A New Amine as an Uncoupler of Chloroplast Electron Transport

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Abbreviations used: DAD-diaminodurene; DBMIB-2,5,8-dibromo-3-methyl-6-isopropyl-p-benzoquinone; DCMU-dichlorophenyl-dimethylurea; DMBQ-2, 5-dimethylbenzoquinone; DNP-INT -2, 4-dinitrophenylether of iodonitrothymol; FCCP-carbonylcyanide-p-trifluoromethoxyphenylhydrazone; MV-methylviologen; TMPD-N-tetramethyl-p-phenylenediamine.

Introduction

In isolated chloroplasts electron transport is coupled to photophosphorylation (1,2). To study electron transport rates in Photosystem I and II, certain chloroplast reactions require an uncoupler to be present. The common uncouplers used for this purpose are FCCP, ammonia and such ionophores as gramicidin (2).

In this study we describe a new amine-type uncoupler, N-[bis-(3,5-trifluoromethyl)-phenyl]-2,4-dinitro(3-trifluromethyl)-benzamine (DPA, Figure 1), which appears to work

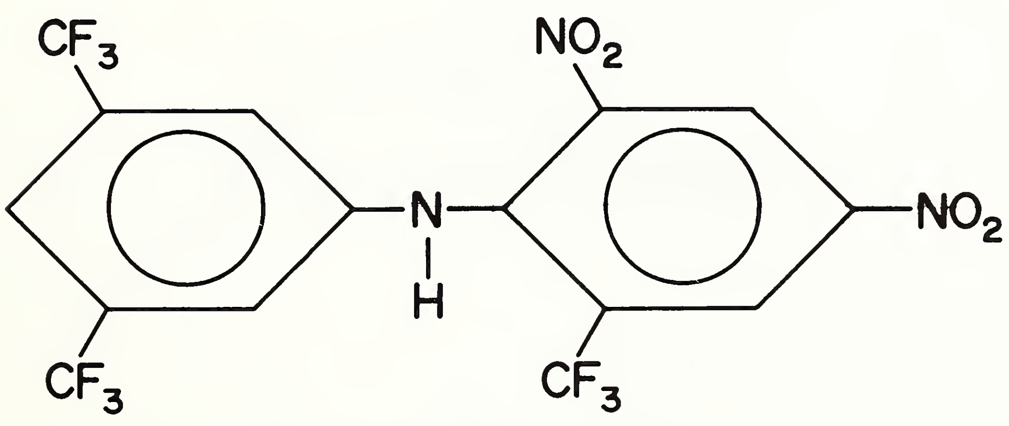

FIGURE 1. The Chemical Composition of the Uncoupler, DPA.

best at coupling site 1, located between the two photosystems in the chloroplast electron transport chain. We show that low concentrations (1×10^{-7}) are required to stimulate electron transport 60% or to inhibit the proton gradients associated with photophosphorylation.

Materials and Methods

Spinach or lettuce chloroplasts were prepared from commercially available sources by methods previously reported (3). Briefly, about 20g of leaves were ground in a Waring blender in 100 ml sucrose-NaCl (0.4 M sucrose, 0.05 M NaCl) with 6 on-and-off bursts of energy. The resulting green suspension was filtered through 10 layers of cheesecloth and a single layer of Miracloth into 2 50-ml centrifuge tubes. Heavy particles, such as the remains of cell walls and nuclei, were pelleted after centrifugation at 600 x g for 2 min and discarded. The supernatant was filtered through Miracloth into clean tubes and centrifuged at 1,200 x g for 10 min. to collect chloroplasts, which were suspended in 5 ml SN. Chlorophyll was determined according to Arnon (4).

Oxygen uptake or evolution were measured with a Clark-type electrode connected to a Yellow Springs Instrument oxygen monitor. Reaction rates were recorded with a Sargent-Welch SRG recorder. Chloroplast proton pump was assayed by the methods of Dilley (5).

DPA was synthesized in the Eli Lilly Laboratories and made available through the courtesy of Dr. Hollingsworth, Purdue Department of Entomology.

Results and Discussion

An uncoupler should stimulate electron transport reactions in low concentrations (1×10^{-6} to 1×10^{-9}). As Figure 2 and 3 show, DPA meets this criterion. Partial reactions, which are known to involve coupling site 1, such as $H_2O \rightarrow$ MV (+ azide) and $H_2O \rightarrow$ FeCN (pH 6 or 8) are stimulated from 30-60%, whereas $H_2O \rightarrow$ FeCN with DNP-INT or $H_2O \rightarrow$ DDMBQ with DBMIB show little stimulation of electron

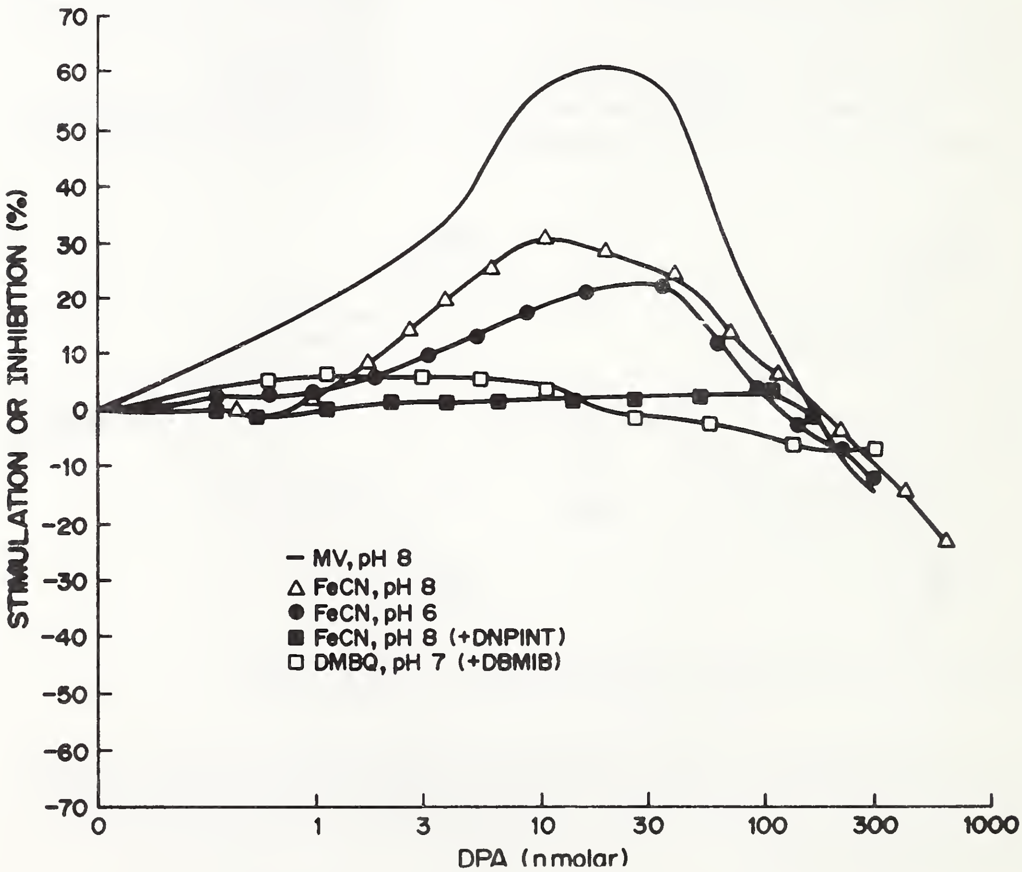

FIGURE 2. Uncoupling of Photosystem I and II Reactions by the Uncoupler, DPA. Reaction mixtures contained chloroplasts (0.05 mg chlorophyll), 25 mM Tris-Mes, pH 6, 7, or 8, as shown and electron acceptors or inhibitors in concentrations indicated below: DMBQ-10mM; DCMU-5μM; DNP-INT; 10μM; and FeCN 250 or 500 μM.

transport, since they accept electrons before coupling site 1 (Figure 4). Likewise, PS I reactions, which involve this site also stimulate electron transport rates from 40-60% (Figure 3).

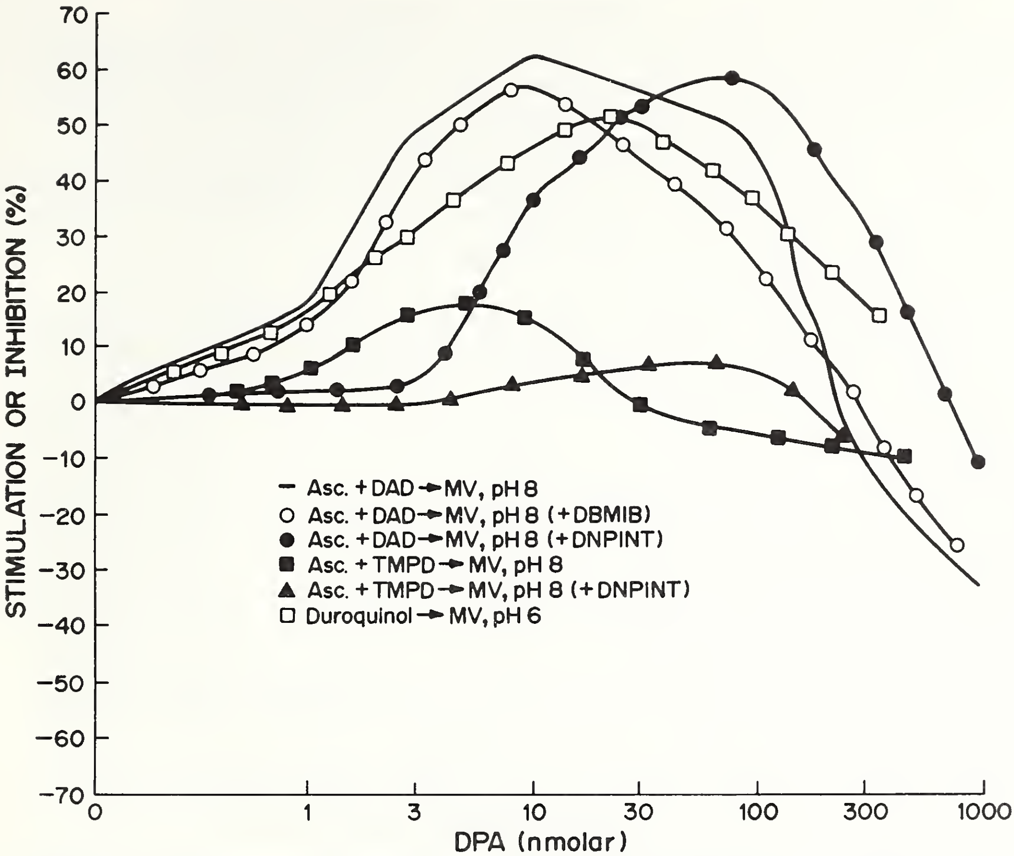

FIGURE 3. Uncoupling of Photosystem I Reactions by the Uncoupler, DPA. Reaction mixtures as in Fig. 1 with additional reaction components in concentrations indicated below: ascorbate, 1mM; DAD, 0.5mM; TMPD, 5μM; and duroquinol, 0.5mM.

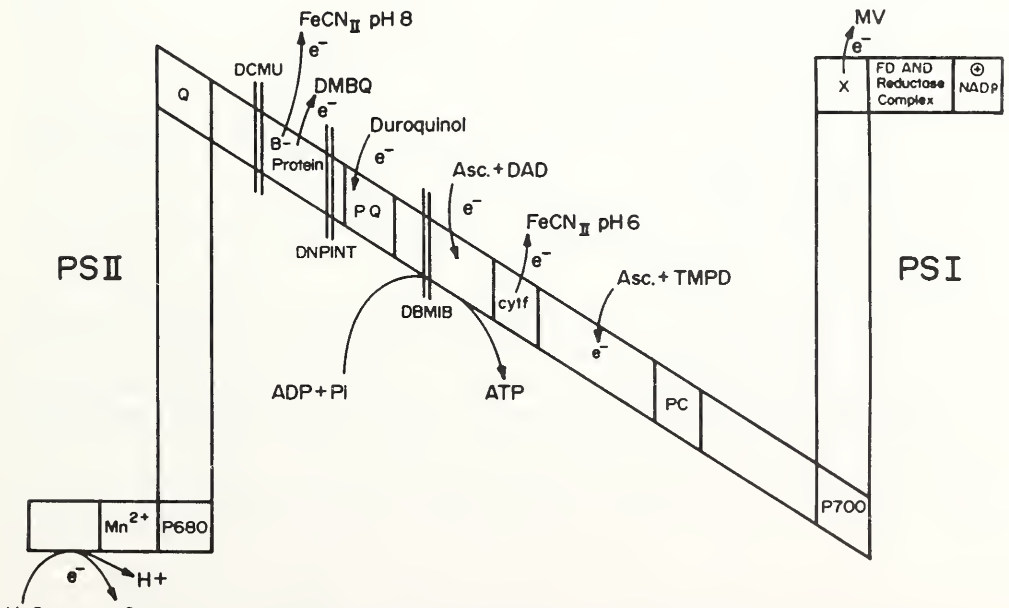

FIGURE 4. The Z-scheme of Chloroplast Electron Transport, Showing Coupling Site 1, Uncoupled by DPA.

The second criterion for establishing a compound as an uncoupler is to show that it inhibits the light-dependent proton pump associated with photophosphorylation. According to Table I, 100 nanomolar DPA (1×10^{-7} M) inhibits the chloroplast proton pump 49%. Higher concentrations of the uncoupler lead to > 90% inhibition.

These criteria establish DPA as a potent new uncoupler. Only a few known uncouplers, such as FCCP (6,7) or TTFB (8) uncouple in lower concentrations than DPA (1×10^{-8} M versus 1×10^{-7} M, respectively). This new uncoupler involves coupling site 1, located between the 2 photosystems (Figure 4).

TABLE I. Inhibition of Chloroplast Proton Pump Associated with ATP Formation

DNP conc. (nM)	ATP (μMOLES/mg CHL•HR)	INHIBITION (%)
0	420	—
10	362	14
30	315	25
60	252	40
100	213	49
300	174	59
600	142	76
1000	20	95

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