THE ACTION OF DIO-9 ON PHOTOPHOSPHORYLATION

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Oxygen evolution occurs when a suspension of isolated chloroplasts is illuminated in the presence of the electron acceptor, ferricyanide. The rate of oxygen evolution is governed by the presence or absence of phosphate acceptor. The increase in the rate of oxygen evolution which occurs on addition of phosphate acceptor and subsequent decrease on the acceptor's exhaustion has been termed photosynthetic control [1]. Photosynthetic control is thus analogous to respiratory control. The characteristics of oligomycin as an inhibitor of mitochondrial energy transfer (i.e. phosphorylation of ADP) [2,3] are usually established using mitochondria which show good respiratory control [4]; by measuring photosynthetic control, inhibitors of photosynthetic energy transfer can be studied in a similar manner. This report describes the action of Dio-9 (an antibiotic introduced into the study of biological energy transfer reactions by Guillory [5] and manufactured by Royal Netherlands Fermentations Industry Company, Delft) as an inhibitor of energy transfer during non-cyclic photophosphorylation in isolated pea chloroplasts.

Phosphorylation in pea chloroplasts is tightly coupled to electron flow. Fig. 1b shows that light initially induced a slow rate of oxygen evolution in the presence of ferricyanide. On the addition of ADP, the rate increased sharply and subsequently decreased. The stimulated rate in the presence of ADP has been termed the state 3 rate, the slower rate after ADP has been phosphorylated the state 4 rate, and the ratio

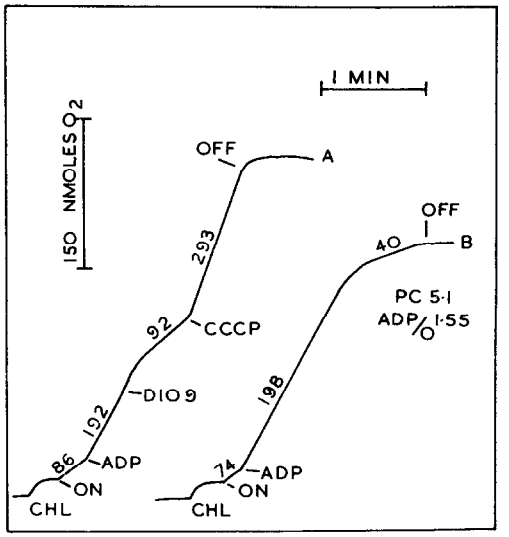

Fig. 1. Polarographic tracing of oxygen evolution (shown by an upward deflection of the trace) by isolated pea chloroplasts showing inhibition of electron flow by Dio-9 (curve A). Curve B shows a control experiment. The reation medium (2.1 ml) contained sucrose (250 mM), K_2HPO_4-KH_2PO_4 buffer pH 7.6 (10 mM), $MgCl_2$ (5 mM), HEPES - KOH buffer 7.6 (10 mM) and potassium ferricyanide (1.4 mM). Additions were ADP (0.3 mM), Dio-9 (44 μg/ml) and CCCP (0.6 μM). The experimental methods for measuring photosynthetic control were as described previously [1]. Chloroplasts were prepared as described previously omitting the final centrifuging. Chloroplasts (CHL) were present in each experiment at 52 μg/ml. Numbers along trace are nmoles O_2/min.

Abbreviations: DNP, 2,4-dinitrophenol; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; FCCP, carbonyl cyanide *p*-trifluoromethoxy phenyl hydrazone. HEPES, *N*-2-Hydroxyethylpiperazine-*N*-2-ethanesulphonic acid.

state 3/state 4 the photosynthetic control ratio. The value of the photosynthetic control ratio (in this experiment 5.1) indicates the degree of coupling be-

tween electron flow and phosphorylation. An ADP/O ratio is also given.

Fig. 1a shows that Dio-9, when added during state 3 electron flow, slowed the rate of oxygen evolution. The inhibited state 3 rate ("pseudo" state 4 rate) is faster than the state 4 rate observed in the control experiment. Subsequent addition of CCCP at 0.6 μM released the inhibition of electron flow caused by Dio-9. This result could be interpreted as showing that Dio-9 acts as an energy transfer inhibitor and that CCCP acts as an uncoupler. However, addition of this concentration of CCCP to chloroplasts during normal state 4 electron flow, did not stimulate oxygen evolution (i.e. uncouple) to the same degree (fig. 2). Dio-9 added after CCCP, gave a fast rate of oxygen evolution (268 nmoles O_2/min) which was similar to that seen in fig. 1 curve A (293 nmoles O_2/min) thus though Dio-9 alone inhibited state 3 electron flow (energy transfer inhibition) and CCCP at 0.6 μm uncoupled the state 4 rate slightly, the combination of the two compounds gave a synergistic uncoupling of photophosphorylation *.

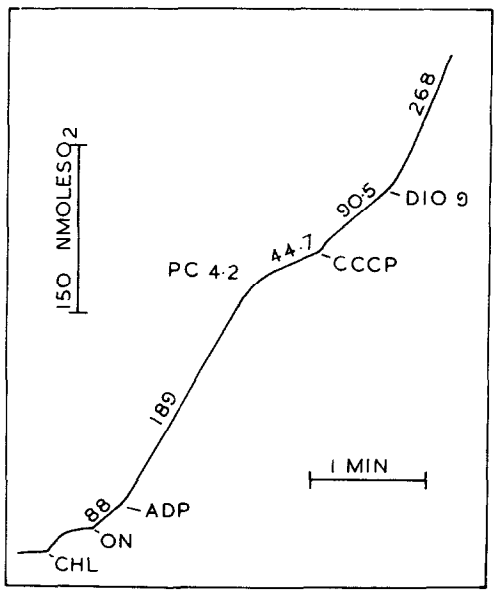

Fig. 2. Polarographic tracing of oxygen evolution by pea chloroplasts showing the effect of CCCP (0.6 μM) and Dio-9 (44 μg/ml) on state 4 rate of oxygen evolution. The conditions for the experiment were the same as described for fig. 1.

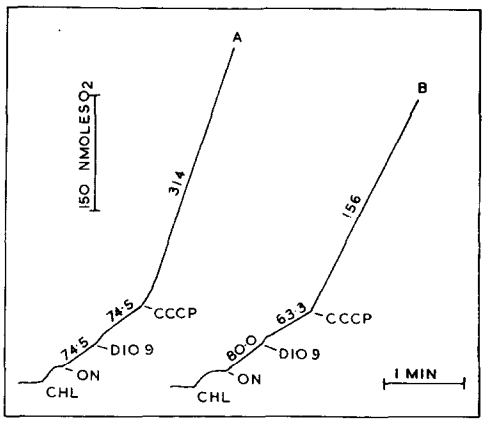

Fig. 3. Polarographic tracing of oxygen evolution by pea chloroplasts showing the effect of Dio-9 at 44 μg/ml (curve A) and 22 μg/ml (curve B). CCCP (0.6 μM) was added in each experiment following the addition of Dio-9. The conditions for the experiment were the same as described for fig. 1.

This suggestion is confirmed by the experiments outlined in fig. 3. Oxygen evolution in the presence of 44 μg/ml Dio-9 and 0.6 μM CCCP (curve A) was twice as fast as that in the presence of 22 μg/ml Dio-9 and 0.6 μM CCCP (curve B). Dio-9 at 22 μg/ml inhibited the Hill reaction rate, (oxygen evolution in the absence of ADP) but there was no change in the rate at the higher Dio-9 concentration. Two separate stocks of Dio-9 were used in these experiments and with both the concentrations of Dio-9 necessary to inhibit state 3 electron flow were higher than those quoted by McCarty, Guillory and Racker [6]. The reason for this discrepancy is not clear.

In a similar series of experiments, it was found that the combination of valinomycin with CCCP and of Dio-9 with DNP resulted in greater uncoupling than when any of these compounds was added alone. In contrast to the effect of Dio-9, valinomycin alone had no effect on chloroplast activity. Karlish and

* To enable direct comparisons all results shown were obtained with a single preparation of chloroplasts. Similar results were obtained with other preparations of chloroplasts.

Avron [7], using different methods, have also reported a similar uncoupling of ATP synthesis from photosynthetic electron flow with valinomycin and DNP. They have proposed that the synergistic uncoupling action of DNP or FCCP and valinomycin, gramicidin or nigericin, is caused by an increase in the permeability of the chloroplast to protons and cations. The synergistic uncoupling action of Dio-9, with concentrations of CCCP which are subcptimal for uncoupling, may also involve altered membrane permeability to protons and ions. Valinomycin alone has little effect on photophosphorylation and Dio-9 alone acts as an inhibitor of energy transfer yet both can stimulate the uncoupling action of suboptimal concentrations of uncouplers (CCCP or DNP).

It is known that valinomycin increases the permeability of mitochondrial membranes to potassium [8]. The mechanism of action of Dio-9 is unknown. It is possible that Dio-9 acts as an inhibitor of photosynthetic energy transfer by preventing proton translocation into the chloroplast. In the presence of Dio-9, protons might be bound to the membrane surface and hence the Hill reaction rate of oxygen evolution might be slower (fig. 3b). Addition of Dio-9 during state 3 electron flow would also restrict proton translocation leading to energy transfer inhibition (fig. 1a). Protons bound to the membrane surface may be "ferried" (the terminology of Mitchell [9]) more efficiently by a low concentration of CCCP thus accounting for uncoupling.

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