Phytochemistry, 1973, Vol. 12, pp. 1207 to 1209. Pergamon Press. Printed in England.

SULPHITE INHIBITION OF ATP FORMATION IN PLANT MITOCHONDRIA

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(*Revised Received* 25 *October* 1972. *Accepted* 11 *December* 1972)

Key Word Index—Mitochondria; ATP production; sulphite inhibition; reversal by oxidized glutathione.

Abstract—Adenosine triphosphate production in mitochondria of bean hypocotyls and maize coleoptiles is inhibited by sulphite. Oxidized glutathione decreases the inhibition, probably by reducing the sulphite concentration in the reaction mixture.

SULPHUR dioxide, a common air pollutant, can be extremely toxic to plants and may have a considerable effect on plant metabolism.[1] Sulphur dioxide fumigations may reduce respiration in plants.[2,3] Sulphite is formed when sulphur dioxide is dissolved in plant tissue,[4] and it may be oxidized by plant mitochondria.[5]

The purpose of the experiments reported here was to determine whether sulphite could inhibit the phosphorylating activity of plant mitochondria, and if so, whether this inhibition could be reversed. Mitochondria prepared from bean hypocotyls and maize coleoptiles were tested because corn has been considered relatively resistant to sulphur dioxide injury while beans have been considered susceptible.[1]

TABLE 1. EFFECT OF Na_2SO_3 ON ATP FORMATION IN MITOCHONDRIA OF BEAN HYPOCOTYLS AND MAIZE COLEOPTILES

Conc. of Na_2SO_3 (mM)	Source of mitochondria		Conc. of Na_2SO_3 (mM)	Source of mitochondria	
	Bean hypocotyl	Maize coleoptile		Bean hypocotyl	Maize coleoptile
1	100*	77	30	41	—
3	77	70	50	31	—
5	77	68	70	23	—
7	75	52	100	15	—
10	56	44			

* Values expressed as % of ATP production in control mitochondria.

Sulphite (as Na_2SO_3) inhibited adenosine triphosphate (ATP) formation in both bean and corn mitochondria, and corn mitochondria are as sensitive to sulphite as bean mitochondria (Table 1). However, sulphite may not directly inhibit ATP production in plant

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mitochondria. The differences in sulphur dioxide injury between bean and maize plants cannot be explained by differences in the sensitivity of mitochondria of the two species to sulphite. Inhibition of plant mitochondria following sulphur dioxide fumigations could occur if sulphite came into contact with the mitochondria of sensitive species. At the pH employed, 7·9, the equilibrium of bisulphite/sulphite is about 1:9·8.[4] Sodium metabisulphite (10 mM) was 1·4 times more inhibitory than 10 mM Na_2SO_3 and not twice as inhibitory as reported for carboxydimutase.[4] Sulphate (10 mM Na_2SO_4) had little effect on ATP formation in bean mitochondria, even though it is known to inhibit photophosphorylation in chloroplasts.[6]

TABLE 2. EFFECT OF OXIDIZED GLUTATHIONE ON ATP FORMATION IN BEAN HYPOCOTYL MITOCHONDRIA

Additions at following times (min)			ATP formation
1	2	3	(% of control)
Control	Mitochondria	10 mM Oxidized Glutathione	110
10 mM Na_2SO_3		Control	42
10 mM Na_2SO_3		10 mM Oxidized Glutathione	62
10 mM Oxidized Glutathione		Control	118
10 mM Oxidized Glutathione		10 mM Na_2SO_3	73

Since 10 mM oxidized glutathione can reduce the concentration of Na_2SO_3 from 10 to 3·4 mM (which is still inhibitory but less so than 10 mM) it was added before and after the addition of mitochondria to determine whether the sulphite inhibition could be reversed or whether there was permanent damage to the mitochondria. The sulphite inhibition could be partially reversed by adding oxidized glutathione to the reaction mixture following addition of mitochondria (Table 2). It has been suggested that sulphur dioxide fumigations may induce a change in the ratio of oxidized to reduced sulphydryl compounds.[1] Perhaps various environmental factors influencing the phytotoxicity of sulphur dioxide[7] may themselves act by altering the ratio of oxidized to reduced sulphydryl compounds, and hence, alter levels of sulphite within the plant cell.

Sulphite, metabisulphite and sulphate had little effect on the ATP assay employed. Oxidized glutathione had a slight inhibiting effect on the ATP assay.

EXPERIMENTAL

Mitochondrial preparations were made from the hypocotyls of week-old etiolated bean seedlings (*Phaseolus vulgaris* cv. Tendergreen Improved) and coleoptiles of week-old etiolated maize seedlings (*Zea mays* cv. Iochief) grown in vermiculite at 30°. Tissues (100 g) were cut up and ground in 200 ml of chilled medium consisting of mannitol, 0·3 M; tricine (*N*-Tris-hydroxymethyl methyl glycine), 0·05 M; EDTA, 0·001 M; $MgCl_2$, 0·0005 M; bovine serum albumen, 0·1%; and cysteine hydrochloride, 0·05%; and adjusted to pH 7·9. The macerate was squeezed through four layers of cheesecloth. The resulting filtrate was centrifuged at 0° at 2500 *g* for 8 min. The pellet was discarded and the supernatant layer was centrifuged again at 30000 *g* for 12 min. The mitochondrial pellet was suspended in mannitol, 0·3 M; tricine, 0·05 M; and $MgCl_2$ 0·0005 M; and adjusted to pH 7·9.

ATP formation was measured by the method of Stenlid.[8] The mitochondrial preparation (*ca* 0·2 mg protein in the case of beans and 0·3 mg protein in the case of maize) was added to a reaction mixture consisting

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of mannitol, 0·3 M; KF, 0·005 M; disodium succinate, 0·005 M; $MgCl_2$, 0·0005 M; KH_2PO_4, 0·0025 M; adenosine diphosphate, 5×10^{-4} M; and tricine, 0·05 M; and adjusted to pH 7·9. This pH value was employed because of the bisulphite–sulphite equilibrium at pH 7·9,[4] and because there is little difference in ATP production of bean mitochondria between pH 7·6 and pH 7·9.

About 0·3 ml of mitochondrial preparation were added to 3 ml of reaction mixture. ATP was measured by the firefly luciferin–luciferase method after 2 min reaction time. 50 μl were removed from the reaction mixture and were added to 1 ml of luciferin–luciferase preparation (containing $MgSO_4$ and arsenite buffer and obtained from Worthington Biochemical Corporation) in an Aminco Photofluorometer. The maximum signal was directly proportional to ATP concentration. Sulphite and protein concentrations were estimated.[9,10] All determinations were replicated four times and involved four separate mitochondrial preparations.

Acknowledgements—Financial support from the University of Victoria Faculty Research Fund is gratefully acknowledged. Some of the facilities employed in this study were provided by the National Research Council of Canada.

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