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## *p*-NITROACETOPHENOXIME *N*-METHYLCARBAMATE, A NEW ELECTRON ACCEPTOR IN ISOLATED THYLAKOIDS

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*p*-Nitroacetophenoxime *N*-methylcarbamate (MCPNA) is a rather potent inhibitor of the electron transfer in spinach class A chloroplasts. In isolated thylakoids, MCPNA is an electron acceptor at the level of photosystem I (PS I). It inhibits O<sub>2</sub> evolution in the presence of NADP and ferredoxin but not the reduction of ferricyanide. MCPNA is active as an acceptor between 3 μM and 100 μM. At concentrations higher than 300 μM, inhibition of photosystem II (PS II) occurs. MCPNA has no uncoupling effect on photophosphorylation. Reduction of MCPNA by thylakoids in the presence of light is in accordance with the  $E'_0$  of this compound ( $>-0.57$  V) and is followed by an electron transfer to O<sub>2</sub>. This reaction probably explains the inhibitory effect of MCPNA on class A chloroplasts.

**Key words:** *N*-methylcarbamate of *p*-nitroacetophenoxime; chloroplasts; electron transfer; methylviologen; thylakoids; spinach

### Introduction

The aim of this investigation was to study the action of MCPNA on isolated thylakoids. An oxime function is present in this compound as in the well-known insecticide aldicarb. MCPNA is related to phenylcarbamate and phenylurea herbicides (Scheme II). The synthesis of MCPNA has already been described [1].

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; Diuron (DCMU), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPIP, dichlorophenolindophenol; I<sub>50</sub>, concentration of the product giving a 50% inhibition; MCPNA, *p*-nitroacetophenoxime *N*-methylcarbamate; MOPS, morpholinopropane sulfonic acid; MV, methylviologen; PS I, photosystem I; PS II, photosystem II; TMPD, *N*-tetramethyl-*p*-phenylenediamine.

### Materials and Methods

#### *Isolation and purification of chloroplasts*

Spinach leaves were cut into small pieces directly within the ice-cold chloroplast extraction medium (330 mM mannitol, 2 mM EDTA, 0.2 g · l<sup>-1</sup> bovine serum albumin, 30 mM sodium pyrophosphate (pH 7.9); tissue/medium ratio 1:3.3). Leaves were homogenized using a Waring Blendor for 3 × 2 s. The brei was filtered through 8 layers of muslin and intact chloroplasts were prepared by differential centrifugation according to Nakatani and Barber [2]. The chloroplasts thus obtained (chlorophyll concentration: 7 to 8 mg · ml<sup>-1</sup>) were purified by isopycnic centrifugation in Percoll, a non-toxic silica sol gradient (Pharmacia); isoosmotic conditions were thus maintained throughout the isolation procedure [3]. The intact purified

chloroplasts (class A chloroplasts) were diluted with the extraction medium (1:10, v/v) and recovered as a pellet after centrifugation ( $5500 \times g$ , 90 s, SS 34 rotor, Sorvall RC5).

#### *Chlorophyll concentration*

Chlorophyll concentration was measured according to Arnon [4].

#### *Photosynthesis assay*

$O_2$  evolution was followed polarographically at  $25^\circ C$  using a Clark-type electrode system. The reaction medium contained: 330 mM mannitol, 50 mM morpholinopropane sulfonic acid (MOPS)—NaOH (pH 7.6), 2 mM EDTA, 5 mM  $NaHCO_3$ , 100  $\mu M$   $Na_2HPO_4$  and chloroplasts in an amount equivalent to 70–80  $\mu g$  of chlorophyll in a total volume of 1 ml. The reaction medium was gassed with argon before chloroplast addition and equilibrated with the  $O_2$  electrode at  $25^\circ C$ . The light source was a 150 W xenon arc lamp giving an irradiance of  $1700 W \cdot m^{-2}$  at the surface of the vessel.

#### *Percentage of broken chloroplasts*

Broken chloroplasts were determined from ferricyanide-dependent  $O_2$  evolution in the presence of  $NH_4Cl$  (2 mM) before and after an osmotic shock [5] and under reduced irradiance ( $700 W \cdot m^{-2}$ ). Thylakoids (class C chloroplasts) were obtained by an osmotic shock (10 s in distilled water) and diluted in the reaction medium. Reduction of NADP was followed by  $O_2$  evolution at pH 7.9 in a medium containing 1 mM NADP, 10  $\mu M$  ferredoxin.

#### *Determination of $E'_0$ . Cyclic voltametry*

Electrochemical measurements were made using a Princeton Applied Research (PARC) Model 173D-179 potentiostat/galvanostat coupled to a PARC Model 175 universal programmer. Voltamograms were recorded on a Hewlett Packard recorder 7045A. The working electrode was made of glass coated with indium oxide. Such an electrode allows the

electrochemical study of many compounds on a large range of negative potentials. The reference electrode was an Ag/AgCl electrode in saturated potassium chloride solution. The counter electrode was a simple platinum wire [6,7].

## Results

MCPNA is a rather potent inhibitor of  $O_2$  evolution by isolated class A chloroplasts in the presence of  $NaHCO_3$ ,  $Na_2HPO_4$  and light. A 50% inhibition of  $O_2$  evolution was observed in the presence of 1 mM sodium bicarbonate and 100  $\mu M$  phosphate for a concentration of MCPNA between 2  $\mu M$  and 4  $\mu M$ , when the chloroplast preparation was composed of 80% class A and 20% class C chloroplasts (chlorophyll concentration  $\leq 10^{-2} mg \cdot ml^{-1}$ ) (Fig. 1). When chlorophyll

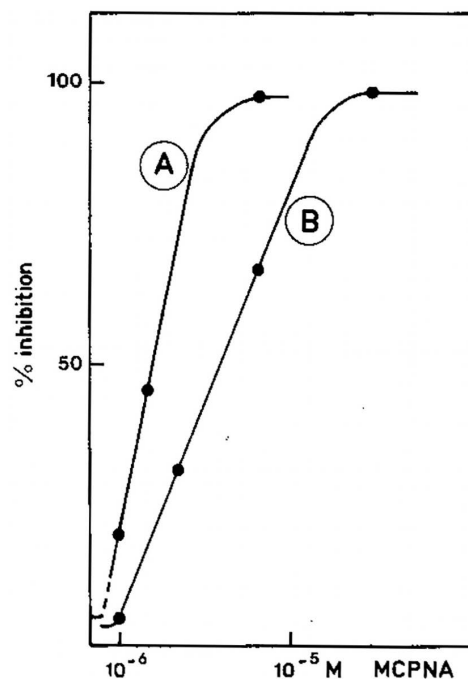


Fig. 1. Effect of MCPNA on light-dependent  $O_2$  evolution by Spinach class A chloroplasts in the presence of  $NaHCO_3$ , 1 mM,  $Na_2HPO_4$ , 0.1 mM. (A) chlorophyll concentration reaches  $10 \mu g \cdot ml^{-1}$  in the reaction medium; (B) chlorophyll concentration is  $40 \mu g \cdot ml^{-1}$ .

concentration increased from  $10^{-2}$  to  $4.10^{-2}$   $\text{mg} \cdot \text{ml}^{-1}$ , the  $I_{50}$  for MCPNA increased only from  $4 \mu\text{M}$  to  $6 \mu\text{M}$ . With more purified class A chloroplast suspensions (95% class A obtained on Percoll gradients)  $\text{O}_2$  evolution was not as sensitive to MCPNA and  $20 \mu\text{M}$  were needed to induce a 50% inhibition at a  $10^{-2}$   $\text{mg} \cdot \text{ml}^{-1}$  chlorophyll concentration. The chloroplasts were insensitive to the presence of small amounts of Percoll which originated from the gradient: up to 2%, Percoll had no effect on the electron transfer in thylakoids and on the inhibitory action of MCPNA.

PS I electron transfer was studied using ascorbate-reduced *N*-tetramethyl-*p*-phenylenediamine (TMPD) as electron donor and methylviologen (MV) as electron acceptor. Although  $300 \mu\text{M}$  MCPNA markedly inhibited the electron transfer between  $\text{H}_2\text{O}$  and MV, no major inhibition appeared at the level of

PS I:  $1 \text{ mM}$  MCPNA only induced a 14% decrease in the electron flow in PS I (Table I). PS II electron transfer was then investigated between  $\text{H}_2\text{O}$  and  $100 \mu\text{M}$  *p*-phenylenediamine in the presence of  $5 \text{ mM}$  potassium ferricyanide and after addition of  $3 \mu\text{M}$  2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) as a plastoquinone antagonist [8–10]. Under these conditions,  $500 \mu\text{M}$  MCPNA gave rise to an 80% inhibition of the electron flow (Table I). Then, the electron transfer was studied between reduced duroquinone [11] and MV, this test allowing the analysis of inhibitions located in the plastoquinone region. MCPNA was completely ineffective in this test, even at a  $200 \mu\text{M}$  concentration.

An electron transfer between  $\text{H}_2\text{O}$  as donor and NADP as acceptor was then studied in the presence of ferredoxin. This transfer took place in the light with freshly

**Table I.** Intensity of the photosynthetic electron flow in class C chloroplasts with different electron donors and acceptors when increasing MCPNA concentration. Columns 1–6: percentage of the value obtained without MCPNA. Columns 7–8: percentage of the maximum value of the oxygen consumption when MCPNA was electron acceptor.

Experimental conditions: (1)  $\text{H}_2\text{O} \rightarrow \text{NADP}$ : oxygen evolution measured in the presence of  $1 \text{ mM}$  NADP and  $10 \mu\text{M}$  Fd with freshly broken chloroplasts (class C). (2)  $\text{H}_2\text{O} \rightarrow \text{MV}$ : oxygen consumption measured in the presence of  $100 \mu\text{M}$  MV and  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ . (3) Asc + DPIP  $\rightarrow$  MV: oxygen consumption measured in the presence of  $100 \mu\text{M}$  MV,  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ ,  $10 \mu\text{M}$  DCMU,  $500 \mu\text{M}$  ascorbate and  $100 \mu\text{M}$  DPIP. (4) Dq  $\rightarrow$  MV: oxygen consumption measured in the presence of  $100 \mu\text{M}$  reduced duroquinone,  $100 \mu\text{M}$  MV and  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ . (5)  $\text{H}_2\text{O} \rightarrow \text{FeCN}_6^{3-}$ : oxygen evolution measured in the presence of  $5 \text{ mM}$   $\text{FeCN}_6^{3-}$  and  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ . (6)  $\text{H}_2\text{O} \rightarrow \text{PD}$ : oxygen evolution measured in the presence of  $5 \text{ mM}$   $\text{FeCN}_6^{3-}$ ,  $3 \mu\text{M}$  DBMIB,  $100 \mu\text{M}$  PD and  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ . (7)  $\text{H}_2\text{O} \rightarrow \text{MCPNA}$ : oxygen consumption measured in the presence of MCPNA as electron acceptor, with  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ . (8) Asc + DPIP  $\rightarrow$  MCPNA: oxygen consumption measured in the presence of  $10 \text{ mM}$   $\text{NH}_4\text{Cl}$ ,  $10 \mu\text{M}$  DCMU,  $500 \mu\text{M}$  ascorbate,  $100 \mu\text{M}$  DPIP and MCPNA. For tests 1–6 see Refs. 12,13. Asc, ascorbate; Dq, reduced duroquinone;  $\text{FeCN}_6^{3-}$ , potassium ferricyanide; PD, *p*-phenylene-diamine.

MCPNA	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	$\text{H}_2\text{O} \rightarrow$ NADP %		Asc + DPIP $\rightarrow$ MV %	Dq $\rightarrow$ MV %	$\text{H}_2\text{O} \rightarrow$ $\text{FeCN}_6^{3-}$ %	$\text{H}_2\text{O} \rightarrow$ PD %	$\text{H}_2\text{O} \rightarrow$ MCPNA %	Asc + DPIP $\rightarrow$ MCPNA %
$10 \mu\text{M}$	48	100	100	100	100	100	100	100
$20 \mu\text{M}$	12	100	100	100	100	100	100	100
$100 \mu\text{M}$	0	100	100	100	97	90	100	100
$500 \mu\text{M}$	0	32	92	90	22	20	20	88
$1 \text{ mM}$	0	0	86	81	0	0	0	80

broken chloroplasts (Table I). MCPNA at a concentration close to  $25 \mu\text{M}$  fully inhibited  $\text{O}_2$  evolution in these conditions. Furthermore,  $100 \mu\text{M}$  MCPNA induced a very marked decrease of the  $\text{O}_2$  concentration in the reaction vessel, showing a significant light-dependent  $\text{O}_2$  consumption which was never seen in the presence of classic inhibitors such as DBMIB or DCMU. Addition at this stage of small amounts of ferricyanide ( $500 \mu\text{M}$ ) was followed by a transient  $\text{O}_2$  evolution which stopped when ferricyanide was fully reduced.

As suggested from the preceding experiment, MCPNA alone ( $10 \mu\text{M}$ ) induced an intense  $\text{O}_2$  consumption ( $150 \mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  chlorophyll) by class C chloroplasts in the presence of light (Table I). This consumption was inhibited by  $5 \mu\text{M}$  3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron, DCMU) and was thereafter restored by addition of ascorbate reduced TMPD or dichloropheno-

lindophenol (DPIP). The  $\text{O}_2$  consumption appeared when MCPNA concentration reached  $2 \mu\text{M}$  and its intensity increased thereafter for concentrations between  $2 \mu\text{M}$  and  $10 \mu\text{M}$ . Between  $10 \mu\text{M}$  and  $100 \mu\text{M}$ , a constant value of  $150 \mu\text{M O}_2 \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  chlorophyll was obtained. At concentrations greater than  $150 \mu\text{M}$ , the intensity of the electron transfer gradually decreased (Fig. 2).

A comparative study of the redox systems of MCPNA and MV has been carried out through cyclic voltametry (Fig. 3). The voltamogram of MV (curve A, Fig. 3) indicates that it is a perfect monoelectronic, fast and reversible electrochemical system [14]. In this case, the potential difference  $\Delta E_p$  between the cathodic and anodic peaks at  $20 \text{ mV} \cdot \text{s}^{-1}$  is almost  $60 \text{ mV}$  and the  $E'_o$  of MV is  $-0.53 \pm 0.01 \text{ V}$ . For MCPNA (curve B, Fig. 3) the electrochemical reaction is much more complex. The cathodic and corresponding anodic peaks of the voltamo-

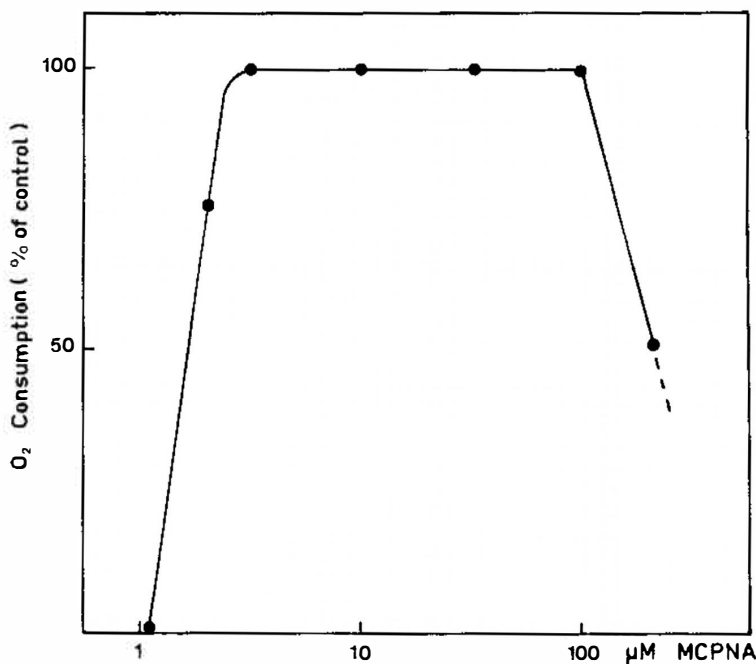


Fig. 2. Relation between the oxygen consumption rate with MCPNA as electron acceptor and MCPNA concentration. One hundred percent refers to  $160 \mu\text{mol O}_2$  consumed  $\cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  chlorophyll.

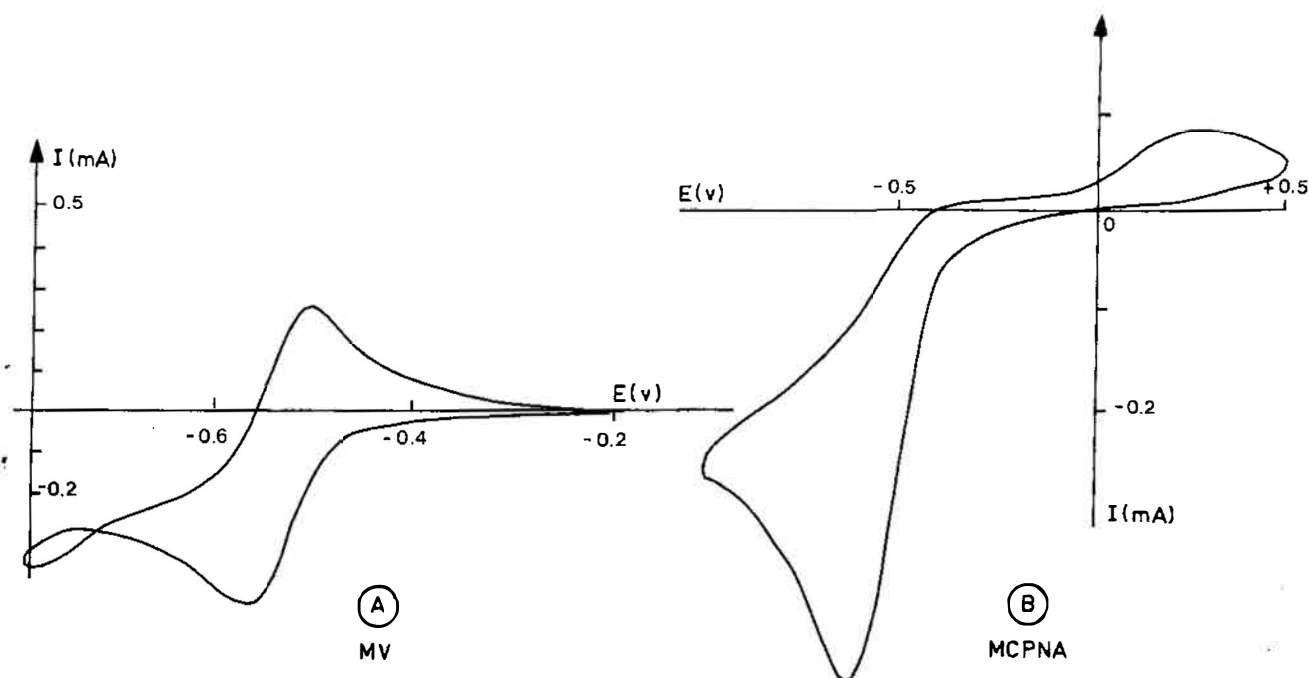


Fig. 3. Cyclic voltammetry in water with 0.2 M MOPS of (A) methylviologen 3 mM at  $20 \text{ mV} \cdot \text{s}^{-1}$  and (B) MCPNA 2 mM at  $200 \text{ mV} \cdot \text{s}^{-1}$ . Presence of residual  $\text{O}_2$  in the solution can explain the low value of the anodic peak at the oxidized state.

gram are of  $-0.62$  and  $+0.27 \text{ V}$  at  $200 \text{ mV} \cdot \text{s}^{-1}$ , respectively. The  $\Delta E_p$  is  $890 \text{ mV}$ . This strongly suggests a slow overall transfer of several particles. According to the structure, it must be  $2e^-$  and  $2\text{H}^+$ . In such a case, it is probably an ECEC mechanism [14] where a proton transfer is a determining step. In this situation, it is not possible to give a quasi thermodynamic standard potential value  $E'_o$  for MCPNA. We can give an  $E''_o$ -value for the reduction of MCPNA ox to MCPNA red which is a maximum value:  $E''_o = -0.57 \text{ V} \pm 0.03 \text{ ox} \rightarrow \text{red}$ . In homogeneous oxidoreduction transfer we probably have to take into account a lower value. In the inverse direction red  $\rightarrow$  ox, the potential  $E''_o$  is about  $+0.20 \pm 0.03 \text{ V}$ . In homogeneous phase with different intermediates, owing to proton acceptor or to donor effect, the potential that we have to take into account can be between  $-0.57$  and  $+0.2 \text{ V}$ .

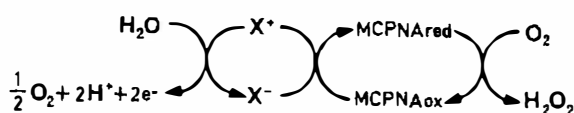
The  $E''_o$  for MCPNA ox  $\rightarrow$  MCPNA red is in accordance with the acceptance of  $e^-$  generated by PS I.

### Discussion

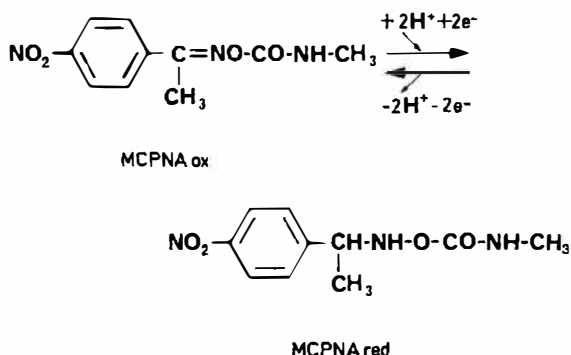
The  $\text{O}_2$  uptake induced by MCPNA indicates that MCPNA from 2 to  $100 \mu\text{M}$  is able to accept electrons emitted by PS I in the light: when the two photosystems are operating, the electron transfer to MCPNA is inhibited by DCMU and restored by addition of ascorbate-DPIP. As can be predicted when considering the  $E'_o$  of MCPNA, ascorbate and DPIP, these two latter compounds are not able to transfer  $e^-$  directly to MCPNA without thylakoids acting in the presence of light.

Addition of less than  $10 \mu\text{M}$  MCPNA is sufficient to obtain complete oxygen consumption in our conditions. This suggests that MCPNA does not accumulate in the

reduced form. The fact that the  $e^-$  transfer to this compound is followed by an oxygen consumption, and that MCPNA does not accumulate in the reduced form during the course of water photolysis in the thylakoids, shows that an electron transfer takes place between the reduced form of MCPNA and  $O_2$ , in a way which can probably be compared to that affecting  $\gamma, \gamma'$  dipyridyl salts and low potential quinones such as anthraquinone sulfonates [13] (Scheme I).



The oxido-reduction activity of MCPNA appears to be associated with the presence of a =NO-structure, as suggested by Scheme II.



We have studied elsewhere the effects of amides and phenylcarbamates [15,16] on photosynthesis. Such compounds, the structures of which are close to that of MCPNA, are unable to transfer  $e^-$  to  $O_2$  in the presence of thylakoids.

In the case of MCPNA itself, the addition of an electron withdrawing region on the phenyl ring, which is represented by a *p*- $NO_2$ , seems to increase the reactivity of the oxime function.

At concentrations higher than 300  $\mu M$ , MCPNA inhibits the electron transfer in the

thylakoids. This inhibition can be seen with the PS II acting alone, with PD and  $FeCN_3^{3-}$  as acceptors, after addition of DBMIB. PS II inhibition by MCPNA could be related to the occurrence of the carbaomyl function  $-CO-NH-CH_3$  and could thus be compared to that by DCMU [17]. An *s*-triazine-like structure might also account for the effect of MCPNA on PS II. It should be noted that the inhibitory effect of MCPNA on PS II is low, as in the case of comparable structures of the phenylamide or phenylcarbamate families [15,16]. For this action, *p*-substitution by  $NO_2$  on the phenyl ring is not favourable.

Addition of MCPNA to thylakoids does not prevent the acceleration of the electron transfer induced by  $NH_4Cl$  (result not shown). MCPNA is therefore devoid of uncoupling activity. The inhibitory effect of MCPNA at low concentration, on the whole photosynthetic mechanism in class A chloroplasts can be explained neither by the inhibition of the electron transfer in the thylakoids, nor by an uncoupling effect. On the other hand, the MCPNA concentration needed for electron transfer to  $O_2$  and for the inhibitory action on class A chloroplasts are almost the same. This suggests that MCPNA is able to enter into the class A chloroplasts and compete with electron transfer from PS I to NADP.

Nevertheless, we have to note that the presence of small amounts of class C chloroplasts in class A suspensions increases the  $O_2$  consumption associated with MCPNA action and leads to a reduction of the oxygen concentration in the medium. This explains why the apparent inhibitory effect of MCPNA is greater on preparations of A plastids containing a small amount of thylakoids than on very pure class A preparations.

The demonstration of the redox properties of the  $-C=N-O-CO-NH-CH_3$  chain of MCPNA and of its correlative effect on photosynthesis leads to further studies in the chemical family of the systemic insecticide aldicarb in which the same chain is also present.

## References

- 1 G. Mrlina and J.P. Calmon, *J. Agric. Food Chem.*, 28 (1980) 605.
- 2 H.Y. Nakatani and J. Barber, *Biochim. Biophys. Acta*, 461 (1977) 510.
- 3 H. Pertoft and T.C. Laurent, in: N. Catsimpoulas (Ed.), *Plenum Publishing Corp.*, New York, 1977, 25.
- 4 D.I. Arnon, *Plant Physiol.*, 24 (1949) 1.
- 5 U.W. Heber and K.A. Santarius, *Z. Naturforsch.*, 25b (1970) 718.
- 6 J.W. Stroiek, T. Kuwana and S.W. Feldberg, *J. Am. Chem. Soc.*, 90 (1968) 1353.
- 7 G. Cauquis, M. Genies and E. Vieil, *Nouv. J. Chim.*, 1 (1977) 307.
- 8 R. Ouitrakul and S. Izawa, *Biochim. Biophys. Acta*, 305(1) (1973) 105.
- 9 A. Trebst, E. Hart and W. Draber, *Z. Naturforsch.*, 25b (1970) 1157.
- 10 H. Böhme, S. Reimer and A. Trebst, *Z. Naturforsch.*, 26b (1971) 341.
- 11 S. Izawa and G.L. Pan., *Biochem Biophys. Res. Commun.*, 3 (1978) 1171.
- 12 A. Trebst, *Methods Enzymol.*, 24 (1972) 146.
- 13 S. Izawa, *Methods Enzymol.*, 69C (1980) 413.
- 14 M.M. Baizer, *Organic Electrochemistry, an Introduction and a Guide*, Marcel Dekker, New York, 1973.
- 15 M. Tissut, D. Aspe, P. Meallier and C. Coste, *Physiol. Veg.*, 21 (1983) 689.
- 16 D. Macherel, *Thèse Doct 3ème cycle*, Université I de Grenoble, France, 1983.
- 17 D.E. Moreland and K.L. Hill, *J. Agric. Food Chem.*, 7 (1959) 832.