EFFECT OF DBMIB, DCMU AND ANTIMYCIN A ON CYCLIC AND NONCYCLIC ELECTRON FLOW IN C_4 MESOPHYLL CHLOROPLASTS

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1. Introduction

Noncyclic electron transport from H_2O to NADP is thought to require the combined action of two photoacts [1]. Cyclic electron flow requires only photosystem 1 and is not inhibited by noncyclic inhibitors such as DCMU. Usually cyclic electron flow is studied with the use of an artificial cofactor, such as PMS, or the more natural catalyst, ferredoxin [2]. A participation of PQ in the latter, but not the former, was suggested [3] on the basis of sensitivity to the PQ antagonist, DBMIB [4]. Forti and Rosa [5], however, working with broken chloroplasts, concluded that PQ was not involved in cyclic electron flow, on the basis of differential sensitivity to DBMIB of low rates of 'endogenous' cyclic phosphorylation to noncyclic phosphorylation. The pathway of electron flow in cyclic phosphorylation still remains open to question.

In the present investigation we have used CO_2 fixation by mesophyll chloroplast preparations of the C_4 plant *Digitaria sanguinalis* as an indication of ATP synthesis. We have recently shown that with C_4 mesophyll chloroplast preparations coupled to cytoplasmic PEP carboxylase, high rates of CO_2 fixation can be induced by pyruvate [6]. CO_2 fixation in our system can be broken down into the following partial reactions [7]:

(a) pyruvate + 2 ATP + P_i $\xrightarrow[\text{adenylate kinase}]{\text{pyruvate } P_i \text{ dikinase}}$ PEP + 2 ADP + PP

(b) PEP + CO_2 $\xrightarrow{\text{PEP carboxylase}}$ OAA + P_i

(c) OAA + NADPH $\xrightarrow{\text{malate dehydrogenase}}$ Malate + NADP

In the system studied, reaction (c) is not required for CO_2 fixation. The ATP for reaction (a) can come from cyclic or noncyclic phosphorylation, which can be distinguished on the basis of sensitivity to specific inhibitors. In this report, we show that the ATP for initiation of pyruvate induced CO_2 fixation comes from cyclic phosphorylation while with CO_2 fixation induced by pyruvate + OAA, the ATP comes principally from noncyclic phosphorylation. When OAA is present, the NADPH is rapidly turned over (by reaction c), such that noncyclic electron flow can proceed. The level of enzymes involved in the CO_2 fixation process are present in high levels, such that the rate of

Abbreviations: C_4 plant, plant having Kranz anatomy and the enzymes of both the C_4 pathway and the Calvin-Benson pathway; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; PMS, phenazinemethosulphate; PQ, plastoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; Chl, chlorophyll; OAA, oxalacetic acid; PEP, phosphoenolpyruvate.

CO_2 fixation is dependent on the amount of ATP formed. DBMIB, a potent inhibitor of PQ-dependent reactions, completely inhibits both cyclic and non-cyclic dependent systems. With the pyruvate induction, which requires cyclic electron flow, DCMU stimulates the apparent rate of cyclic phosphorylation and also overcomes the DBMIB inhibition. The cyclic phosphorylation in the presence of DCMU is also shown to become less sensitive to antimycin A. It is concluded that normal cyclic electron flow involves PQ and that in the presence of DCMU, the flow of electrons is diverted to bypass PQ. This may account for some of the stimulation of cyclic phosphorylation by DCMU.

2. Methods

Mesophyll protoplasts were isolated from fully mature leaves of *Digitaria sanguinalis* by the method of Kanai and Edwards [8,9] using the optimum conditions of Huber and Edwards (submitted to Physiol. Plant). Protoplasts were gently broken to release mesophyll chloroplasts [6]. The total protoplast homogenate was used in order to couple chloroplast reactions to extrachloroplastic PEP carboxylase for $^{14}CO_2$ fixation [6,7]. Protoplast homogenates are referred to as chloroplast preparations. Chloroplasts prepared in this manner were judged to be about 80% intact, on the basis of enzyme retention [10] and the rate of ferricyanide reduction before and after osmotic shock (Ku and Edwards, unpublished). All reactions were run under 2% O_2 to eliminate any possibility of pseudocyclic electron flow. DBMIB was obtained through the courtesy of Dr A. Trebst.

3. Results and discussion

3.1. *Effect of antimycin A and DCMU on CO_2 fixation*

As shown in table 1, the induction of CO_2 fixation by pyruvate and pyruvate + OAA responds differently to inhibitors of electron transport. Antimycin A, an inhibitor of cyclic electron flow [2,3], inhibited the pyruvate induction almost completely while the pyruvate + OAA induction was only slightly inhibited. This suggests that the pyruvate induction utilizes cyclic ATP exclusively. DCMU, which blocks noncyclic electron flow, drastically inhibited the pyruvate + OAA induction while the same concentration stimulated the pyruvate induction several fold. This suggests that ATP for the pyruvate + OAA induction comes largely from noncyclic electron flow. The stimulation of pyruvate induced CO_2 fixation is compatible with other reports that DCMU stimulates cyclic electron flow [2,11]. DCMU at 0.8 μM completely blocked noncyclic electron flow in our system, as O_2 evolution induced by pyruvate + OAA was completely inhibited (table 1).

3.2. *Effect of DBMIB*

As shown in figure 1, both the cyclic electron flow (pyruvate induced CO_2 fixation) and noncyclic electron flow (pyruvate + OAA induced CO_2 fixation)

Table 1
Effect of antimycin A and DCMU on CO_2 fixation by mesophyll chloroplast preparations of *D. sanguinalis*

Substrates	10 μM Antimycin A % control	0.8 μM DCMU	Control rate (μmoles · mg Chl^{-1} h^{-1})
Pyruvate – CO_2 fixation	5	340	39
Pyruvate, OAA – CO_2 fixation	88	10	243
Pyruvate, OAA – O_2 evolution	98	2	126

Conditions: 0.3 M sorbitol, 2 mM $MgCl_2$, 1 mM KH_2PO_4, 4 mM $NaH^{14}CO_3$, mesophyll chloroplast preparations containing 3–6 μg Chl, and where used, 2.5 mM pyruvate and 0.5 mM OAA. Temperature: 37°C. Illumination: 80 nE/cm^2 · s^{-1} between 400 and 700 nm. Rates were calculated after 3 min of fixation on the basis of cpm fixed into acid-stable products.

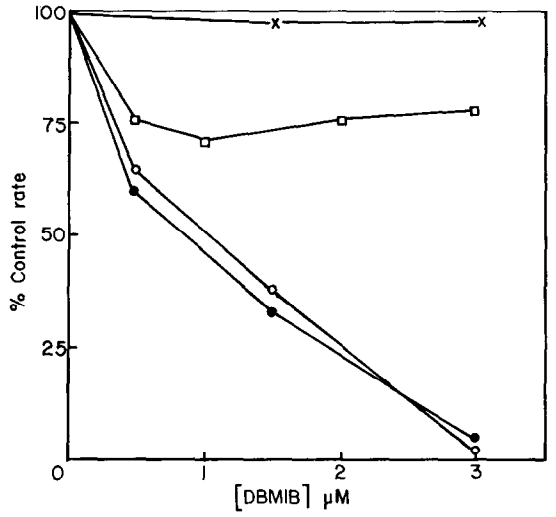

Fig.1. Effect of DBMIB on CO_2 fixation by mesophyll chloroplast preparations of *D. sanguinalis* induced by pyruvate (o——o), pyruvate + OAA (•——•), pyruvate + 20 μM PMS (□——□) and pyruvate + 0.2 μM DCMU (X——X), Conditions as in table 1.

were completely inhibited at 3 μM DBMIB, whereas CO_2 fixation catalyzed by PMS was not. This suggests that both cyclic and noncyclic electron flow require PQ since DBMIB is a potent inhibitor of PQ-dependent reactions. Fig.1 also demonstrates that PMS catalyzed 'cyclic' electron flow is not dependent on PQ; the inhibition observed could be due to an inhibition of the path that involves PQ or to some nonspecific inhibition. The partial inhibition observed parallels that obtained by Than Nyunt and Wiskich [12] and may reflect that part of the PMS catalyzed pathway which involves b-type cytochrome through PQ [13]. CO_2 fixation induced by pyruvate in the presence of DCMU was completely insensitive to DBMIB (fig.1), suggesting that PQ is not an obligatory intermediate in the presence of DCMU.

The effect of DCMU was investigated further. As shown in fig.2, cyclic phosphorylation, as indicated by pyruvate induced CO_2 fixation, was strongly stimulated by DCMU. The rate in the absence of DCMU is taken to indicate the normal level of cyclic electron flow. In the absence of DCMU, both the DBMIB and antimycin A-inhibited reactions show little or no CO_2 fixation, which would be expected since the cyclic system is sensitive to both inhibitors. However, DCMU allows CO_2 fixation to proceed in the presence of either inhibitor, suggesting that in the presence of DCMU, the path of electron flow bypasses the DBMIB block and partially the antimycin A block, i.e., PQ

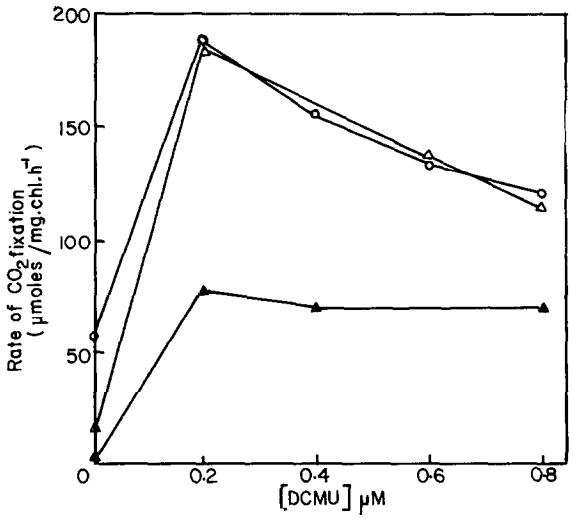

Fig.2. Effect of DCMU on CO_2 fixation by mesophyll chloroplast preparations of *D. sanguinalis* induced by pyruvate (o——o), pyruvate + 5 μM antimyc in A (△——△) and pyruvate + 3 μM DBMIB (▲——▲). Conditions as in table 1.

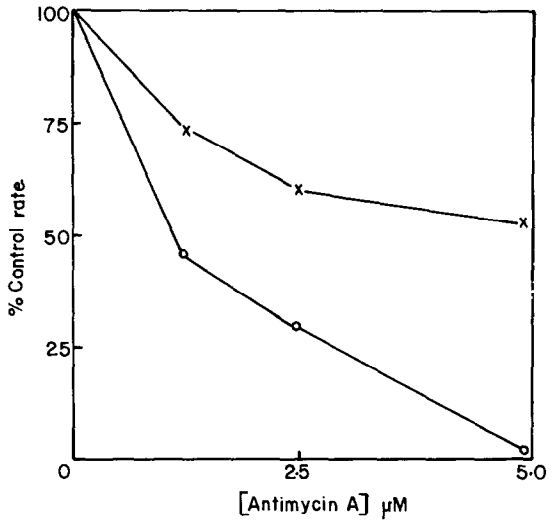

Fig.3. Effect of antimycin A on CO_2 fixation induced by pyruvate (o——o) and pyruvate + 0.2 μM DCMU (X——X). Conditions as in table 1.

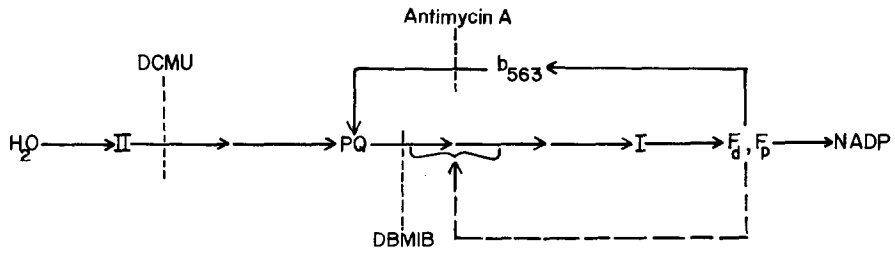

Fig.4. Scheme of electron transport in cyclic and noncyclic phosphorylation (solid lines) and the effect of DCMU on cyclic electron flow (broken line).

and cyt b_6 are no longer obligatory intermediates. That the DCMU reversal of the inhibition by DBMIB and antimycin A is not due to permeability problems in the presence of DCMU, was shown by first adding either DBMIB or antimycin A to demonstrate inhibition, and then adding DCMU to the same preparation to reverse the inhibition (data not shown).

Normal cyclic electron flow is completely antimycin A sensitive, as shown in Fig.3. However, in the presence of DCMU, the phosphorylation was strikingly less sensitive to the inhibitor. At 5 μM antimycin A, slightly more than 50% of the control rate remained in the presence of DCMU, suggesting that at least part of the electron flow bypasses the antimycin A block at cytochrome b_6.

4. Conclusion

The investigations reported here support the following conclusions: a) PQ is involved in both cyclic and noncyclic electron flow in intact mesophyll chloroplasts of *D. sanguinalis*, a C_4 plant, as suggested by sensitivity to DBMIB (fig.1). b) DCMU, in addition to inhibiting noncyclic electron flow (table 1), stimulates cyclic electron flow (fig.2, table 1) and causes the electron flow to bypass PQ (fig.1,3) and the antimycin A block (fig.3). Both pathways contain sites of energy conservation. This shift in the flow of cyclic electrons to a shorter path may be involved in the stimulation of cyclic phosphorylation observed, perhaps in addition to the 'overreduction' phenomenon proposed by Hauska [14]. Fig.4 summarizes the above conclusions.

Acknowledgments

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