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# SULPHITE OXIDATION BY MITOCHONDRIA FROM GREEN AND ETIOLATED PEAS

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**Key Word Index**—*Pisum sativum*; Leguminosae; pea mitochondria; sulphite oxidation; etiolated peas; green peas.

**Abstract**—Sulphite oxidation by mitochondria prepared from green pea epicotyls had a higher $K_m$ than did the sulphite oxidation of mitochondria prepared from etiolated pea epicotyls. Mitochondrial sulphite oxidation from green and etiolated tissues was inhibited by cyanide but not by azide, rotenone, antimycin A or oligomycin. Mitochondria from green and etiolated tissues were able to oxidize glyoxal-bisulphite, but not as effectively as sulphite.

## INTRODUCTION

Sulphite oxidation has been observed in mitochondrial fractions prepared from the etiolated shoots of *Avena* [1, 2] and *Pisum* [3] and the roots of *Pisum* [4]. While mitochondrial preparations from green, light grown peas have been successful [5, 6], sulphite oxidation has not been described for this system. However, if sulphur dioxide becomes sulphite as it enters plant cells such a system could play a rôle in understanding the response of plants to sulphur dioxide pollution. Sulphite is an effective inhibitor of adenosine triphosphate formation in plant mitochondria [7] and the toxicity of sulphite to plant mitochondria may be alleviated by sulphite oxidation [8]. The purpose of this study was to compare the sulphite oxidation of mitochondria isolated from green and etiolated tissues of *Pisum sativum* because green tissues are more significant than etiolated tissues in considering pollution damage or the resistance of plant tissues to sulphur dioxide.

## RESULTS AND DISCUSSION

There were considerable similarities in sulphite oxidation in mitochondria prepared from etiolated and green pea epicotyls (Table 1). The sulphite oxidation of mitochondria prepared from etiolated epicotyls appeared to be far more sensitive to substrate (sulphite) than that of mitochondria prepared from green epicotyls as indicated by the values for $K_m$ and $V_{max}$. The $V_{max}$ values may merely reflect a higher content of non-mitochondrial protein in the green tissues. While the $K_m$ values were determined for relatively crude preparations, they were quite consistent for the four replications employed. The phosphorylation rates of green pea mitochondria have been reported to be lower than the phosphorylation rates of mitochondria prepared from etiolated tissues [5]. Both systems were inhibited by cyanide but not by azide. In the case of oat mitochondria there was a stimulation of sulphite oxidation by azide [2] and an inhibition by cyanide [1]. Probably cytochrome oxidase is involved in sulphite oxidation.

When increasing amounts of mitochondrial preparation were added to reaction mixtures containing sulphite concentration representing 50 or 95% substrate saturation the response was linear from 5.1 mg (0.3 ml) to 17.0 mg (1.0 ml) mitochondrial protein in the case of green tissues. For etiolated tissues the response was

Table 1. Sulphite oxidation by mitochondria of green and etiolated epicotyls of *Pisum sativum* cv. Improved Laxton's Progress†

| | Green | Etiolated |
|---|---|---|
| $V_{max}$ | 4.9$\mu$ $MO_2$/mg protein/min | 26.7$\mu$ $MO_2$/mg protein/min |
| $K_m$ | 6.2 mM $SO_3^{2-}$ | 2.1 mM $SO_3^{2-}$ |
| % inhibition (−) or stimulation (+) due to | | |
| 0.01 M NaCN | −71%*‡ | −85%*§ |
| 0.001 M NaCN | −17%‡ | −62%*§ |
| 0.01 M $NaN_3$ | +8%‡ | +8%§ |
| 0.001 $NaN_3$ | −8%‡ | +15%§ |
| 0.01 M NaCl | −8%‡ | +8%§ |
| 0.001 M NaCl | −21%‡ | −8%§ |
| 20$\mu$M Rotenone | 0%‡ | −6%§ |
| 3$\mu$M Antimycin A | 0%‡ | 0%§ |
| 20$\mu$g Oligomycin/6 ml | +3%‡ | −6%§ |
| 1 mM Adenosine diphosphate | −8%‡ | −8%§ |
| Replacing $SO_3^{2-}$ with Glycoxal-bisulphite | −62%*‡ | −38%*§ |
| pH 7.5 | +10%‡¶ | +8%§¶ |
| pH 7.0 | −2%‡¶ | −8%§¶ |
| pH 6.5 | −2%‡¶ | 0%§¶ |

* Significance from the control at the 5% level. † Reactions carried out at pH 7.9 unless otherwise indicated. ‡ 6.2 mM $SO_3^{2-}$ added. §2.1 mM $SO_3^{2-}$ added. ¶ Compared with a pH of 8.0.

linear from 0.75 mg (0.3 ml) to 5.0 mg (2.0 ml) mitochondrial protein. In the case of mitochondrial preparations from green tissues there was a curvilinear response and little increase in oxygen uptake at 3.75 mg (1.5 ml) and 5.00 mg (2.0 ml) mitochondrial protein, indicating the presence of endogenous inhibitors in the green preparation [9]. No endogenous activators could be detected in preparations from etiolated or green tissues by this procedure [9].

Unlike mitochondria prepared from tall peas, adenosine diphosphate did not stimulate sulphite oxidation [3, 4] (A dwarf cultivar was employed in these studies). Rotenone, antimycin A and oligomycin had little influence on sulphite oxidation. Glyoxal-bisulphite, formed in the leaves of $SO_2$-fumigated wheat plants [10], could be oxidized by mitochondria, of both green and etiolated tissues, although not as rapidly as sulphite. As the pH decreased there was little change in the rate of sulphite oxidation of mitochondria from etiolated or green peas. Perhaps the mitochondria of peas are as sensitive to bisulphite formed under increasing acidity [11] as they are to sulphite.

The sulphite oxidation system of mitochondria of green and etiolated peas were similar. Because we are principally concerned with the influence of sulphur dioxide on green plants rather than etiolated plants, the sulphite oxidation system of green peas may play a role in understanding the responses of green plants, especially peas, to $SO_2$.

## EXPERIMENTAL

Mitochondrial preparations were made from the epicotyls of 17-day-old green and etiolated pea seedlings (*Pisum sativum* cv. Improved Laxton's Progress) were grown in a 1:1 mixture of coarse peat moss and sand. Pea seedlings were grown in the dark at 18° or in a greenhouse or, in a lathhouse during July, August and September. The preparative procedures of Livne and Levine [6] for mitochondria from green peas were employed together with media similar to those of Israelstam and Fukumoto [12] and a centrifugation schedule similar to that of Malhotra and Spencer [13]. 100 g pea tissue were ground in a chilled Waring Blender for three 5-sec runs in 200 ml 0.3 M mannitol, 0.07 M sucrose, 0.05 M Tricine, 1.0 mM EDTA, 0.1% BSA (Sigma Fraction V), and 0.05% cysteine adjusted to pH 7.9 with KOH. Homogenate was filtered through 2 layers of cheesecloth followed by a filtration through 4 layers of cheesecloth. Filtered homogenate was centrifuged at 2500 *g* for 10 min and then at 10000 *g* for 10 min. The suspending medium was similar to the grinding medium but did not contain cysteine. The pellet was suspended to give approximately 2.5 mg protein/ml in the case of etiolated epicotyls and 17 mg protein/ml in the case of green epicotyls. The reaction medium was 0.3 M mannitol, 0.07 M sucrose, 0.01 M KCl, 0.01 M $KH_2PO_4$, 5.0 mM $MgCl_2$ and 0.05 M Tricine adjusted to pH 7.9 with KOH except in the case of the pH series. In the pH study the tricine concentration was 0.01 M in the suspending medium. In the reaction medium MES buffer was used for pH 6.5. HEPES for pH 7.0 and 7.5 and tricine for pH 8.0. All buffers were 0.05 M. A pH of 7.9 was employed except in the case of the pH series because at this pH the equilibrium of bisulphite/sulphite is about 1:9.8 [14]. Oxygen uptake was measured at 30° with the Clark oxygen electrode and YSI Model 53 oxygen monitor. All determinations were replicated four times and involved four separate mitochondrial preparations. Protein concentrations were estimated by the method of Lowry *et al.* [15] and protein was precipitated with 5% trichloroacetic acid [6].

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