Preliminary Communication

Dio-9, an Inhibitor of Coupled Electron Transport and Phosphorylation in Chloroplasts*

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Oxidative phosphorylation and photophosphorylation have been found to respond differently to various inhibitors. Ammonia inhibits photophosphorylation at low concentrations (1), whereas high concentrations are required to uncouple oxidative phosphorylation (2). Dinitrophenol, on the other hand, is a more potent uncoupler of oxidative phosphorylation than of photophosphorylation (3). Oligomycin inhibits respiration in tightly coupled mitochondria in the presence of a phosphate acceptor system. Respiration is restored by the addition of an uncoupler such as dinitrophenol. In loosely coupled preparations, oligomycin inhibits phosphorylation, but it has little effect on respiration (4). Photophosphorylation, in contrast, is not affected by oligomycin. In fact, no compound is known which inhibits photophosphorylation and coupled electron transport in the manner that oligomycin acts on oxidative phosphorylation (5).

Recently, the antibiotic Dio-9 was found to inhibit the respiration of rat liver mitochondria only in the presence of P1 (6). This inhibition did not require added adenine nucleotides and it was not reversed by dinitrophenol. In the absence of Pi, Dio-9 stimulated respiration. It is the purpose of this communication to report that Dio-9 has effects on chloroplasts analogous to those of oligomycin on mitochondria.

Fig. 1 illustrates the effect of Dio-9 on oxygen evolution accompanying ferricyanide reduction in chloroplasts. Although some inhibition of oxygen evolution occurred in the absence of ADP, Pi, and Mg++ (Experiment A), the stimulation of oxygen evolution obtained upon the addition of the phosphate acceptor system was completely abolished by Dio-9 (Experiment B). With five different chloroplast preparations, the inhibition of either oxygen evolution or ferricyanide reduction by 3.5 μg of Dio-9 per ml, in the presence of the phosphate acceptor system, averaged 64.8%, whereas in its absence it averaged 23.8%. The inhibition of oxygen evolution by Dio-9 was reversed by the addition of NH₄Cl (Experiments A and B). Results similar to those presented in Fig. 1 were obtained with TPN⁺ as the electron acceptor. Experiment C shows that oxygen evolution by chloroplasts that had been treated with EDTA, a procedure which uncouples electron transport from photophosphorylation (7), was not inhibited by Dio-9. Carbonyl cyanide trifluoromethoxyphenylhydrazone was also found to reverse Dio-9 inhibition of electron transport, but atebrin, octylguanidine, and dinitrophenol did not.

In contrast to the observations with rat liver mitochondria in which P1 alone was required for the Dio-9 inhibition of respiration, pronounced inhibition of oxygen evolution in chloroplasts occurred only in the presence of ADP, Pi, and Mg++. Arsenate replaced P1, but the presence of ADP and Mg++ was required. The latter results are reminiscent of the observation that ADP and Mg++ were required for the stimulation of ferricyanide reduction by arsenate (6).

**Fig. 1. Inhibition of oxygen evolution by Dio-9.** Oxygen evolution was assayed polarographically in a volume of 1.0 ml at room temperature, with a Clark-type electrode. The reaction mixtures contained: Experiment A: 50 mM Tris-HCl, pH 8.0, 70 mM NaCl, 1.2 mM K₃Fe(CN)₆, and spinach chloroplasts containing 40 μg of chlorophyll; Experiment B: the reagents were the same as in Experiment A, but 2.5 mM ADP, 10 mM potassium phosphate buffer, pH 8.0, and 5 mM MgCl₂ were also added; Experiment C: the reaction mixture was as in Experiment B, but the chloroplasts were incubated at 0°C for 5 min in the presence of 0.5 mM EDTA at a concentration of 0.2 mg of chlorophyll per ml. Each vessel was illuminated with a 300-watt reflector flood lamp which provided approximately 5000 foot candles after passage of the light through 8 cm of water. The slopes in the figure are expressed as microatoms of oxygen per min.

Phosphorylation coupled to ferricyanide reduction was also inhibited by Dio-9. Fig. 2 shows that in the presence of Dio-9 (3.5 μg per ml), which inhibited phosphorylation 90%, inhibited ferricyanide reduction 67%. In the presence of 0.8 mM NH₄Cl, which partly uncoupled phosphorylation, ferricyanide reduction was not inhibited by Dio-9 but phosphorylation was. These results indicate, in conjunction with those presented in Fig. 1, that only electron transport which is tightly coupled to phosphorylation is inhibited by Dio-9. Cyclic phosphorylation in chloroplasts (40 μg of chlorophyll), supplemented with n-methyl-
Inhibition of latent, Ca++-activated ATPase activity by Dio-9

Spinach chloroplasts containing 80 μg of chlorophyll were incubated 6 min with 200 μg of trypsin, and the Ca++-activated ATPase activity was determined. Purified Ca++-activated ATPase (15 μg) was treated with 40 μg of trypsin. An aliquot containing about 0.75 μg of ATPase protein was assayed for ATPase activity. Trypsin treatment and ATPase assays were performed as previously described (8).

<table>
<thead>
<tr>
<th>Chloroplasts</th>
<th>Purified enzyme</th>
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<tbody>
<tr>
<td>Dio-9 (μg/ml)</td>
<td>ATPase activity</td>
</tr>
<tr>
<td>0</td>
<td>0.63</td>
</tr>
<tr>
<td>1.61</td>
<td>0.33</td>
</tr>
<tr>
<td>3.22</td>
<td>0.20</td>
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<tr>
<td>6.44</td>
<td>0.06</td>
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which was activated by treatment with trypsin. Table I shows that the Ca++-dependent ATPase activity catalyzed by either the purified enzyme or by chloroplasts was inhibited by low concentrations of Dio-9.

REFERENCES