.0	38258	8.7
asparagine											1393	0.7		
glutamine											2265	1.1	11160	2.5
glutamate							141	0.8	216	0.4	7843	3.7	25475	5.8
alanine							684	4.1	792	1.3	1665	0.8	5075	1.2
glycine/serine							5661	33.8	16036	26.5	29468	14.0	41595	9.5
citrulline									404	0.7	515	0.2	2745	0.6
isoleucine											330	0.2		
malate							77	0.5	238	0.4	565	0.3		
glycerate/citrate	381	30.1	77	15.2	85	5.3	574	3.4	3670	6.1	17868	8.5	19555	4.5
3-phospho-glyceric acid	234	18.5	218	42.9	163	10.1	336	2.0	1642	2.7	3145	1.5	6875	1.6
sugar mono-phosphates	420	33.2	67	13.2	847	52.7	5163	30.9	27866	46.1	72950	34.6	75540	17.2
fructose bisphosphate									546	0.9	1028	0.5	2690	0.6
sucrose											3390	1.6		
phospho-glycolate									80	0.1	200	0.1	575	0.1
glycolate							51	0.3	114	0.2	1533	0.7	4750	1.1
uridinbisphos-phate glucose									1832	3.0	1883	0.9		
phosphoenol-pyruvate									30	0.0	170	0.1	540	0.1

the interrupted Krebs-cycle. Aspartate is very heavily labelled (20 to 75%) which we suggest to a CO_2 fixation of PEP carboxylation (anapleurotic function of PEP carboxylase) and a sequence of interrupted Krebs cycle.

The nearly parallel slops of the curves for glycerate/citrate and 3-phosphoglyceric acid and the relatively high radioactivity of glycine/serine indicate an operation of the glycolate metabolism. Cochromatographic investigations of the glycerate/citrate spots showed that more than 80% of the labelling was glycerate. Therefore, we think that the route of glycolate metabolism followed mainly the tartronic semialdehyde pathway [15]. But this does not rule out the operation via glycine/serine in *Anabaena cylindrica.* On the other hand, serine could be synthesized via 3-phosphoglyceric acid. Codd and Stewart [15] found all enzymes which are necessary for this route of glycolate metabolism. For this reason we studied the effect of N_2 and 100% O_2 on the ^{14}C-incorporation into the photosynthetic products (see Tables II and III).

In short term experiments of dark/light transients we observed under anoxygenic conditions (N_2 + 0.04% CO_2) a very strong labelling of sugar monophosphates until 90 sec and relatively low radioactivity in aspartate. In an oxygen atmosphere (100% O_2) in comparison to N_2 an enhanced radioactivity in aspartate and a decrease in sugar phosphates could be detected. Under both conditions a late labelling of glycine/serine (after 90 sec) was found. On the other hand, an initial and strong labelled photosynthetic product was glycerate; percentage proportion of phosphoglycolate and glycolate was very low and radioactivity could be detected very late (after 1 or 2 min photosynthesis). This can be interpreted that the tartronic semialdehyd pathway is the main route of glycolate and metabolism in *Anabaena cylindrica.*

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