UNCOUPLING OF CORN SHOOT MITOCHONDRIA BY *N*-(PHOSPHONOMETHYL)GLYCINE

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1. Introduction

Very few derivatives of α-amino acids produce deleterious effects on mitochondrial energy-linked functions. Sarcolysine, an antitumour agent, has been shown to uncouple mitochondria by an undefined mechanism [1]. Although, the thyroid hormones are known to exert a direct effect upon mitochondria by stimulating oxygen consumption [2] and protein synthesis [3], thyroxine acts as an uncoupler of oxidative phosphorylation [4–7] if preincubated with mitochondria.

The *N*-phosphonomethyl derivative of glycine, a broad-spectrum, non-selective herbicide [8] when administered i.p. to rats at a sublethal dosage of 350 mg/kg causes an elevation in rectal temperature, asphyxial convulsion [9] and rigor, effects which are similar to those produced by a number of chemicals which uncouple mitochondrial oxidative phosphorylation [10–12].

In this paper, the 'in vitro' effects of *N*-(phosphonomethyl)-glycine on oxidative phosphorylation in corn shoot mitochondria were investigated to establish whether the substitution of an NH_2-hydrogen by a phosphonomethyl group ($-CH_2.H_2PO_3$) could confer on glycine a deleterious biological effect involving the process of energy metabolism in the mitochondrion.

2. Materials and methods

N-(phosphonomethyl)glycine (isopropylamine salt) was a gift from the Monsanto Company, Brussels. Isolation of corn shoot mitochondria (CSM) was carried out essentially by the method in [13]. Mitochondrial protein was estimated by the method in [14]. Oxygen consumption was measured polarographically by using a Clark-type oxygen electrode (supplied by Yellow Springs Instrument Co. Ohio) linked to a Perkin-Elmer recorder, Model 56. Respiratory control ratios of mitochondria were computed as the ratio of rate of ADP-stimulated respiration (state 3) to rate of ADP-limited respiration (state 4) according to [15,16]. ATPase activity was measured by the method in [17].

3. Results and discussion

Several classes of foreign compounds (xenobiotics) such as the dinitrophenol and hydroxybenzonitrile pesticides [18,19] are known to uncouple mitochondrial oxidative phosphorylation. Some α-amino acids or their derivatives, e.g., sarcolysine [1] and thyroxine [2–5] have been shown to uncouple mitochondrial respiration.

The results obtained by using intact corn shoot mitochondria (CSM) are presented in fig.1,2 which are typical oxygen electrode tracings showing the effect of the *N*-phosphonomethyl derivate of glycine (PMG) on state 4 oxidation of succinate and malate, respectively, by CSM. As seen here, state 4 oxidation of succinate by CSM was stimulated by factors of 1.9 and 2.6 at 7.80×10^{-5} M and 6.25×10^{-4} M PMG, respectively. When the mitochondria were respiring on malate the rate of oxygen consumption was similarly enhanced at 3.95×10^{-5} M to 2.50×10^{-3} M PMG. Maximal stimulations, 154% and 136% respectively, for succinate and malate oxidation were obtained at 1.56×10^{-4} M PMG.

Table 1 summarises the effect of PMG on state 3 oxidation of succinate by CSM. The data obtained

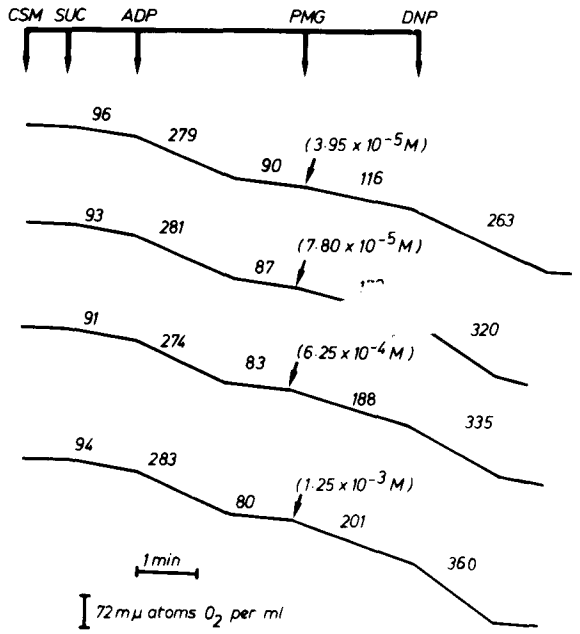

Fig.1. Oxygen electrode tracings showing the effect of PMG on the rate of oxygen uptake by corn shoot mitochondria (CSM). Arrows indicate points of successive additions of CSM (4 mg protein), succinate (3.3 mM), PMG and 2,4-dinitrophenol (DNP, 0.08 mM) in total vol. 3 ml. The numbers along the tracings represent the rates of oxygen consumption in mμatoms O_2/min. Temp. 26°C.

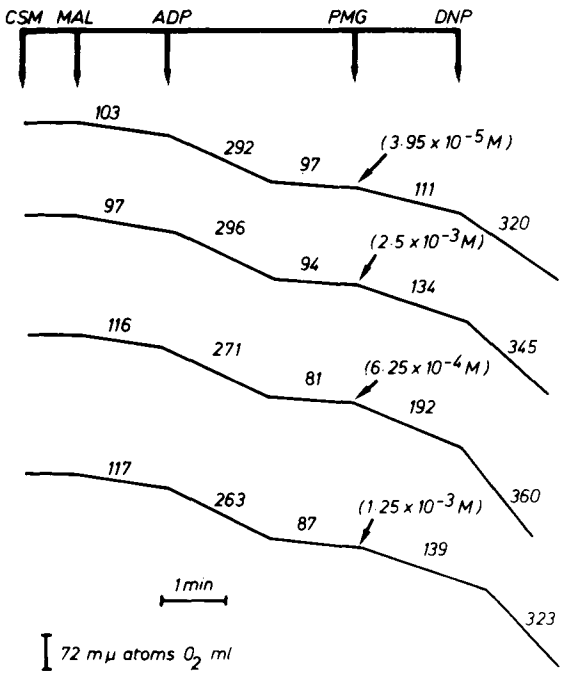

Fig.2. Oxygen electrode tracings showing the effect of PMG on the rate of oxygen uptake by corn shoot mitochondria (CSM). Arrows indicate points of successive additions of CSM (4 mg protein), malate (3.3 mM), PMG and 2,4-dinitrophenol (DNP, 0.08 mM) in total vol. 3 ml. The numbers along the tracings represent the rates of oxygen consumption in mμatoms O_2/min. Temp. 26°C.

Table 1
Effect of PMG on the respiratory control ratio of corn shoot mitochondria (CSM) respiring on succinate

[PMG] (M)	Resp. control[a]	Ratio[b]	% Reduction
3.95×10^{-5}	3.10 ± 0.14	2.81 ± 0.17	9.3
7.80×10^{-5}	3.31 ± 0.15	2.84 ± 0.14	14.2
1.56×10^{-4}	3.61 ± 0.21	2.19 ± 0.19	39.3
3.12×10^{-4}	3.80 ± 0.19	2.14 ± 0.16	43.4
6.25×10^{-4}	3.37 ± 0.17	1.49 ± 0.16	55.8
1.25×10^{-3}	3.09 ± 0.15	2.46 ± 0.14	20.4
2.50×10^{-3}	3.36 ± 0.19	2.76 ± 2.79	16.9
5.00×10^{-3}	3.15 ± 0.20	217. ± 0.18	31.1
10.00×10^{-3}	3.68 ± 0.16	1.61 ± 0.13	56.2

[a] When PMG was not added
[b] When PMG was present

Each value is the mean of 5 determinations ± SD

Table 2
Effect of PMG on the respiratory control ratio of corn shoot mitochondria (CSM) respiring on malate

[PMG] (M)	Resp. control[a]	Ratio[b]	% Reduction
3.95×10^{-5}	3.31 ± 0.19	2.99 ± 0.17	9.7
7.80×10^{-5}	3.41 ± 0.17	2.81 ± 0.16	17.6
1.56×10^{-4}	3.10 ± 0.17	2.00 ± 0.17	35.5
3.12×10^{-4}	3.24 ± 0.19	1.67 ± 0.17	48.4
6.25×10^{-4}	3.13 ± 0.15	1.51 ± 0.14	51.7
1.25×10^{-3}	3.29 ± 0.13	2.37 ± 0.15	27.9
2.50×10^{-3}	3.11 ± 0.17	2.40 ± 0.15	22.8
5.00×10^{-3}	2.21 ± 0.16	1.92 ± 0.18	40.2
10.00×10^{-3}	3.24 ± 0.14	1.66 ± 0.16	48.8

[a] When PMG was not present
[b] When PMG was added

Each value is the mean of 5 determinations ± SD

Table 3
Effect of various concentrations of PMG on ATPase activity of isolated corn shoot mitochondria (CSM)

[PMG] (M)	ATPase act. (mol P_i/ mg protein/min)
Control	1.61 ± 0.09
9.70×10^{-6}	1.91 ± 0.07
1.95×10^{-5}	2.20 ± 0.10
3.90×10^{-5}	2.58 ± 0.11
7.80×10^{-5}	2.91 ± 0.13
1.50×10^{-4}	3.20 ± 0.09
3.12×10^{-4}	3.99 ± 0.15
6.25×10^{-4}	3.69 ± 0.18
1.25×10^{-3}	3.30 ± 0.11
2.50×10^{-3}	2.16 ± 0.10
5.00×10^{-3}	1.90 ± 0.08
10.00×10^{-3}	1.40 ± 0.08
8.00×10^{-4} DNP	9.60 ± 0.41

Each value is a mean of 5 determinations ± SD

Each reaction vessel contained 65 mM Tris–HCl buffer (pH 7.4) 0.5 mM KCl, 1 mM ATP, 25 mM sucrose and the test compound or distilled water in final vol. 2.0 ml. The reaction which was started by the addition of the mitochondrial fraction was allowed to proceed for 5 min with constant shaking at 27°C. The reaction was terminated by the addition of 8 ml 10% trichloroacetic acid.

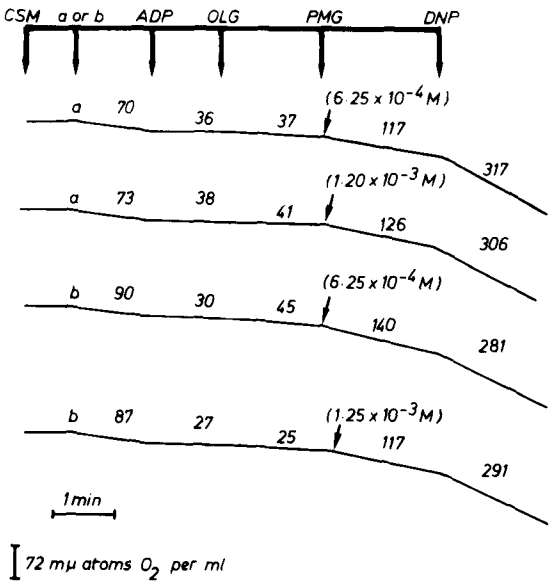

Fig.3. Oxygen electrode tracings showing the effect of PMG on oligomycin-inhibited respiration of corn shoot mitochondria (CSM). Arrows indicate points of additions of CSM (4 mg protein), succinate (a) or malate (b) (3.3 mM), oligomycin (10 μg),ADP (0.15 mM) PMG and 2,4-dinitrophenol (DNP, 0.08 mM) in total vol. 3 ml. Numbers along tracings are the rates of oxygen consumption in mμatoms O_2/min. Temp. 26°C.

shows that the additions of varying concentrations of PMG (3.95×10^{-5} M to 2.50×10^{-3} M) resulted in decreases in the respiratory control ratio of CSM. Maximal reduction, 55.8%, was obtained at 6.25×10^{-4} M PMG. When malate was used as substrate, the pattern (table 2) of the effect of PMG was similar to that obtained for succinate. In this respect, the maximal reduction, 51.7%, was also obtained at 6.26×10^{-4} M PMG.

The data in table 3 reveal that PMG enhanced ATPase activity in CSM. Maximal enhancement, 2.5-fold, was obtained at 6.25×10^{-4} M PMG. The enhancement by 2,4-dinitrophenol was about 3-fold that of PMG (table 3).

It has been demonstrated that the inhibition of respiration by oligomycin, an antibiotic and a potent fungicide, represents an important property of mitochondrial oxidative phosphorylation [20,21]. One important feature of oligomycin-inhibited respiration is the fact that respiration is restored by the addition of an uncoupler [20,21]. Figure 3 represents the pattern of the effects of PMG on oligomycin-inhibited oxygen uptake by CSM respiring on succinate and malate, respectively. As seen here, PMG stimulated the rate of oxygen consumption by oligomycin-inhibited mitochondria. Maximal enhancements, at least 2-fold, were obtained at 6.25×10^{-4} M PMG.

The evidence discussed here suggests that PMG uncouples oxidative phosphorylation in corn shoot mitochondria. This suggestion is supported by the consideration that:

(i) An increase in state 4 mitochondrial respiration (when ADP is absent from the medium);
(ii) A reduction of state 3 respiration or respiratory control ratio;
(iii) An enhancement of mitochondrial ATPase activity;
(iv) The restoration of respiration to oligomycin-inhibited mitochondria are among the common effects exhibited by uncouplers of mitochondria [22–24].

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