THE ROLE OF PLASTOQUINONE IN THE IN VIVO PHOTOSYNTHETIC CYCLIC ELECTRON TRANSPORT PATHWAY IN ALGAE

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1. Introduction

The existence of a cyclic electron transport pathway in the photosynthesis of plants and algae in vivo is now well documented [1] and recently reports have appeared regarding the regulation of this pathway during the assimilation of carbon dioxide [2, 3]. Now it would appear crucial to accurately ascertain the components of the cyclic pathway and relate this electron transport to the numerous types in vitro which are mediated by redox agents such as PMS, DAD, and DCPIP*.

There is general concurrence that the cyclic pathway shares photosystem I, P_{700}+ and X^- with the non-cyclic pathway, but there is a paucity of information concerning the components and their interaction between the two primary photoproducts in the cycle. Ferredoxin, cytochrome b_{563}, PQ, cytochrome *f* and plastocyanin have all been implicated in this regard albeit with some discordance [4, 5].

Trebst and associates [6, 7] have reported that the benzoquinone derivative, dibromothymoquinone (DBMIB), is a competitive antagonist of PQ and that it is an effective inhibitor of electron transport between the two photoacts. On the basis of investigations with DBMIB, Böhme et al. [7] have classified the various cyclic photophosphorylations in vitro and Izawa et al. [8] and Trebst and Reimer [9] have established the existence of an energy coupling site within photosystem II.

The present report concerns the effect of DBMIB on the cyclic pathway in algae assayed in vivo by cytochrome *f* turnover during CO_2 photosynthesis and by anaerobic substrate photoassimilation. The data indicate that PQ is a component of the in vivo cyclic pathway and, therefore, that the intermediary electron transport chain between the two photoacts is shared with the non-cyclic pathway.

* *Abbreviations*: PMS, methyl-phenazoniummethosulfate; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DCPIP, dichlorophenolindophenol; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone.

2. Materials and methods

Phorphyridium cruentum was obtained from the Indiana Culture Collection, Bloomington and was grown as described previously [3]. *Chlorella pyrenoidosa* Chick (Emerson strain #3) was kindly provided by Dr. Jerome Schiff (Waltham) and grown photoautotrophically on the medium of Schiff and Levinthal [10].

Cytochrome *f* turnover was determined in whole cells by averaging the repetitive absorption transient at 420 nm in response to modulated actinic light of low frequency using a single beam spectrophotometer as described previously [3]. The photomultiplier (EMI 9558) was blocked with a glass cut-off filter (Corning 5.60) and a 420 nm interference filter (Baird Atomic).

The far-red photoassimilation of glucose and acetate by algae was performed in a thermostated chamber and the cells were continuously sparged with nitrogen. D-[^{14}C]glucose(U) or Na-[1,2-^{14}C]acetate

(New England Nuclear) was added after a 5 min-dark anaerobic pre-incubation in the presence of 10 μM DCMU. Far-red illumination ($\lambda \geq 695$ nm) was provided by a 500 W tungsten iodide lamp filtered through water and two long-pass filters (Schott RG 695) at an incident intensity of 3×10^3 ergs/cm^2-sec. One ml aliquot samples were withdrawn from the reaction chamber at the times indicated, the cells were collected by ultrafiltration and rapidly washed free of reaction mixture with water. Routinely the washed cells and filter were dispersed in 3 ml of scintillation fluid (Aquasol or toluene–methoxyethanol, 3:2 containing 0.4% Omnifluor) and the total radioactivity determined by scintillation counting.

DBMIB was obtained through the courtesy of Dr. Achim Trebst (Bochum).

3. Results and discussion

Fig. 1 shows the absorption change at 420 nm in *P. cruentum* in response to photoactivation by light absorbed primarily by photosystem I. As was shown previously [3], this change is due to cytochrome *f* which turns over in the DCMU resistant in vivo cyclic pathway. The post-illumination recovery represents a reduction of the cytochrome with a first order rate constant of approx. 4.6 sec^{-1}. The addition of the PQ antagonist, DBMIB, considerably decelerates the reduction indicating that PQ is a component of the in vivo cyclic electron transport pathway. The reversible absorption change could not be totally abolished even at the highest DBMIB concentrations possible suggesting that the quinone pool is by-passed by a slower electron transport pathway. Bohme et al. [7] have also reported that DBMIB modifies the DAD- and PMS-mediated cyclic pathways in isolated spinach chloroplasts. The results are at variance with those of Böhme and Cramer [11] who found that DBMIB accelerates the dark reduction of cytochrome *f* in isolated chloroplasts.

Fig. 2 shows data from a similar experiment when the cells were excited by light which activates photosystems I and II. Under these conditions both the cyclic and the non-cyclic pathways were expected to be active. The post-illumination reduction of cytochrome *f* was biphasic; the fast component (k = 28 sec^{-1}) is associated with non-cyclic transport and is abolished by DCMU, and the slow component (k = 4.6 sec^{-1}) is associated with cyclic transport as is obtained in photosystem I light alone [3]. Also it was previously shown in this laboratory [3] that the ratio of cyclic and non-cyclic transport is dependent on the

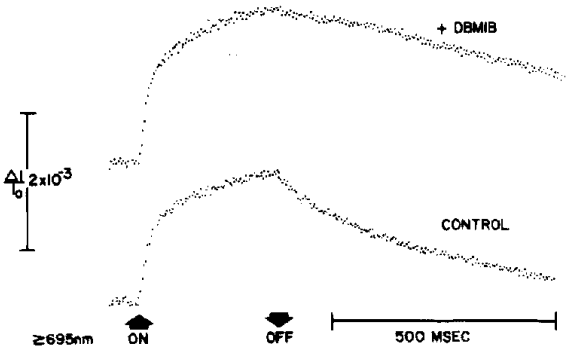

Fig. 1. Photoinduced absorption transients at 420 nm in *P. cruentum* as a result of activation by photosystem I. The wavelengths of the excitation flash were 695 nm and longer (Schott RG 695). DBMIB was added to give a final concentration of 2 μM and the chlorophyll concentration was 8.2 μg/ml. The averaged absorption transient (128 sweeps) was 1 sec and the overall cycle time between flashes was 2 sec.

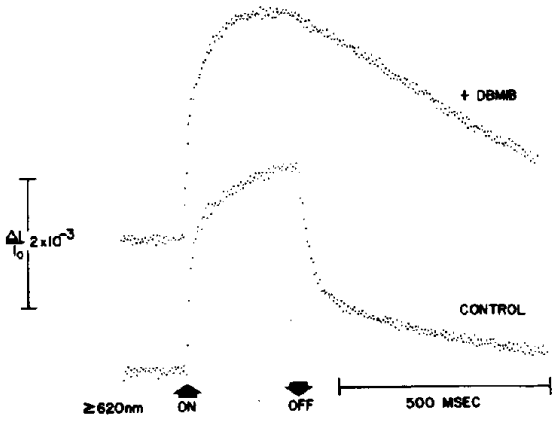

Fig. 2. Photoinduced absorption transients at 420 nm in *P. cruentum* as a result of activation by photosystem I and photosystem II. The wavelengths of the excitation flash were 620 nm and longer (Schott RG 630). The chlorophyll concentration was 11.7 μg/ml and the flash and averaging conditions were as in fig. 1. DBMIB was added to give a final concentration of 2 μM.

intensity and spectral composition of the exciting light such that at saturation the cyclic pathway does not contribute to photosynthesis. In this particular experiment the cyclic pathway represented about 14% of the total electron transport flux. The addition of DBMIB resulted in a more rapid oxidation of cytochrome *f* followed by a much slower dark recovery similar to that presented in fig. 1. The amplitude and kinetics of the cytochrome redox change in the presence of DBMIB was unaffected by DCMU indicating that the cytochrome turnover was totally driven by the cyclic pathway. These results confirm those of Gimmler and Avron [12] and Böhme and Cramer [11] who also showed that DBMIB prevents the reduction of cytochrome *f* by electrons generated from photosystem II. The in vivo cyclic pathway, therefore, includes plastoquinone as an intermediate and must also share the intermediary electron transport chain between PQ and cytochrome *f* with the non-cyclic pathway. This section of the non-cyclic pathway has been shown to be a site of energy coupling in the electron transport chain of chloroplasts [13].

Because of the current interest in the details of the numerous cyclic pathways in vitro and the contribution of the in vivo pathway to the various phases of cellular metabolism in algae it was of interest to investigate the effect of DBMIB on the anaerobic assimilation of organic substrates by photosystem I driven reactions. Urbach and Kaiser [14] have already reported that the rate of synthesis and the steady state level of intracellular ATP induced by photosystem I light in anaerobic conditions in *Ankistrodesmus braunii* is reduced in the presence of DBMIB.

Fig. 3 curve A shows the DCMU-resistant anaerobic incorporation of glucose by *C. pyrenoidosa* in far-red light of low intensity. No induction period was found for this species in contrast to the previous report on glucose uptake by *C. vulgaris* [15] and the incorporation was found to be linear for at least 90 min under these conditions. Curve B shows that DBMIB inhibits the photoassimilation but does not affect the intracellular level of glucose already assimilated and curve C shows a control experiment conducted in darkness. Essentially the same results were found for the photoassimilation of acetate, but the inhibition by DBMIB was not as complete. Similar experiments were attempted using *P. cruentum* but all were negative. It was not possible to demonstrate the

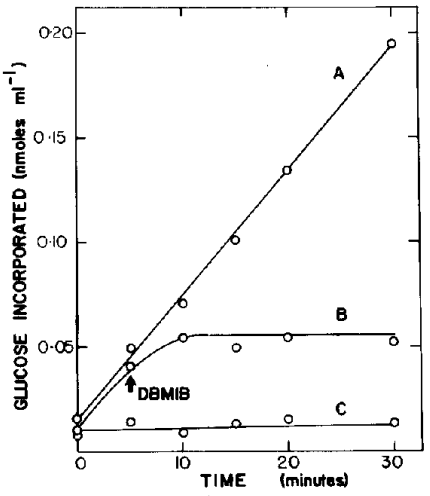

Fig. 3 Photoassimilation of D-glucose by *C. pyrenoidosa*. The reaction mixture contained algae equivalent to 10 μg chlorophyll/ml growth medium, 10 μM DCMU and 10 mM glucose (0.5 μCi/ml) at 25°C. (A) light control; (B) as A but DBMIB was added after 5 min to give a final concentration of 2 μM; (C) as A but in complete darkness. All samples were pre-incubated anaerobically in darkness for 5 min prior to the glucose addition and onset of illumination at zero time.

photoassimilation or the aerobic utilization of acetate or glucose in this red alga even after dark starvation or after long periods of growth in the presence of these organic substrates. Thus the basis for the obligate autotrophy of red algae would appear to be different from that of the blue-green algae which do show some very limited photoheterotrophy [16, 17].

These results are in agreement with those of Urbach and Kaiser [14] and I also conclude that the in vivo cyclic pathway for organic substrate photoassimilation in algae includes PQ and that the pathway is most likely identical to the cyclic transport which is utilized during certain phases of CO_2 fixation. Based upon experiments with isolated Class I chloroplasts, Forti and Rosa [5] reported that DBMIB had no effect on the endogenous cyclic photophosphorylation. The endogenous cyclic pathway is often construed to be representative of the pathway in vivo [1]. This anomaly, and that on the kinetics of cytochrome *f* reduction reported by Böhme and Cramer [11] illustrate the major differences between investigations with higher plant chloroplasts and those with intact algae on this particular problem.

Acknowledgments

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References

[1] Simonis, W. and Urbach, W. (1971) Ann. Rev. Plant Physiol. 24, 89–114.
[2] Klob, W., Kandler, O. and Tanner, W. (1973) Plant Physiol. 51, 825–827.
[3] Biggins, J. (1973) Biochemistry 12, 1165–1170.
[4] Amesz, J., Pulles, M.P.J., Visser, J.W.M. and Sibbing, F.A. (1972) Biochim. Biophys. Acta 275, 442–452.
[5] Forti, G. and Rosa, L. (1971) FEBS Letters 18, 55–58.
[6] Trebst, A., Harth, E. and Draper, W. (1970) Z. Naturforsch. 25b, 1157–1159.
[7] Böhme, H., Reimer, S. and Trebst, A. (1971) Z. Naturforsch 26b, 341–352.
[8] Izawa, S., Gould, J.M., Ort, D.R., Felker, P. and Good, N.E. (1973) Biochim. Biophys. Acta 305, 119–128.
[9] Trebst, A. and Reimer, S. (1973) Biochim. Biophys. Acta 305, 129–139.
[10] Schiff, J.A. and Levinthal, M. (1968) Plant Physiol. 43, 547–554.
[11] Böhme, H. and Cramer, W.A. (1971) FEBS Letters 15, 349–351.
[12] Gimmler, H. and Avron, M. (1972) in Proc. 2nd Int. Congr. Photosynthesis Research, Stresa, 1971 (Forti, G., Avron, M. and Melandri, A., eds), Vol. 1, pp. 789–800, Dr. W. Junk, The Hague.
[13] Böhme, H. and Cramer, W.A. (1972) Biochemistry 11, 1156–1160.
[14] Urbach, W. and Kaiser, W. (1972) in: Proc. 2nd Int. Congr. Photosynthesis Research, Stresa, 1971 (Forti, G., Avron, M. and Melandri, A., eds), Vol. 2, pp. 1401–1411, Dr. W. Junk, The Hague.
[15] Tanner, W., and Kandler, O. (1967) Z. Pflanzenphysiol. 58, 24–32.
[16] Hoare, D.S., Hoare, S.L. and Moore, R.B. (1967) J. Gen. Microbiol. 49, 351–370.
[17] Smith, A.J., London, J. and Stanier, R.Y. (1967) J. Bacteriol. 94, 972–983.