SULPHITE OXIDATION BY A PLANT MITOCHONDRIAL SYSTEM

I. PRELIMINARY OBSERVATIONS

by

J. M. TAGER AND NIILO RAUTANEN

Plant Physiological Research Institute, University of Pretoria (South Africa)

In the biological oxidation of many sulphur-containing organic compounds, the sulphur is ultimately converted to inorganic sulphate (for reviews, see FROMAGEOT[1] and GREENBERG[2]). The oxidation of cysteine, for example, can lead to the formation of inorganic sulphite and finally, inorganic sulphate (PIRIE[3], MEDES[4]). Since the *in vitro* oxidation of sulphite is catalyzed by traces of heavy metal ions, it is possible that the final step in the biological oxidation of the sulphur of cysteine is non-enzymic (KEARNEY AND SINGER[5]). HEIMBERG *et al.*[6], however, have demonstrated that preparations from mammalian liver and kidney and from several microorganisms catalyze an enzymic oxidation of sulphite. A similar enzyme had been extracted from rat liver mitochondria by SINGER AND KEARNEY[7].

In a recent study (RAUTANEN AND TAGER[8]) it was shown that oat mitochondria bring about the oxidation of several amino acids, including cysteine. During the course of the study, neutralized sodium bisulphite was used as a ketone fixative in certain experiments, and a vigorous oxygen uptake was observed when mitochondria were incubated in the presence of this reagent. After establishing that the oxidation is enzymic, we began a study of the properties of this system. In this paper, the results of experiments on the cofactor requirements, inhibitors and stoichiometry of the sulphite oxidation system are presented.

MATERIAL AND METHODS

Etiolated seedlings of Algerian oats, *Avena sterilis* (L.) Mal. ssp. *byzantina* (C. Koch) Thell., were grown as previously described (TAGER[9]), and the aerial portions used as a source of mitochondria. The material was harvested 6 days after sowing, when the aerial portions, consisting of the coleoptiles, first leaves and first internodes, were about 6 cm high. The material was washed in tap water, rinsed with distilled water, dried superficially and kept at 5° C for 2 to 48 hours.

Isolation of mitochondria

Approx. 60 g of the plant material were placed in a cold mortar with 90 ml 0.5 *M* sucrose-0.01 *M* ethylenediaminetetraacetic acid, pH 7.0, cut finely with scissors and ground with a pestle for about 30 seconds. The suspension was filtered through a layer of muslin and centrifuged for 5 minutes at approx. 500 × *g* in a Servall SS-1 centrifuge. The residue was discarded and the supernatant centrifuged for a further 15 minutes at approx. 20,000 × *g*. The sedimented mitochondria were suspended in 40 ml 0.5 *M* sucrose with the aid of a Potter-Elvehjem homogenizer and recentrifuged for 20 minutes at approx. 20,000 × *g*. The washed mitochondria were suspended in 0.5 *M* sucrose and the volume of the suspension adjusted so that the total nitrogen content was 0.35 to 0.60 mg

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per 0.5 ml. All manipulations were performed in a cold room maintained at approx. 2° C. The mitochondrial suspension was used immediately after preparation.

Manometric method

Oxygen uptake was determined at 30° C by the conventional Warburg method (UMBREIT *et al.*[10]). The side-arm of each Warburg vessel contained 0.2 ml 10% potassium hydroxide and folded filter paper. In the experiments with azide and cyanide, potassium hydroxide was omitted in order to minimize changes in the concentrations of the inhibitors. The same values for oxygen uptake during the oxidation of sulphite were obtained with or without potassium hydroxide in preliminary experiments. Flasks and manometers were equilibrated for 5 minutes with stopcocks open and 5 minutes with stopcocks closed. The activity is expressed as Q_{O_2} (μl O_2 per mg mitochondrial N in 60 minutes).

Nitrogen determination

Nitrogen was determined by micro-Kjeldahl digestion, using mercuric sulphate as catalyst and hydrogen peroxide to hasten the digestion. The ammonia produced was steam-distilled into 2% boric acid and titrated with 0.01 *N* sulphuric acid, using bromocresol green-methyl red as indicator.

Sulphite and sulphate determination

Sulphite and sulphate in the reaction mixture were determined after proteins had been removed. Uranyl acetate was used to precipitate proteins, as described by LETONOFF AND REINHOLD[11]. Sulphite was determined iodometrically. For the determination of sulphate, we first tried the benzidine method of LETONOFF AND REINHOLD[11]. HEIMBERG *et al.*[6] used this method in their study of sulphite oxidation. We found, however, that in the presence of sulphite, this method gives irregular values for sulphate. We finally determined sulphate turbidimetrically after precipitation of the sulphate in acid solution as barium sulphate, as described by ZAHN[12].

Reagents

Analytical grade sodium bisulphite was used; solutions were neutralized with potassium hydroxide and made up daily. Cytochrome *c*, adenosine-5-phosphoric acid (AMP), disodium adenosine triphosphate (ATP) and 95% diphosphopyridine nucleotide (DPN) were obtained from Nutritional Biochemicals Corporation. A preparation of liver coenzyme concentrate, containing 4% triphosphopyridine nucleotide (TPN), 7% DPN and 13 Lipmann units of coenzyme A per mg, was obtained from Armour and Co. Quartz glass double-distilled water was used in all the metabolic experiments.

RESULTS

Enzymic nature of the oxidation

From the results presented in Table I, it can be seen that oat mitochondria catalyze a vigorous oxidation of sulphite, whereas inactivation by heating at 90° C for 1 min resulted in abolition of the oxidation. It was confirmed that manganese ions catalyze a non-enzymic oxidation of sulphite, and in addition, it was observed that cobaltous ions have the same effect.

TABLE I

THE EFFECT OF HEAT-INACTIVATION OF OAT MITOCHONDRIA ON THE OXIDATION OF SULPHITE

Mitochondrial preparation	*Sulphite M*	Q_{O_2}
Untreated	$2 \cdot 10^{-2}$	95
	$3 \cdot 10^{-2}$	198
	$4 \cdot 10^{-2}$	335
Inactivated	$2 \cdot 10^{-2}$	12
	$3 \cdot 10^{-2}$	23
	$4 \cdot 10^{-2}$	28

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; magnesium chloride, $2 \cdot 10^{-3}$ *M*; cytochrome *c*, $3 \cdot 10^{-5}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml.

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TABLE II

THE EFFECT OF ADDITION OF COFACTORS ON SULPHITE OXIDATION BY OAT MITOCHONDRIA

Experiment	Additions					Q_{O_2}
	Magnesium chloride $2 \cdot 10^{-3}$ *M*	*Cytochrome c* $3 \cdot 10^{-5}$ *M*	*DPN* 10^{-4} *M*	*ATP* 10^{-3} *M*	*Liver coenzyme concentrate* 0.6 *mg*	
1	—	—	—	—	—	38
	—	+	—	—	—	122
	+	—	—	—	—	88
	+	+	—	—	—	185
2	—	+	+	+	—	103
	+	+	+	—	—	169
	+	+	+	+	—	172
3	+	—	+	+	—	130
	+	+	—	+	—	214
	+	+	+	+	—	233
4	+	+	—	—	—	239
	+	+	—	—	+	237

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; sulphite, $2 \cdot 10^{-2}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 3 ml.

Cofactor requirements

The results of experiments to determine the effect of various cofactors on the oxidation of sulphite by oat mitochondria are presented in Table II. In the absence of added cofactors, the rate of oxidation of sulphite was low. For the maximal rate of oxidation, both magnesium and cytochrome *c* were necessary. ATP at 10^{-3} *M* was without effect and DPN at 10^{-4} *M* caused only a slight increase in oxygen uptake. Liver coenzyme concentrate, which contains DPN, TPN, and coenzyme A, was without effect at a concentration of 0.6 mg per vessel. AMP increased the rate of sulphite oxidation. As shown in Fig. 1, the degree of stimulation of sulphite oxidation was a function of the AMP concentration. No disappearance of inorganic phosphate could be detected during the oxidation of sulphite in the presence of 10^{-2} *M* AMP.

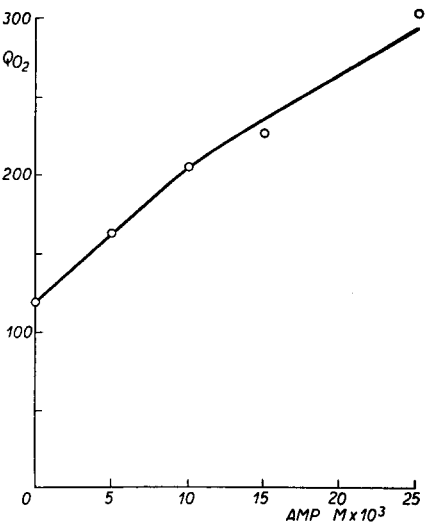

Fig. 1. Influence of AMP concentration on the rate of oxidation of sulphite. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; magnesium chloride, $2 \cdot 10^{-3}$ *M*; cytochrome *c*, $3 \cdot 10^{-5}$ *M*; sulphite, $2 \cdot 10^{-2}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 3 ml.

Effect of sulphite concentration on the rate of oxidation

It may be calculated from the second dissociation constant of sulphurous acid ($5 \cdot 10^{-6}$) that the ratio of sulphite to bisulphite ions in a neutral solution is 50:1. Since the pH of the reaction mixtures, in which the activity of the sulphite oxidation system was measured, was approx. 7.1, it may be

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assumed that the sulphite concentration was approx. equal to the concentration of neutralized sodium bisulphite used.

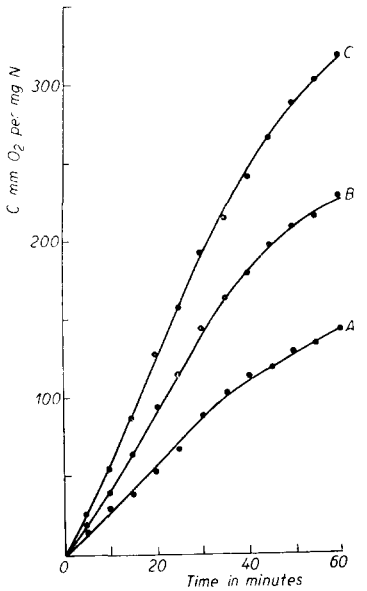

Fig. 2. Rate of oxidation of sulphite with time measured at three sulphite concentrations. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; magnesium chloride, $2 \cdot 10^{-3}$ *M*; cytochrome *c*, $3 \cdot 10^{-5}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml. Sulphite concentration: curve A, $2 \cdot 10^{-2}$ *M*; curve B, $3 \cdot 10^{-2}$ *M*; curve C, $4 \cdot 10^{-2}$ *M*.

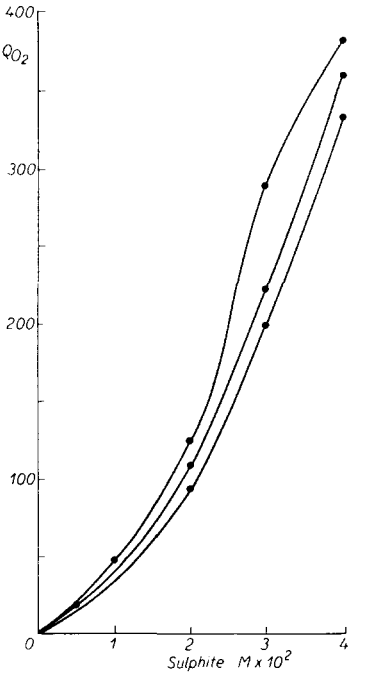

Fig. 3. Rate of sulphite oxidation as a function of substrate concentration. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; magnesium chloride, $2 \cdot 10^{-3}$ *M*; cytochrome *c*, $3 \cdot 10^{-5}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml. Each curve represents the results of a separate experiment.

A typical experiment in which oxygen uptake was measured at three sulphite concentrations is presented graphically in Fig. 2. Some decline in activity during the second 30 minute period was found in all experiments. A slight lag period in the oxidation of sulphite was often observed. The activity of the sulphite oxidation system as a function of substrate concentration is shown in Fig. 3. The maximum rate of sulphite oxidation was not obtained even at highest concentration of sulphite ($4 \cdot 10^{-2}$ *M*) used in the experiments.

Effect of magnesium and cytochrome c *concentration*

As shown in Figs. 4 and 5, the activity of the sulphite oxidation system is a function of the concentration of magnesium ions and of cytochrome *c* in the reaction mixture. The system was found to be saturated with magnesium ions at a concentration of approx. $2 \cdot 10^{-3}$ *M* and with cytochrome *c* at a concentration of approx. $6 \cdot 10^{-5}$ *M*. For comparison, the effect of these cofactors on the oxidation of hydroquinone and of succinate by oat mitochondria is shown in Table III. Hydroquinone oxidation by mitochondrial cytochrome oxidase was found to be almost completely dependent on the presence of exogenous cytochrome *c*. There was a slight inhibition of hydroquinone oxidation when mag-

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nesium ions were added in addition to cytochrome *c*. Succinate oxidation was unaffected by added cytochrome *c*.

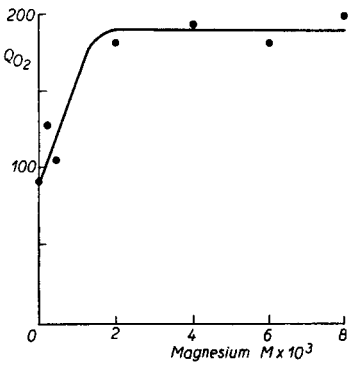

Fig. 4. Effect of magnesium chloride concentration on the rate of oxidation of sulphite by oat mitochondria. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; cytochrome *c*, $3 \cdot 10^{-5}$ *M*; sulphite, $2 \cdot 10^{-2}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml.

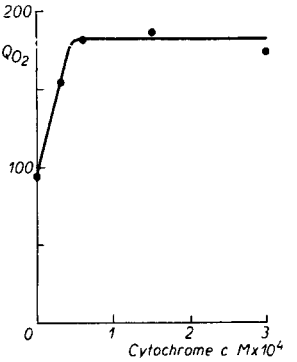

Fig. 5. Effect of cytochrome *c* concentration on the rate of oxidation of sulphite by oat mitochondria. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; magnesium chloride, $2 \cdot 10^{-3}$ *M*; sulphite, $2 \cdot 10^{-2}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml.

TABLE III

THE EFFECT OF MAGNESIUM IONS AND CYTOCHROME *c* ON THE OXIDATION OF HYDROQUINONE AND SUCCINATE BY OAT MITOCHONDRIA

Substrate	*Additions*		Q_{O_2}
	Magnesium chloride $2 \cdot 10^{-3}$ *M*	*Cytochrome c* $3 \cdot 10^{-5}$ *M*	
Hydroquinone, $9 \cdot 10^{-3}$ *M*	—	—	14
	+	—	26
	—	+	194
	+	+	161
Succinate, $2 \cdot 10^{-2}$ *M*	+	—	214
	+	+	226

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ *M*; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 3 ml.

Several other divalent cations were tested. As mentioned above, manganese and cobaltous ions catalyze a non-enzymic oxidation of sulphite. Calcium and zinc ions inhibited the sulphite oxidation system (Table IV).

Inhibitor studies

Since the sulphite oxidation system is stimulated by added cytochrome *c*, it was expected that heavy metal reagents would inhibit the reaction. It was found that cyanide did in fact inhibit oxygen uptake (Table V). Contrary to expectations, however, azide did not inhibit sulphite oxidation. Cytochrome oxidase, as measured by the oxidation of hydroquinone plus cytochrome *c*, and succinoxidase were both inhibited by azide; but the sulphite oxidation system was greatly stimulated by azide (Table VI).

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TABLE IV

THE EFFECT OF CALCIUM, MAGNESIUM AND ZINC IONS ON SULPHITE OXIDATION BY OAT MITOCHONDRIA

Experiment	Additions: Calcium chloride $2 \cdot 10^{-3}$ M	Additions: Zinc sulphate $2 \cdot 10^{-3}$ M	Additions: Magnesium chloride $2 \cdot 10^{-3}$ M	Q_{O_2}	Inhibition or stimulation %
1				95	
	+			66	− 30
			+	157	+ 65
	+		+	73	− 23
2				249	
			+	397	+ 59
		+		123	− 51

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ M; cytochrome *c*, $3 \cdot 10^{-5}$ M; mitochondrial suspension, 0.5 ml; sucrose, 0.4 M. Experiment 1: sulphite, $2 \cdot 10^{-2}$ M and final volume, 3 ml. Experiment 2: sulphite, $4 \cdot 10^{-2}$ M and final volume, 1 ml.

TABLE V

CYANIDE INHIBITION OF SULPHITE OXIDATION BY OAT MITOCHONDRIA

Cyanide, M	Q_{O_2}	Inhibition %
0	254	
10^{-4}	152	40
10^{-3}	138	46

Assay mixture as described for Fig. 1.

TABLE VI

THE EFFECT OF AZIDE ON THE OXIDATION OF SULPHITE, HYDROQUINONE AND SUCCINATE BY OAT MITOCHONDRIA

Substrate	Azide M	Q_{O_2}	Inhibition or stimulation %
Sulphite	0	227	
$2 \cdot 10^{-2}$ M	10^{-3}	293	+ 22
	10^{-2}	438	+ 72
Hydroquinone	0	166	
$9 \cdot 10^{-3}$ M	10^{-3}	61	− 63
	10^{-2}	24	− 85
Succinate	0	181	
$2 \cdot 10^{-2}$ M	10^{-3}	118	− 35
	10^{-2}	42	− 77

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2}$ M; magnesium chloride, $2 \cdot 10^{-3}$ M; cytochrome *c*, $3 \cdot 10^{-5}$ M; mitochondrial suspension, 0.5 ml; sucrose, 0.4 M. Final volume, 3 ml.

Further study of this phenomenon showed that the degree of stimulation is a function of the azide concentration (Fig. 6). At a sulphite concentration of $3 \cdot 10^{-2} M$, the system became saturated with azide at approx. $2 \cdot 10^{-2} M$ and the activity declined very slightly as the azide concentration was increased from $2 \cdot 10^{-2} M$ to $4 \cdot 10^{-2} M$. Heat inactivation of mitochondria abolished sulphite oxidation in the absence and in the presence of azide.

Pyrophosphate was an effective inhibitor of sulphite oxidation, while fluoride had no effect even at a concentration of $10^{-2} M$ (Table VII). Dinitrophenol did not effect sulphite oxidation significantly (Table VII).

At concentrations ranging from $10^{-5} M$ to $10^{-3} M$, monoiodoacetate had no significant effect on the sulphite oxidation system; but at $10^{-2} M$, it greatly stimulated oxygen uptake (Table VII).

TABLE VII

THE EFFECT OF PYROPHOSPHATE, FLUORIDE, 2,4-DINITROPHENOL AND MONOIODOACETATE ON SULPHITE OXIDATION BY OAT MITOCHONDRIA

Inhibitor	*Concentration* M	Q_{O_2}	*Inhibition or stimulation* %
Pyrophosphate	0	167	
	$6.7 \cdot 10^{-4}$	177	+ 6
	$6.7 \cdot 10^{-3}$	115	−31
	$1.3 \cdot 10^{-3}$	72	−57
Fluoride	0	217	
	10^{-4}	216	0
	10^{-3}	217	0
	10^{-2}	207	− 7
Dinitrophenol	0	374	
	10^{-6}	391	+ 4
	10^{-5}	411	+ 10
	10^{-4}	402	+ 7
	10^{-3}	344	− 8
Iodoacetate	0	365	
	10^{-5}	273	+ 2
	10^{-4}	394	+ 8
	10^{-3}	338	− 7
	10^{-2}	651	+ 78

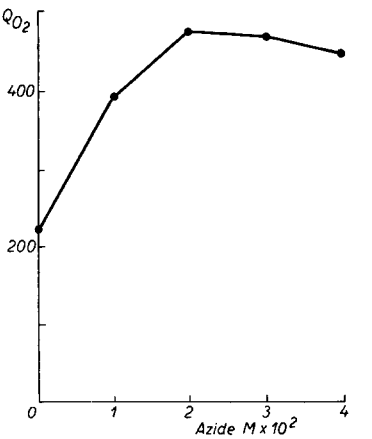

Fig. 6. Effect of azide concentration on the rate of sulphite oxidation by oat mitochondria. Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2} M$; magnesium chloride, $2 \cdot 10^{-3} M$; cytochrome *c*, $3 \cdot 10^{-5} M$; sulphite, $3 \cdot 10^{-2} M$; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml.

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2} M$; magnesium chloride, $2 \cdot 10^{-3} M$; cytochrome *c*, $3 \cdot 10^{-5} M$; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml. Sulphite concentration: $2 \cdot 10^{-2} M$ in pyrophosphate experiment and $4 \cdot 10^{-2} M$ in other experiments.

Effect of sulphydryl compounds

In a preliminary experiment, it was observed that glutathione at a concentration of $10^{-3} M$ caused a 33% inhibition of the sulphite oxidation system. Two other sulphydryl compounds, L-cysteine and thioglycollate, were tested for their effect on the system. The results of two experiments are presented in Fig. 7. As cysteine itself is oxidized by oat mitochondria, the values for sulphite oxidation were corrected for the oxygen uptake in the presence of cysteine alone. Thioglycollate is not oxidized by oat mitochondria. Both L-cysteine and thioglycollate strongly inhibited the sulphite oxidation system.

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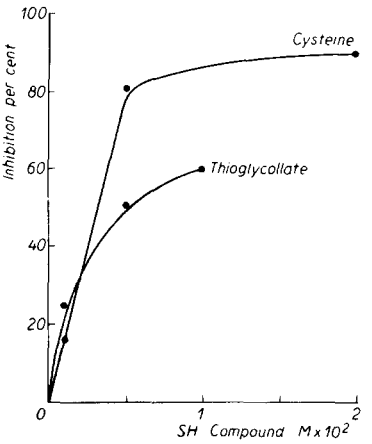

Fig. 7. Effect of sulphydryl compounds on the rate of sulphite oxidation by oat mitochondria. Assay mixture as described in Table VIII.

TABLE VIII

THE EFFECT OF ASCORBIC ACID ON SULPHITE OXIDATION BY OAT MITOCHONDRIA

Ascorbic acid *M*	Q_{O_2} *Sulphite*	*Sulphite*	*Difference*
0	348	27	321
10^{-3}	73	35	38
$5 \cdot 10^{-3}$	81	143	62
10^{-2}	81	211	130

Assay mixture: potassium phosphate buffer at pH 7.4, $2 \cdot 10^{-2} M$; magnesium chloride, $2 \cdot 10^{-3} M$; cytochrome *c*, $3 \cdot 10^{-5} M$; sulphite, $4 \cdot 10^{-2} M$; mitochondrial suspension, 0.5 ml; sucrose, 0.4 *M*. Final volume, 1 ml.

Effect of ascorbic acid and pyruvate

Since the inhibitory effect of sulphydryl compounds may have been due to their acting as reducing agents, a reducing compound of different nature, ascorbic acid, was tested. The results of the experiment are presented in Table VIII. Ascorbic acid itself was oxidized by oat mitochondria, possibly through cytochrome oxidase. At $10^{-3} M$, oxidation of ascorbic acid was negligible, and there was an 88% inhibition of sulphite oxidation in the presence of this concentration of ascorbic acid. At the two higher concentrations of ascorbic acid, both sulphite oxidation and ascorbic acid oxidation were inhibited when the two compounds were added together.

Since there is an equilibrium in aqueous solution between the enol and 3-keto forms of ascorbic acid, an addition compound should be formed between ascorbic acid and bisulphite. The inhibition of sulphite oxidation by ascorbic acid could therefore be due to its action as a reducing agent, to the formation of an inhibitory addition compound, or to both. An experiment with the α-keto compound, pyruvate, in which inhibition of sulphite oxidation was obtained (Table IX) lends support to the idea that bisulphite-keto compound addition products may be inhibitory to the sulphite oxidation system.

TABLE IX

THE EFFECT OF PYRUVATE ON SULPHITE OXIDATION BY OAT MITOCHONDRIA

Pyruvate *M*	Q_{O_2}	*Inhibition* %
0	351	
10^{-3}	277	21
$5 \cdot 10^{-3}$	209	40
10^{-2}	142	59

Assay mixture as described for Table VIII.

Stoichiometry of the reaction

In Table X, the results of an experiment are given in which sulphite disappearance, oxygen uptake and sulphate production were measured simultaneously. For each mole

of sulphite disappearing, half a mole of oxygen was taken up and one mole of sulphate was formed, in accordance with the equation

$$SO_3^{--} + 1/2\ O_2 \longrightarrow SO_4^{--}$$

With heat inactivated mitochondria, sulphite disappearance, oxygen uptake and sulphate production were low.

TABLE X

STOICHIOMETRY OF THE SULPHITE OXIDATION SYSTEM

Mitochondrial preparation	*Per mg mitochondrial N in 60 minutes*		
	O_2 absorbed μ atoms	*Sulphite disappearing μ moles*	*Sulphate formed μ moles*
Untreated	35.8	35.6	33.3
Heat-inactivated	3.5	6.0	3.7

Assay mixture as described for Table VIII.

DISCUSSION

The sulphite oxidation system of oat mitochondria differs in at least some respects from the "sulphite oxidase" of rat liver described by HEIMBERG *et al.*[6]. The cofactor requirements of the rat liver enzyme were not studied. Experiments with inhibitors, however, indicated that heme or copper groups were not involved in the animal enzyme. The oat mitochondrial system exhibits a requirement for exogenous cytochrome *c*, and is strongly inhibited by cyanide. It may thus be inferred that cytochrome oxidase is part of the plant sulphite oxidation system. In addition, the plant system requires magnesium ions for maximal activity, while it is not clear if the animal enzyme has this requirement. It is not stated whether the oxidation of sulphite by rat liver residue is stimulated by azide and monoiodoacetate or not. The plant sulphite oxidation system is greatly stimulated by both azide and monoiodoacetate.

Oat mitochondria, like those from other sources, contain an integrated complex of enzymes and coenzymes. The endogenous coenzymes may mask the coenzyme requirements of the sulphite oxidation system, when the usual test is carried out of demonstrating increased enzyme activity on the addition of one or more coenzymes. Thus although it is well known that succinate oxidation is linked to the cytochrome system, added cytochrome *c* has no effect on the oxidation of succinate by oat mitochondria. Similarly, the oxidation of pyruvate by oat mitochondria through the Krebs cycle is unaffected by added cytochrome *c* or DPN (TAGER[9]). A detailed study of the cofactor requirements of the oat sulphite oxidation system will have to await purification of the enzymes involved. At this stage we can only state that magnesium ions are essential and that cytochrome oxidase is involved.

The lack of inhibition of the oat sulphite oxidation system by monoiodoacetate indicates that sulphydryl groups are not necessary for the activity. Indeed, the inhibition of the enzyme system by glutathione, cysteine and thioglycollate suggests that disulphide linkages may be essential. PETERS AND WAKELIN[13] found an inhibition of trypsin, chymotrypsin and chymotrypsinogen by thiol compounds and interpreted this to be due

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to the scission of essential disulphide linkages. On the basis of the observation that rhodanese is protected by glutathione and cysteine against a slow inhibition by cyanide, SÖRBO[14] suggested that the prosthetic group of this enzyme contains a disulphide linkage.

The inhibition of the oat sulphite oxidation system by keto compounds seems to be due to the formation of addition products between the keto compounds and bisulphite. At first sight this inhibition may appear to be due to a reduction of the concentration of sulphite in the reaction mixture. Yet pyruvate inhibits the system effectively when the ratio of the concentration of pyruvate to the concentration of neutralized sodium bisulphite is approx. 1 to 40, and the effective concentration of sulphite is reduced only by about 2.5% in this case. Thus it is clear that the inhibition by compounds like pyruvate cannot be explained in this way. A possible explanation is that the addition product competes with sulphite for essential groups in the enzyme system.

A peculiar feature of the oat sulphite oxidation system is the marked stimulation of activity by azide and AMP. From the relationship between concentration of azide or AMP and stimulation of activity, it appears that these compounds do not act catalytically, and are effective at concentrations stoichiometrically similar to the concentrations of sulphite used. In experiments which will be reported on in a later paper, we have found that a similar stimulation is given by creatinine, histidine and thiamine. This has led us to believe that the stimulation is non-specific and is given by certain heterocyclic N-containing compounds.

The significance of the sulphite oxidation system in the metabolism of plants is not clear. It is known that plants obtain their sulphur from the soil in the form of inorganic sulphate; but very little is known of the mechanism by which inorganic sulphate is reduced and converted to sulphur-containing compounds of metabolic importance (for review, see BERSIN[15]). Nevertheless, it is of interest to note that the reduction of sulphate in *Desulphovibrio* involves a cytochrome (POSTGATE[16]), as has been shown in the case of our sulphite oxidation system, which requires cytochrome *c*. Therefore there is the possibility that the sulphite oxidation system described in this paper may play a role in the assimilation of sulphate by catalyzing the reduction of sulphate to sulphite. This possibility is being investigated.

ACKNOWLEDGEMENTS

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SUMMARY

1. Mitochondria isolated from etiolated oat seedlings contain an enzyme system which oxidizes inorganic sulphite to sulphate.

2. For the maximal rate of oxidation of sulphite, the mitochondria must be supplemented with magnesium ions and cytochrome *c*.

3. The sulphite oxidation system is inhibited by cyanide and pyrophosphate, but not by fluoride, dinitrophenol or low concentrations of monoiodoacetate. High concentrations of monoiodoacetate greatly stimulate the sulphite oxidation system.

4. Azide causes a marked stimulation of the sulphite oxidation system. Maximum stimulation

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is obtained at azide concentrations of the same order of magnitude as the concentration of sulphite used. Adenosine monophosphate behaves similarly to azide.

5. The sulphite oxidation system is inhibited by sulfhydryl compounds, indicating a possible role of disulphide linkages in the system.

6. The sulphite oxidation system is inhibited by ascorbic acid and by pyruvate.

RÉSUMÉ

1. Les mitochondries isolées de germes d'avoine étiolés contiennent un système enzymatique qui oxyde le sulfite minéral en sulfate.

2. Pour obtenir la vitesse maximum d'oxydation du sulfite, il faut ajouter aux mitochondries des ions magnésium et du cytochrome *c*.

3. Le système oxydant le sulfite est inhibé par les cyanures et les pyrophosphates, mais non par les fluorures, le dinitrophénol ou de faibles concentrations de monoiodoacétate. Des concentrations élevées de monoiodoacétate stimulent fortement le système d'oxydation du sulfite.

4. Les asides provoquent une stimulation nette du système d'oxydation du sulfite. La stimulation maximum est obtenue à des concentrations en azide du même ordre de grandeur que la concentration en sulfite employé. L'adénosine monophosphate a le même comportement que l'azide.

5. Le système oxydant le sulfite est inhibé par les corps sulfhydrylés, ce qui indique un rôle possible de liaisons disulfures dans le système.

6. Le système oxydant les sulfites est inhibé par l'acide ascorbique et par le pyruvate.

ZUSAMMENFASSUNG

1. Aus verkümmerten Gerstesetzlingen isolierte Mitokondrien enthalten ein Enzymsystem, welches durch Oxydation inorganisches Sulfit in Sulfat verwandelt.

2. Um die Höchstgeschwindigkeit der Oxydation von Sulfit zu erreichen, ist es notwendig, den Mitokondrien Magnesiumionen und Cytochrom *c* hinzuzufügen.

3. Das Sulfit-Oxydationssystem wird durch Cyanid und Pyrophosphat, jedoch nicht durch Fluorid, Dinitrophenol oder niedrige Konzentrationen von Monoiodoazetat gehemmt. Hohe Konzentrationen von Monoiodoazetat verursachen eine grosse Steigerung des Sulfit-Oxydationssystems.

4. Säureazid verursacht eine bedeutende Steigerung des Sulfit-Oxydationssystems. Der grösste Steigerungseffekt wurde bei Säureazidkonzentrationen derselben Grössenordnung wie die angewandte Sulfitkonzentration beobachtet. AMP verhält sich ähnlich wie Säureazid.

5. Das Sulfit-Oxydationssystem wird durch Sulfhydrylgruppen gehemmt, wodurch man auf die Möglichkeit schliessen könnte, dass Disulfidbindungen eine Rolle in dem System spielen.

6. Das Sulfit-Oxydationssystem wird durch Askorbinsäure und Brenztraubensäure gehemmt.

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