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Inhibition of photophosphorylation and electron transport by *N,N*-dimethylformamide

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Abstract

The basal electron transport of pea chloroplasts was inhibited by 78% by 7% (v/v) *N,N*-dimethylformamide; the inhibition was partially reversed by NH_4Cl. *N,N*-Dimethylformamide also inhibited the P_i–ATP exchange, ATP synthesis and to a smaller extent Mg^{2+}-ATPase activity. Light induced proton uptake was not affected by up to 30% (v/v) *N,N*-dimethylformamide. Uncoupled electron transport in photosystem II was inhibited to a larger extent by *N,N*-dimethylformamide than in photosystem I. These results indicate that *N,N*-dimethylformamide acts as an inhibitor of energy transfer and electron transport.

Keywords: *N,N*-dimethylformamide; Energy transfer inhibitor; Electron transport inhibitor; Mg^{2+}-ATPase inhibition; Photosystem II inhibition

1. Introduction

The synthesis of ATP by chloroplasts is coupled to light-dependent electron flow by an H^+-electrochemical gradient [1–3]. Reagents that prevent the formation of the H^+-gradient (inhibitors of electron flow) or promote its dissipation (uncouplers) abolish photophosphorylation. Photophosphorylation may also be inhibited by reagents that block phosphorylation, i.e. energy transfer inhibitors. Some of the latter are Dio-9 [4], dicyclohexylcarbodiimide [5], phlorizin [6], synthalin [7] and antisera to CF1 [8]. During the course of the studies on the effect of various inhibitors on photophosphorylation in chloroplasts, it was observed that the solvents used for dissolving the inhibitors affected the rate of ATP synthesis and hydrolysis, as well as electron transport. In particular DMF was found to have a marked inhibiting effect of the phosphorylation reaction at relatively low concentration, whereas at high concentrations, it was a powerful inhibitor of electron transport.

2. Materials and methods

Intact chloroplasts were isolated from 15–20 days old Pisum sativum plants as described else-

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where [9]. Electron transport from water to methylviologen was determined with an oxygraph YSI Model 5300. For illumination the light of a projector lamp (Gaf 2660) was passed through a filter of 5 cm of a 1% (w/v) $CuSO_4$ solution [10].

ATP hydrolysis was measured by measuring the release of ($^{32}P_i$) formed from ($\gamma^{32}P$)ATP: whereas phosphorylation was measured by the uptake of ($^{32}P_i$). Radioactive phosphate from ICN was purified as described in ref. [11]. ($\gamma^{32}P$)ATP was prepared from $^{32}P_i$ as reported in [12].

For the assay of ATPase activity, the enzyme was activated by illuminating intact chloroplasts suspensions with saturating white light for 1.5 min provided by two 500 W projectors screened with heat filters in a magnetically stirred test tube maintained at 20 °C [9]. Afterwards the light was turned off and the activated chloroplasts were osmotically lysed by the addition of 6.5 volumes of 5 mM $MgCl_2$, 15 mM K-tricine buffer pH 8.0. The ATPase activity of this preparation was measured as described in the Results section (Section 3). The reaction was stopped with trichloroacetic acid. The amount of $^{32}P_i$ formed was measured in the supernatant by extracting it as a phosphomolybdate complex using isobutanol–benzene as described in [11,13].

The $^{32}P_i$–ATP exchange reaction was measured in the reaction mixtures described in the Results section in chloroplasts that had been illuminated and lysed as described above. The reaction was arrested with trichloroacetic acid. To the supernatant 1 ml of 3.3% ammonium molybdate in 3.75% H_2SO_4 was added. The phosphomolybdate complex was removed by extracting five times with isobutanol : benzene (1 : 1). The radioactivity of the aqueous phase was determined and the amount of $^{32}P_i$ incorporated into ATP was calculated [11,13].

Proton uptake was measured as a pH rise between pH 7.0 and pH 7.1 as described by Dilley [14] using a combination microelectrode connected to a Corning potentiometer with expanded scale. The pH changes were recorded (Gilson recorder). After the illumination period the H^+ produced were quantitated by titration with a calibrated solution of 0.01 *N* HCl.

Total chlorophyll was determined as reported in ref. [15].

3. Results

3.1 Effect of DMF on basal, phosphorylating and uncoupled electron transport from water to methylviologen

DMF inhibited basal, phosphorylating and uncoupled electron transport from water to methylviologen (Fig. 1). The results suggest that DMF

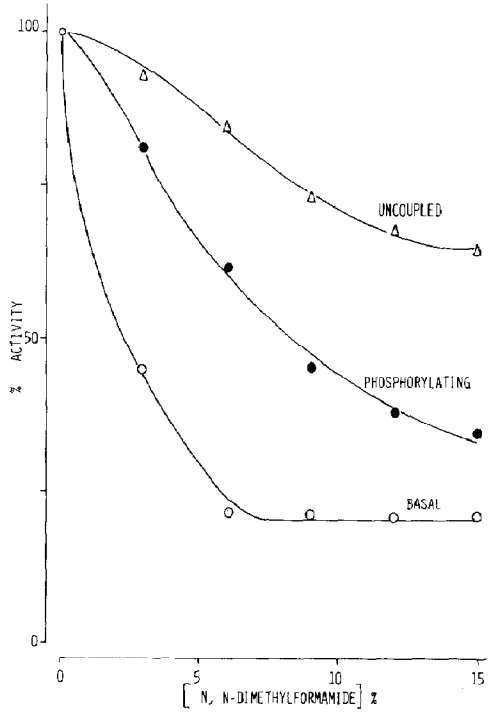

Fig. 1. Effect of DMF on electron transport from water to methylviologen. Intact chloroplast (30 μg of chlorophyll) were incubated in 3.0 ml of the following mixtures. Basal electron transport: 0.1 *M* sorbitol, 0.01 *M* KCl, 1 m*M* $MgCl_2$, 0.05 m*M* methylviologen, 10 m*M* K-tricine, pH 8.0. Phosphorylating electron transport: in addition to the basal mixture, 1 m*M* ADP and 3 m*M* KH_2PO_4 were added; the reaction was initiated by illumination. Uncoupled electron transport: in addition to the phosphorylating mixture 3 m*M* NH_4Cl was included. The temperature was 22 °C. Control values in μmol O_2 mg^{-2} chlorophyll h^{-1} were: basal, 247; phosphorylating, 603; uncoupled, 916. These correspond to 100% in the figure.

acts as an inhibitor of the electron transport chain. However, the low rates of the basal electron transport observed at concentrations of DMF lower than 6.0% (v/v) were increased by ammonia; this suggests that at these concentrations DMF acts also as an energy transfer inhibitor similar to dio-9 [4], phlorizin [6] and synthalin [7]. At higher DMF concentrations, ammonia did not stimulate the rate of electron transport. Thus the overall results suggest that at relatively low concentrations DMF acts at the level of the ATPase, whereas at higher concentrations it also affects the electron transport chain.

3.2 Effect of DMF on photophosphorylation, the P_i–ATP exchange reaction, ATPase activity and the light-induced pH rise

DMF at concentrations lower than 6.0% (v/v) inhibited photophosphorylation, the P_i–ATP exchange reaction and the ATPase activity of chloroplasts (Fig. 2). These results confirm the aforementioned suggestion that at these concentrations DMF acts as an energy transfer inhibitor, ATP formation and the P_i–ATP exchange reaction were inhibited to a very similar extent by concentrations of DMF lower than 8% (v/v). The inhibition of the ATPase activity by these concentrations of the solvent was lower (Fig. 2). The results are similar to the data of Arntzen et al. [16] who showed that Kaempferol mildly affected ATP hydrolysis, whereas reactions involving ATP synthesis were affected to a more important extent. In contrast the effect of DMF was different from that obtained with other energy transfer inhibitors, i.e. phlorizin [6] and chlorotributyltin [17], since these last two compounds strongly inhibited the ATPase activity. For the case of phlorizin the inhibition of ATPase activity paralleled the inhibition of the ATP formation and phosphorylating electron transport; phlorizin, similarly to DMF, did not affect proton uptake (Fig. 2). In

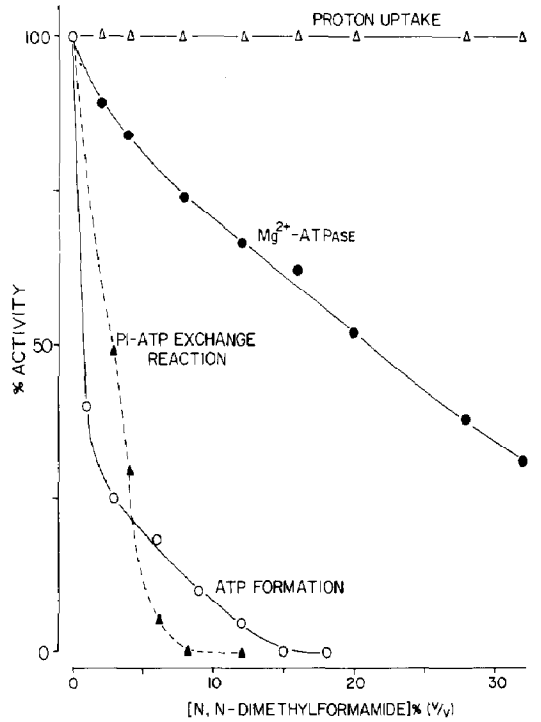

Fig. 2. Effect of DMF on H^+-uptake, Mg^{2+}-ATPase, ATP formation and $^{32}P_i$–ATP exchange reaction. Mg^{2+}-ATPase activity: the activated intact chloroplasts, (0.3 mg of chlorophyll), were resuspended in a volume of 0.25 ml containing: 1.2 *M* sorbitol, 100 m*M* tricine–KOH pH 8.0, The sample was preincubated for 1.5 min under illumination and osmotically lysed as described under Methods, immediately afterwards 0.1 ml of lysed chloroplast were added to 0.9 ml of a medium consisting of 2m*M* $MgCl_2$, 1.1 m*M* NH_4Cl, 2.2 m*M* $^{32}P_i$–ATP (2×10^3 cpm/nmol) and 15 m*M* K-tricine pH 8.0. The incubation was carried out in a shaking water bath at 22 °C in the dark for 1 min and stopped by the addition of 0.5 ml 20% TCA. Precipitated chloroplasts were removed by centrifugation and the amount of ^{32}P formed was determined as described under Methods. The $^{32}P_i$–ATP exchange reaction was measured simultaneously in the same conditions of ATPase activity, except that ammonium chloride was omitted and that the mixture contained 2.2 m*M* ATP and 3 m*M* P_i (^{32}P 2×10^6 cpm/nmol) instead of $^{32}P_i$. ATP formation; intact chloroplasts (40 μg of chlorophyll) were added to 0.3 ml of a medium containing 3 m*M* $^{32}P_i$ (1.5×10^5 cpm/nmol), incubated at 22 °C with shaking and illumination, the reaction was initiated by the addition of 1 m*M* ADP and arrested 1 min after with 0.5 ml of 20% trichloroacetic acid and centrifuged. The uptake of $^{32}P_i$ into ATP was determined in the supernatant described under Methods. Control values for Mg^{2+}-ATPase, ATP formation and $^{32}P_i$–ATP exchange expressed in μmol$\times$mg^{-1} chlorophyll $\times$ h^{-1} were: 280, 450 and 550, respectively. Light induced H^+-uptake was measured with a suspension of intact chloroplasts (30 μg chlorophyll) in an incubation medium similar to that of Fig. 1 except that the buffer was changed to 1 m*M* tricine–KOH pH 8.0.

accordance with the results here described, it has been reported that some organic solvents modify the hydrolytic activity of soluble and particulate H^+-ATPase from various sources [18,19].

When chloroplasts that had been incubated with DMF were centrifuged out of the medium and resuspended in a solution free of DMF, photophosphorylation was restored to values similar to those of the control (Table 1). The latter data indicated that DMF did not bind covalently to the site(s) that produced inhibition of photochemical phosphorylation reactions.

DMF, similarly to Dio-9 [4], DCCD [5], phlorizin [6] and the antiserum to CF1 [8], did not affect the light induced H^+- uptake of chloroplasts, even at concentrations that completely inhibited photophosphorylation, i.e. 32% (v/v) (Fig. 2). DMF did not affect the first order rate constant for either the pH rise in the light, or its

Table 1

Reversibility of the inhibition by *N,N*-dimethylformamide of pea chloroplasts ATP synthase [a]

Experiment	15% DMF	ATP synthase activity (μmol ATP $\times$ mg^{-1} Chlorophyll $\times$ h^{-1})
I	–	750
	+	0
II	–	607
	+	595

[a] Freshly lysed intact chloroplasts containing 30 μg (experiment I) or 100 μg (experiment II) chlorophyll were incubated for 1.5 minutes under the photophosphorylation conditions specified in Fig. 2, with or without 15% (v/v) DMF. In experiment II chloroplasts were diluted (1:11) with 350 mM sorbitol, 2 m*M* EDTA, 1 m*M* $MgCl_2$, 1 m*M* $MnCl_2$, 50 m*M* HEPES (pH 7.6) and centrifuged out of the medium; the pellet was washed two times with the same medium. ATP synthesis was assayed in the conditions of Fig. 2. Photophosphorylation was started by illumination.

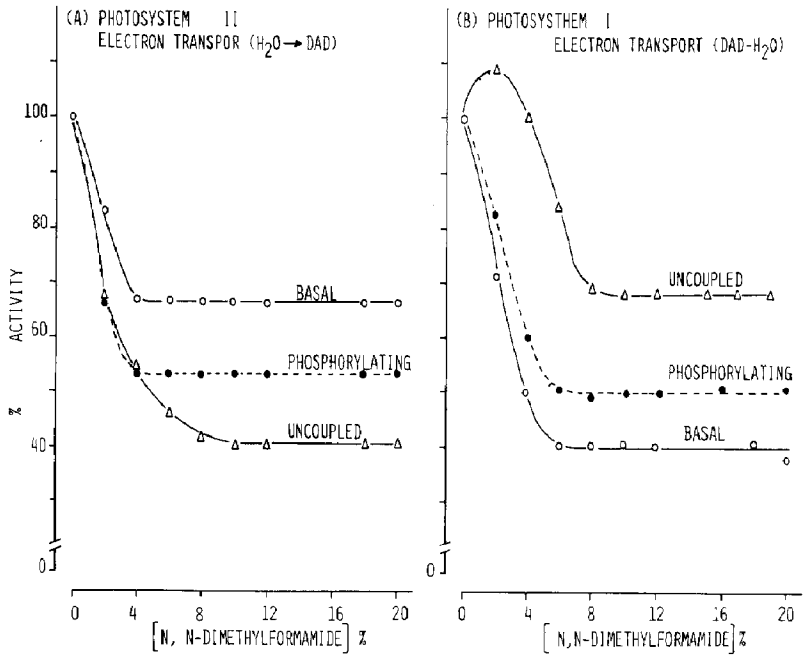

Fig. 3. Effect of increasing the concentration of *N,N*-dimethylformamide on (A) photosystem II and (B) photosystem I electron transport rates. The composition of the reaction mixture is similar to Fig. 1 except that in photosystem II the following reagents were added: (A) 0.4 m*M* DAD, 3 m*M* $K_3Fe(CN)_6$, 1 μ*M* DBMIB and methylviologen was ommited. The reaction mixture of Fig. 1 was used for photosystem I measurment and the following reagents were added: 4 m*M* potassium ascorbate, 100 μ*M* DAD, 10 μ*M* DCMU. Control values of electron transport rate from photosystem I expressed in μmol O_2 mg^{-1} chlorophyll h^{-1} are basal, 218; phosphorylating, 612 and uncoupled, 824 and for photosystem II: basal, 103; phosphorylating 212, and uncoupled 95.

decay in the dark (data not shown). Apparently the proton permeability of the thylakoid membrane is not affected by these relatively high concentrations of DMF.

3.3 Effect of DMF on photosystems I and II

In order to localise the site of action of DMF on electron transport, its effect on the basal, phosphorylating and uncoupled electron transport of photosystems I and II was studied. Uncoupled electron transport from photosystem II was more sensitive to DMF than photosystem I; the highest inhibition of photosystem I was around 30%, whereas that of photosystem II was 60% at concentrations of DMF that ranged between 8 and 20% (v/v) (compare data in Fig. 3, A and B). Basal electron transport rates in photosystems I and II were inhibited by DMF, but photosystem I was more sensitive than photosystem II to inhibition by DMF (Fig. 3). Phosphorylating electron transport was inhibited to the same extent in both photosystems by DMF; within the DMF concentration range tested, the highest inhibition was about 50%. The magnitude and the pattern of inhibition of electron transport by DMF of photosystem I was similar to the inhibition of the Hill reaction from water to methylviologen (compare data in Fig. 1 and Fig. 3B).

4. Discussion

The effect of organic solvents on the activity of large variety of enzyme has been extensively studied (cf. [20] and references therein). Of particular importance is that stimulation of the ATPase activity of mitochondrial F_1 [21,22] and spinach chloroplast CF_1 [23,24] by methanol has been reported. Here it was found that DMF inhibits in a concentration dependent form the ATPase and the ATP synthase activities of chloroplast membrane bound CF_1; however, there were differences in the degree of inhibition by DMF of photophosphorylation and the $^{32}P_i$–ATP exchange reaction, with respect to the effect of DMF on the ATPase activity. The latter results are consistent with those of Tuena de Gómez-Puyou et al. [13], who found that oxidative phosphorylation and the P_i–ATP exchange reaction in submitochondrial particles were differentially inhibited by dimethylsulfoxide, as well as by other water miscible organic solvents. The differential inhibition of ATP synthesis relative to ATP hydrolysis could be explained by differences in the kinetic rate constants of the two reactions [25]. However, there is evidence that indicates that there are different kinetic forms of H^+-ATPase involved in either ATP synthesis or hydrolysis [25,26]. Thus the data obtained with DMF suggests that different enzyme conformations may participate in ATP hydrolysis and ATP synthesis. Alternatively the results may suggest that DMF has more than one target site(s) in CF_1 which manifests differently during synthesis or hydrolysis of ATP.

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