INCREASED RATE OF CYCLIC PHOTOPHOPHORYLATION IN PREPARATIONS FROM *ANABAENA VARIABILIS* CELLS GROWN IN THE PRESENCE OF DIPHENYLAMINE [a]

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Cell free homogenates and membrane fractions prepared from *Anabaena variabilis* cells grown in the presence of diphenylamine have markedly higher activities for cyclic phosphorylation than similar preparations from normal cells. The preparations from diphenylamine-grown cells are also more active in system I mediated electron transport from reduced dichloroindophenol to oxygen or methyl viologen. The light intensity required to saturate phenazine methosulphate-supported cyclic phosphorylation, in such preparations, is higher than for preparations for normal cells.

1. Introduction

Diphenylamine (DPA [d]) is a known inhibitor of carotenoid synthesis in various organisms [1, 2]. Previous studies in this laboratory have shown that cells of the blue-green algae *Anabaena variabilis* grown in the presence of DPA differ from normal cells in pigment composition [3]. Cell free preparations of these cells have higher electron-transport activities and increased fluorescence activity when compared to preparations from normal cells [3]. We report in the present communication that such preparations have also a markedly increased activity in cyclic photophosphorylation.

2. Experimental

Anabaena variabilis cells were grown in the modified Detmer's medium [4] in the absence or presence of diphenylamine, according to the method reported by Ogawa et al. [3]. The cells, which were harvested after nine days of culture, were suspended in a solution containing 0.4 M sucrose, 0.05 M tris buffer (pH 7.5) and 0.01 M NaCl and were sonicated in a 10 kC Raytheon sonic oscillator for 5 min at 0° [5]. The supernates obtained after centrifugation of the sonicated cells at 17,000 *g* for 10 min were used as sonicates. The sonicates prepared from the cells grown in the absence and presence of diphenylamine will be described as normal-*Anabaena* sonicate and diphenylamine-*Anabaena* sonicate, respectively.

Chlorophyll *a* was determined after extraction by methanol using an extinction coefficient of 6.58×10^4 M^{-1} cm^{-1} at 666 nm [6]. ATP formation was measured following ^{32}P incorporation according to Avron [7]. It should be noted that the

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[d] *Abbreviations*: DPA: diphenylamine; PMS: phenazine methosulphate; tricine: tris(hydroxymethyl)methylglycine; DPIP: 2,6-dichlorophenolindophenol; DCMU:3-(3,4-dichlorophenyl)-1,1-dimethylurea.

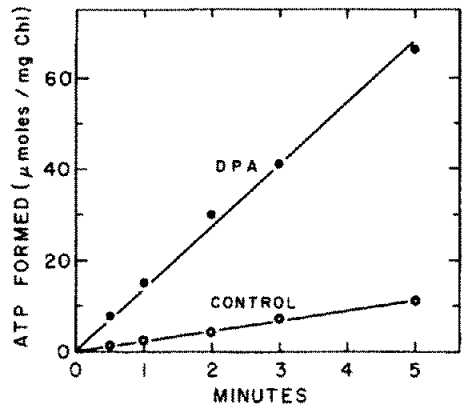

Fig. 1. Time course of cyclic phosphorylation. The reaction mixture contained the following in μmoles in a total volume of 3 ml: 50 tricine; 50 NaCl; 10 $MgCl_2$; 10 NaP_i; 4 ADP; 15 ascorbate and 0.15 PMS. The pH was adjusted to 6.7. The solution also contained ^{32}P and *Anabaena variabilis* sonicates equivalent to 5 μg chlorophyll *a*. Illumination was provided by white light at an intensity of 4×10^5 ergs/ cm^2/ sec.

normal *Anabaena* cells contain 6 mg protein per mg chlorophyll *a*, whereas the DPA-*Anabaena* cells contain twice as much protein.

3. Results and discussion

It has been shown previously that membrane fragments prepared from DPA-*Anabaena* cells have higher activities for NADP photoreduction when $DPIPH_2$ served as electron donor in comparison with fragments prepared from normal cells [3]. Sonicates prepared from DPA-*Anabaena* cells also have a markedly higher activity in PMS cyclic phosphorylation when expressed on the basis of chlorophyll *a* (fig. 1). The activity of both preparations is linear for at least 5 min. In the experiment reported in fig. 1, the control had an activity of 143 μmoles ATP formed per mg chlorophyll per hour and the activity of the DPA sonicate was 828. The ATP forming activity of the normal cells is similar to the lower range of activities found in *Anabaena* particles by Duane et al. [8]. Our experiments were performed under an atmosphere of air which resulted in somewhat lower activities than was observed under a nitrogen atmosphere [8].

Table 1
Effect of various carriers and washings on ATP formation.

Conditions	ATP formation (μmoles/mg chlorophyll/hr)	
	Normal preparations	DPA preparations
1. Sonicate		
No cofactor	7	76
DPIP	28	166
PMS	143	828
2. Sonicate		
PMS	163	1150
Membrane fragment		
0 washings	130	1060
1 washings	114	805
2 washings	116	745
3 washings	100	835

In experiment 1, DPIP was added at 0.13 mM and in this reaction DCMU at 5×10^{-5} M was also included. The PMS reaction performed at pH 6.7 and the other reactions at pH 8.0.

In experiment 2, PMS was used as a carrier. The sonicate was centrifuged for 1 hr at 144,000 *g* to obtain the membrane fragments. These fragments were suspended in the homogenizing medium and recentrifuged. The same procedure was repeated 3 times. The reaction mixtures were illuminated for 3 min, otherwise experimental conditions as in fig. 1.

The higher phosphorylating activity of the DPA sonicates is seen also in the presence of DPIP-ascorbate or with no cofactors (table 1). Each assay was performed at its pH optimum, with PMS at pH 6.8 and with DPIP-ascorbate at pH 8.0, according to Duane et al. [8].

The effect of light intensity on PMS phosphorylation is shown in fig. 2. At all light intensities tested, the sonicates prepared from DPA cells are more active. However, contrary to the controls, the DPA activity does not saturate in the light intensity range which was employed. Consequently, the relative increase in activity in the DPA preparations is larger at higher light intensities.

The cyclic phosphorylation activity of the blue-green algal preparations is rather stable [9]. As can be seen from the results of table 1, repeated washings of membranes in an iso-osmotic medium causes only slight losses in PMS phosphorylation capacity, indicating that the enzymes and pigments involved are

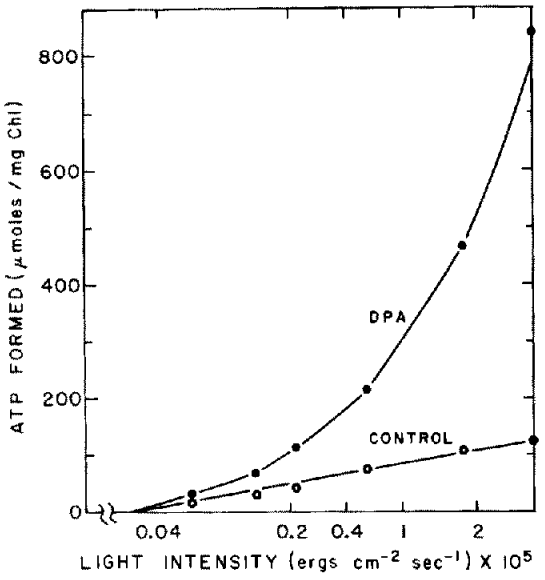

Fig. 2. Effect of light intensity on PMS cyclic phosphorylation. Illumination for 2 min. Otherwise experimental conditions as in fig. 1.

tightly bound to the lamellae. The increased activity of the DPA preparations is retained during washings.

Honeycutt and Krogmann have recently presented detailed evidence that in lamellae prepared from *Anabaena variabilis*, reduced trichlorophenol indophenol can donate electrons at two sites [10]. At a high concentration the donor seems to donate electrons in the proximity of P_{700}, whereas at low concentrations the site of donation is presumably located closer to system II and additional carriers participate in electron transport. A similar experiment was performed with sonicates prepared from two types of cells and the results are presented in table 2. Confirming the results of Honeycutt and Krogmann, it can be seen that homogenates of *Anabaena variabilis* are able to carry out electron transfer presumably mediated by photosystem I because it was carried out in the presence of DCMU, from $DPIPH_2$ to oxygen. The homogenates prepared from DPA cells have a higher activity in this reaction, both in the presence and absence of methyl viologen. The electron transport at low $DPIPH_2$ concentration is stimulated to a larger extent than that at high $DPIPH_2$ levels. The former reaction is supposedly mediated by a larger segment of the electron transport chain [10].

Table 2
Electron transport from $DPIPH_2$ to oxygen.

DPIP concentration (mM)	μmoles O_2 consumed/mg chlor/hr			
	Normal sonicates		DPA sonicates	
	−MV	+MV	−MV	+MV
0.08	147	270	582	1500
0.32	195	635	460	2080

The reaction mixture contained in 1.5 ml the following in μmoles: 50 tricine, pH 8.0; 2 ascorbate ; 0.03 DCMU; 1.25 NaN_3 and 1.25 methyl viologen where indicated. It also contained sonicates of normal or DPA cells equivalent to 5–10 μg chlorophyll *a*. Oxygen uptake was measured by a Gilson KM-C oxygraph with a YSI Clark electrode at 20°. A yellow Corning filter (#3–68) was placed between the light source and the sample to eliminate artifactual responses by the O_2 electrode. The light intensity at the level of the test tubes was 6×10^5 ergs/cm²/sec.

The growth of *Anabaena variabilis* cells in the presence of DPA results in large changes in the chemical composition of the photosynthetic apparatus [3]. The amounts of β-carotene, some xanthophylls and chlorophyll *a* are reduced, whereas the amount of myxoxanthophyll is increased per cell. Recently it has been observed that in DPA cells the amount of $cytochrome_{562}$ and $cytochrome_{554}$ is also markedly increased [11]. The increased rate of cyclic photophosphorylation in preparations from these cells, when expressed on a chlorophyll basis, is presumably due to the change in the chemical composition of the photosynthetic apparatus, implicating the participation of $cytochrome_{562}$ and perhaps $cytochrome_{554}$ in cyclic electron flow. However, removal of a large portion of $cytochrome_{554}$, apparently does not affect cyclic phosphorylation to a large extent [9].

An increase in several photochemical activities has been observed recently by Keck et al. [12] in a soybean mutant. In this mutant there was a decrease in the amount of β-carotene per chloroplast and a 2–3 fold increase in plastoquinone turnover which could account for the increased photochemical activities. It is of interest to note that the higher photoreaction of the preparation from DPA cells and the higher light required for saturation, are properties which have also been observed in some higher plant mutants [13, 14].

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