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LIGHT-DEPENDENT REDUCTION OF SELENITE BY SONICATED PEA CHLOROPLASTS

PETER P. JABLONSKI* and JOHN W. ANDERSON

Botany Department, La Trobe University, Bundoora, Victoria 3083, Australia

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Key Word Index—*Pisum sativum*; Leguminosae; peas; selenite reduction; glutathione reductase; glutathione oxidation; selenodiglutathione reduction; selenium; chloroplasts.

Abstract—Sonicated chloroplasts in the presence of catalytic concentrations of NADP(H) and GSSG supported light-dependent reduction of SeO_3^{2-} with the concomitant evolution of O_2. Neither O_2 evolution nor SeO_3^{2-} reduction were detected in the absence of GSSG or in the presence of inhibitors of GSSG reductase. The O_2 evolution response was specific for GSSG/GSH and NADP(H). In the dark, chloroplasts reduced SeO_3^{2-} in the presence of substrate concentrations of GSH but not GSSG. Subsequent illumination initiated O_2 evolution at rates similar to those using catalytic concentrations of GSSG. Sonicated chloroplasts also supported O_2 evolution in the presence of substrate amounts of selenodiglutathione (GSSeSG) and catalytic concentrations of NADPH. Partially purified GSSG reductase from peas catalysed GSSeSG-dependent oxidation of NADPH with the concomitant production of elemental selenium (Se^0). It was concluded that water serves as the eventual electron donor for light-dependent reduction of SeO_3^{2-} via GSSeSG and that NADPH and GSH serve as intermediate electron donors. The role of GSSG reductase in the process is discussed.

INTRODUCTION

Purified cysteine synthases (EC 4.2.99.8) from leaf tissue of selenium-accumulator and non-accumulator plants catalyse the incorporation of Se^{2-} into selenocysteine in the presence of *O*-acetylserine [1]. Isolated chloroplasts, which contain cysteine synthase [2, 3], also support this reaction [1]. In addition, chloroplasts catalyse a light-dependent incorporation of SeO_3^{2-} into selenocysteine in the presence of *O*-acetylserine [4]. The presumed reduction of SeO_3^{2-} to Se^{2-} was attributed to the GSSG reductase activity [4] associated with chloroplasts [5, 6], as initially proposed by Hsieh and Ganther [7] for yeast. It was concluded from studies of the cofactor requirements for SeO_3^{2-} and SO_3^{2-} incorporation into selenocysteine and cysteine by chloroplasts, and the relative sensitivities of these reactions to zinc chloride and potassium cyanide, that sulphite reductase (EC 1.8.1.2) was not involved in SeO_3^{2-} reduction [4].

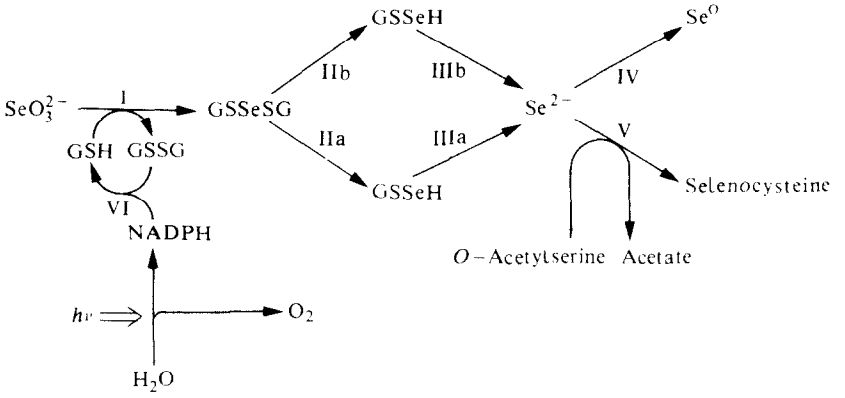
Incorporation of SO_3^{2-} into cysteine by chloroplasts in the presence of *O*-acetylserine is limited by the rate of SO_3^{2-} reduction by light-coupled sulphite reductase [4, 8]. Although the rates of Se^{2-} and SeO_3^{2-} assimilation into selenocysteine in the presence of *O*-acetylserine are much less than the analogous reactions for S^{2-} and SO_3^{2-} [1, 4], the properties and rates of the SeO_3^{2-} reduction mechanism of illuminated chloroplasts in the absence of *O*-acetylserine have not been studied. GSSG reductase activity, implicated in SeO_3^{2-} reduction, can be monitored polarographically in sonicated chloroplasts in the light by GSSG-dependent O_2 evolution in the presence of catalytic concentrations of NADPH [9]. This paper describes a polarographic study of SeO_3^{2-} reduction in sonicated chloroplasts in the presence of catalytic concentrations of GSSG (or GSH) and NADPH (or NADP). Special reference is made to the possible role of light-coupled GSSG reductase activity and selenodiglutathione (GSSeSG) and glutathione selenopersulphide (GSSeH) as intermediates in SeO_3^{2-} reduction (Scheme 1).

RESULTS

(SeO_3^{2-} *plus glutathione*)*-dependent* O_2 *evolution by sonicated chloroplasts*

In the presence of 50 μM NADPH, sonicated pea chloroplasts evolved a small amount of O_2 (<4 nmol/ml) which ceased within 10 sec of illumination. When 0.2 mM GSSG was supplied, chloroplasts catalysed O_2 evolution at a mean rate of 10.2 μmol/mg chl/hr (s.d. 3.6). O_2 evolution ceased after the evolution of 0.43 mol/mol of GSSG supplied; this is consistent with the stoichiometric reduction of GSSG by light-coupled GSSG reductase [9]. Following the cessation of GSSG-dependent O_2 evolution, addition of 0.4 mM SeO_3^{2-} caused resumption of O_2 evolution at a mean rate of 10.0 μmol/mg chl/hr (s.d. 0.94). The (SeO_3^{2-} plus GSSG)-dependent O_2 evolution response was light-dependent and inhibited (100%) by 3.4 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Sonicated chloroplasts in the

*Present address: Department of Agronomy, University of Kentucky, Lexington, KY 40546, U.S.A.



Scheme 1. Proposed pathway for the reductive assimilation of SeO_3^{2-} by illuminated chloroplasts. The reactions, which are not shown stoichiometrically, are adapted from refs. [4] and [7]. Reaction I is non-enzymic but in the presence of trace amounts of GSH/GSSG is dependent on light-coupled GSSG reductase activity [9] (reaction VI). Reactions IIa and IIIa are catalysed by GSSG reductase using NADPH as reductant, whereas reactions IIb and IIIb are non-enzymic and involve GSH as reductant [7]. In theory, reactions II and III can also utilize light-generated reducing equivalents from water with the evolution of O_2. Reaction IV involves non-enzymic oxidation of Se^{2-} and reaction V is catalysed by cysteine synthase [4].

presence of 50 μM NADPH did not catalyse SeO_3^{2-}-dependent O_2 evolution in the absence of GSSG but subsequent addition of 0.2 mM GSSG promoted sustained O_2 evolution in excess of the stoichiometric GSSG-dependent O_2 evolution. Low concentrations of GSSG (20–50 μM) elicited a similar response although the total amount of O_2 evolved per mol of GSSG supplied in the presence of 0.2 mM SeO_3^{2-} generally increased with the concentration of GSSG (Table 1). When GSSG was replaced with a two-fold molar concentration of GSH similar results were obtained except that GSH did not support significant O_2 evolution prior to addition of SeO_3^{2-} (Fig. 1). The mean rate of (SeO_3^{2-} plus GSH)-dependent O_2 evolution was 11.8 μmol/mg chl/hr (s.d. 1.22).

Some properties of (SeO_3^{2-} plus glutathione)-dependent O_2 evolution were examined. Substituting

Table 1. Effect of concentration of GSSG on the total amount of O_2 evolved by sonicated chloroplasts in the presence of 0.2 mM Na_2SeO_3

Experiment*	Concn of GSSG (μM)	O_2 evolved† (μmol)
1	20	0.64
	30	1.35
	50	1.85
2	20	0.89
	30	1.52
	40	1.84
	50	2.05
3	20	0.76
	30	2.11
	40	1.61
	50	1.79

*Chloroplast intactness for experiments 1–3 prior to sonication was 94, 90 and 80% respectively.

†O_2 evolution refers to the total amount evolved in the presence of GSSG and SeO_3^{2-}. It therefore includes both GSSG-dependent O_2 evolution and any additional O_2 evolution following a single addition of 0.2 mM SeO_3^{2-} (see Fig. 1A). O_2 evolution was determined under the conditions described for (SeO_3^{2-} plus GSSG)-dependent O_2 evolution except that the concentration of GSSG was as specified.

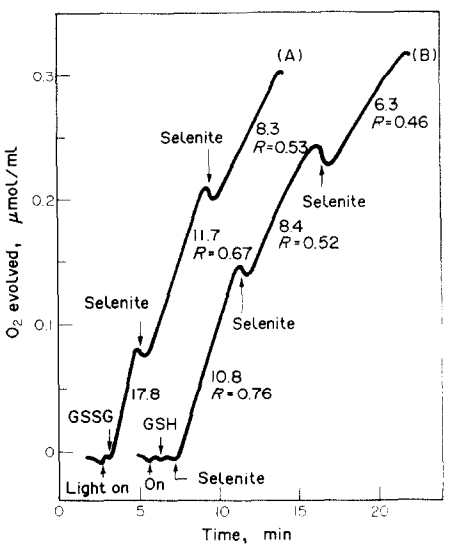


Fig. 1. Effect of repeated additions of 0.2 mM SeO_3^{2-} on O_2 evolution by sonicated chloroplasts in the presence of 0.2 mM GSSG (A) and 0.4 mM GSH (B). All other reaction conditions were as described for (SeO_3^{2-} plus glutathione)-dependent O_2 evolution except that reactions initially contained NADPH (50 μM) and chl in the dark and further treatments were made as shown. Values beside the curves represent the rate of O_2 evolution in μmol/mg chl/hr. *R* denotes the ratio of O_2 evolved to SeO_3^{2-} supplied. Chloroplast intactness prior to sonication, 94%.

NADP for NADPH did not affect the rate of (SeO_3^{2-} plus GSH)-dependent O_2 evolution but in the absence of NADP(H), the rate decreased by 59%. NAD(H) (50 μM) did not substitute for NADP(H) in this reaction. The rate of (SeO_3^{2-} plus GSH)-dependent O_2 evolution was independent of NADPH concentration from 20 to 60 μM. When SeO_3^{2-} (0.2 mM) was supplied to chloroplasts in the presence of 50 μM NADPH and 0.4 mM GSH or 0.2 mM GSSG, O_2 evolution ceased abruptly after the evolution of *ca* 0.6–0.8 mol/mol of SeO_3^{2-} supplied. (Fig. 1). When more SeO_3^{2-} was added, O_2 evolution recommenced implying that SeO_3^{2-} served as the terminal electron acceptor. This could be repeated several times although the rate of O_2 evolution and the O_2:SeO_3^{2-} ratio decreased with each successive addition of SeO_3^{2-}. The thiols L-cysteine, thioglycollate, 2-mercaptoethanol and the monosulphide L-djenkolate (each 0.4 mM) did not support SeO_3^{2-}-dependent O_2 evolution when supplied in place of GSH. Similarly the disulphides L-cystine and L-homocystine (0.2–0.6 mM) did not support SeO_3^{2-}-dependent O_2 evolution or inhibit (SeO_3^{2-} plus GSSG)-dependent O_2 evolution. SO_3^{2-} (1 mM) did not initiate O_2 evolution when supplied in place of SeO_3^{2-}. However, SO_3^{2-} (0.1–1 mM) inhibited both (SeO_3^{2-} plus GSSG)- and GSSG-dependent O_2 evolution (Fig. 2) although 10 mM SO_3^{2-} did not inhibit partially purified GSSG reductase or the uncoupled rate of $Fe(CN)_6^{3-}$-dependent O_2 evolution catalysed by sonicated chloroplasts. Sonicated chloroplasts preincubated with 0.2 mM zinc chloride for 5 min in the dark did not catalyse SeO_3^{2-}-dependent O_2 evolution in the presence of glutathione. These conditions also inhibit light-coupled GSSG reductase [10].

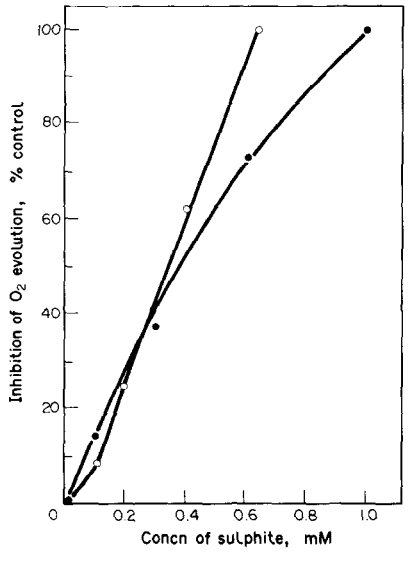


Fig. 2. Effect of SO_3^{2-} concentration on (SeO_3^{2-} plus GSSG)-dependent O_2 evolution (●) and GSSG-dependent O_2 evolution (○) by sonicated chloroplasts. Reaction mixtures were as described in the Experimental except that SO_3^{2-} was added at the concentrations specified after determining the uninhibited rate. Chloroplast intactness prior to sonication, 73%.

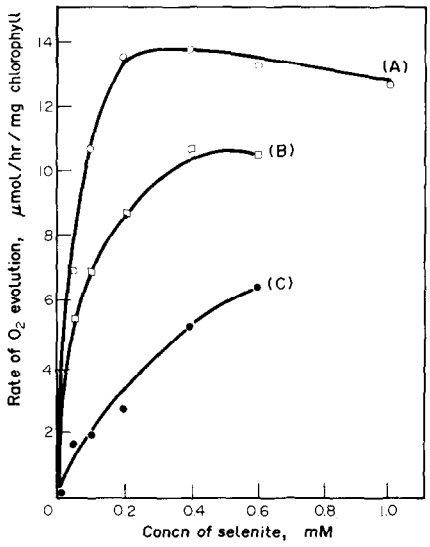


Fig. 3. Effect of concentration of SeO_3^{2-} on (SeO_3^{2-} plus GSH)-dependent O_2 evolution by sonicated chloroplasts in the presence of 0.4 mM (A), 1 mM (B) and 3 mM (C) GSH. All other conditions were as described for (SeO_3^{2-} plus GSH)-dependent O_2 evolution. Chloroplast intactness prior to sonication, 70%.

A concentration of *ca* 0.2 mM SeO_3^{2-} supported optimum rates of O_2 evolution in the presence of 0.4 mM GSH (Fig. 3A). However the apparent affinity of the reaction for SeO_3^{2-} decreased with the concentration of GSH (Fig. 3B and 3C). In the presence of 0.4 mM GSH, the rate of (SeO_3^{2-} plus GSH)-dependent O_2 evolution decreased with SeO_3^{2-} concentrations greater than 1 mM (e.g. 58% of the optimum rate at 2 mM SeO_3^{2-}). In the presence of 0.4 mM SeO_3^{2-} the concentration of GSH supporting $V_{max}/2$ was 70 μM (results not shown).

Relation between (SeO_3^{2-} plus glutathione)-dependent O_2 evolution and SeO_3^{2-} consumption by sonicated chloroplasts

(SeO_3^{2-} plus glutathione)-dependent O_2 evolution was accompanied by the consumption of SeO_3^{2-} in a glutathione-dependent reaction (e.g. Fig. 4). When 0.2 mM GSSG was used as the source of glutathione the ratio of the rate of SeO_3^{2-} consumption to O_2 evolution was 1.6. When illumination was terminated, both SeO_3^{2-} consumption and O_2 evolution ceased (Fig. 4B). Zinc chloride (0.2 mM) completely inhibited both O_2 evolution and SeO_3^{2-} consumption (results not shown). These results demonstrate that O_2 evolution and SeO_3^{2-} consumption are interdependent when GSSG is used as the source of glutathione. Furthermore, they are consistent with the proposal that SeO_3^{2-} serves as the terminal electron acceptor for reducing equivalents from water and that GSH formed from GSSG in the light is an intermediate electron donor. On the other hand, when 1.2 mM GSH was supplied instead of GSSG, SeO_3^{2-} was consumed rapidly in the dark (43 μmol/mg chl/hr) with the concomitant oxidation of 4.2 mol of GSH per mol of SeO_3^{2-} consumed. Illumination enhanced the rate of SeO_3^{2-} consumption *ca* 35%. Zinc chloride

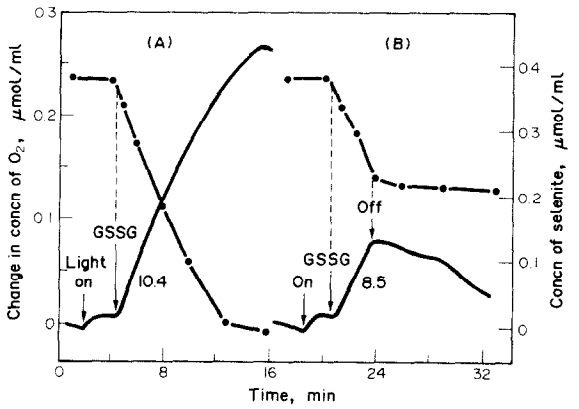


Fig. 4. Correlation between (SeO_3^{2-} plus GSSG)-dependent O_2 evolution (curve without symbols) and SeO_3^{2-} consumption (●) by sonicated chloroplasts during continuous illumination (A) and discontinuous illumination (B). Reaction mixtures initially contained 50 μM NADPH, 0.4 mM Na_2SeO_3 and chl (200 μg chl/ml) in the dark. Light treatments and the addition of 0.2 mM GSSG were made as shown. Values beside the continuous curves denote the rate of O_2 evolution in μmol/mg chl/hr. Chloroplast intactness prior to sonication, 85%.

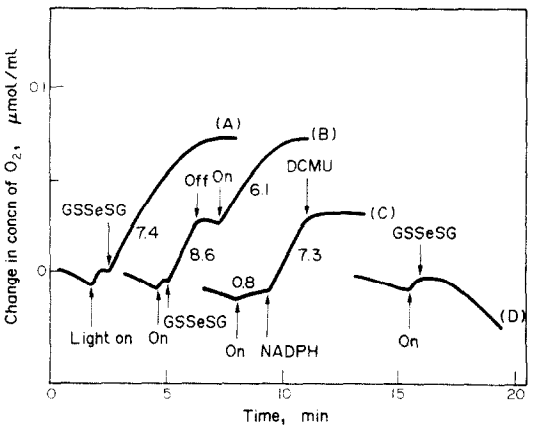


Fig. 5. GSSeSG-dependent O_2 evolution by sonicated chloroplasts. Reaction mixtures (A–D) initially contained 200 μg chl/ml/min in incubating medium in the dark. Reactions A, B and D also contained 50 μM NADPH. In addition, D contained 0.4 mM $ZnCl_2$ and C contained 0.1 mM GSSeSG. Light treatments and the addition of GSSeSG (0.1 mM), NADPH (50 μM) and DCMU (2 μM) were made as shown. Values beside the curves represent the rate of O_2 evolution in μmol/mg chl/hr. Chloroplast intactness prior to sonication, 70%.

(0.2 mM) inhibited SeO_3^{2-} consumption in the light by 20% but not in the dark. The non-enzymic consumption of SeO_3^{2-} with 1.2 mM GSH completely accounted for the consumption of SeO_3^{2-} by sonicated chloroplasts in the dark. The differences between the rates of SeO_3^{2-} consumption by sonicated chloroplasts in the light and dark with GSH as the source of glutathione were attributed to the operation of light-coupled GSSG reductase.

GSSeSG-dependent O_2 evolution by sonicated chloroplasts

Ganther [11] demonstrated that GSSeSG, the first stable intermediate in the reduction of SeO_3^{2-} by GSH (Scheme 1), was reduced by yeast GSSG reductase in the presence of NADPH. Partially purified GSSG reductase from peas also catalysed GSSeSG-dependent oxidation of NADPH with the concomitant formation of Se^0 as determined by turbidity at 400 nm [12] (results not shown); neither process occurred in the absence of the pea enzyme. In the presence of 50 μM NADPH sonicated chloroplasts evolved O_2 in the light when supplied with 0.1 mM GSSeSG. The reaction was light-dependent and completely inhibited by 2 μM DCMU and 0.4 mM zinc chloride (Fig. 5). In the absence of 50 μM NADPH, the rate of GSSeSG-dependent O_2 evolution decreased by 89%. The mean rate of GSSeSG-dependent O_2 evolution was 9.3 μmol/mg chl/hr. After cessation of the reaction, the ratio of O_2 evolved to GSSeSG supplied was 0.60–0.78.

Other experiments

The effect of 1 mM sodium selenite on some activities of illuminated intact chloroplasts was examined. In the presence of 10 mM DL-glyceraldehyde, chloroplasts supported SeO_3^{2-}-dependent O_2 evolution at slow rates (2.4 μmol/mg chl/hr) for *ca* 3–4 min. SeO_3^{2-} inhibited O_2 evolution in the presence of NO_2^-, phosphoglycerate and carbon dioxide plus ribose 5′-phosphate by 12, 56 and 100% respectively. However the uncoupled rate of $Fe(CN)_6^{3-}$-dependent O_2 evolution by osmotically shocked chloroplasts was unaffected by 1 mM sodium selenite.

Reaction mixtures containing ATP sulphurylase and pyrophosphatase from yeast support the reduction of SeO_4^{2-} to Se^0 in the presence of GSH and ATP [12]. Since these enzymes and GSSG reductase have been reported in chloroplasts [5, 13, 14] this raises the possibility that SeO_4^{2-} might initiate O_2 evolution. However, sonicated chloroplasts in the light did not support O_2 evolution in the presence of 20 mM magnesium chloride, 50 μM NADPH, 2–5 mM ATP, 0.6–4 mM GSH and 0.5–3 mM sodium selenate.

DISCUSSION

The dependence of O_2 evolution on the availability of SeO_3^{2-} (Fig. 1, Table 1) and its association with the light-dependent consumption of SeO_3^{2-} (Fig. 4) demonstrates that SeO_3^{2-} serves as the eventual acceptor of electrons emanating from water via a light-dependent mechanism. This implies that the small amounts of NADP(H) and GSH/GSSG required to support this process fulfill a catalytic function, serving as intermediates in electron flow from water to SeO_3^{2-} (Scheme 1). Neglecting the O_2 evolved prior to addition of SeO_3^{2-}, the relatively constant ratio of *ca* 0.66 mol of O_2 evolved per mol of SeO_3^{2-} consumed in the presence of an eight-fold excess of SeO_3^{2-} relative to NADP(H) and a two-fold excess of SeO_3^{2-} relative to GSSG is consistent with this proposal. The reduction of SeO_3^{2-} by chloroplasts in the dark in the presence of GSH (but not GSSG) implies that GSH serves as a reductant of SeO_3^{2-}. This sug-

Table 2. Selenium fluxes in intact and sonicated pea chloroplasts

Reaction(s)	Se flux (μg atoms/mg chl/hr)	Reference
Intact chloroplasts		
$SeO_3^{2-} + OAS^* \xrightarrow{h\nu}$ selenocysteine	0.35	[4]
$Se^{2-} + OAS^* \longrightarrow$ selenocysteine	24	[1]
Sonicated chloroplasts		
$SeO_3^{2-} + GSSG \xrightarrow{h\nu} GSSeSG$†	16	This paper
$SeO_3^{2-} + GSH \longrightarrow GSSeSG$†	66	This paper
$GSSeSG \xrightarrow{h\nu} GSSeH^*$	18	This paper

*O-Acetylserine.

†The nature of these reaction products is assumed from Scheme 1; selenium flux is calculated from the rate of O_2 evolution and/or consumption of the appropriate selenium substrate.

gests that the requirement for light for SeO_3^{2-} reduction in the presence of GSSG is due to light-coupled GSSG reductase activity. Collectively, these properties are consistent with the role of GSSG reductase in the reduction of SeO_3^{2-} as described for yeast [7] except that in the chloroplast system GSSG reductase activity utilizes light-generated reductant, hence the requirement for only catalytic amounts of GSH/GSSG and NADP(H).

The inhibition of (SeO_3^{2-} plus GSSG)-dependent O_2 evolution by SO_3^{2-} is consistent with the reported inhibition of SeO_3^{2-} incorporation into selenocysteine by SO_3^{2-} in illuminated chloroplasts but appears inconsistent with the proposal that the reduction of SO_3^{2-} and SeO_3^{2-} proceed by independent mechanisms [4]. As proposed previously for SeO_3^{2-} incorporation into selenocysteine [4], inhibition by SO_3^{2-} could result from the formation of a selenotrisulphide. However, SO_3^{2-} also inhibited GSSG-dependent O_2 evolution although GSSG reductase activity was unaffected. Perhaps the inhibition of both GSSG- and (SeO_3^{2-} plus GSSG)-dependent O_2 evolution by SO_3^{2-} (Fig. 2) could be attributed to SO_3^{2-}-induced O_2 consumption by sonicated chloroplasts in the light, a process involving the reduction of $O_2^{\cdot-}$ by SO_3^{2-} [15].

The GSSeSG-dependent O_2 evolution catalysed by sonicated chloroplasts in the presence of 50 μM NADPH and the sensitivity of this reaction to DCMU and zinc chloride (Fig. 5) is consistent with the proposed role of GSSeSG in SeO_3^{2-} reduction and the role of GSSG reductase in this process (Scheme 1). The predicted amounts of O_2 evolution for the reduction of 1 mol of GSSeSG to GSSeH ($2e^-$) and Se^{2-} ($4e^-$) according to the equations:

$$GSSeSG + NADPH + H^+ \longrightarrow GSSeH + GSH + NADP^+$$

$$GSSeSG + 2NADPH + 2H^+ \longrightarrow 2GSH + H_2Se + 2NADP^+$$

are 0.5 and 1 respectively. The experimental values (0.60–0.78) infer that at least some of the GSSeSG was reduced beyond the GSSeH stage. Experiments with partially purified GSSG reductase from peas support this proposal: after cessation of NADPH oxidation the ratio of NADPH oxidized to GSSeSG supplied was 1.3 with some Se^0 formation (Scheme 1).

Although the reduction products formed from SeO_3^{2-} and GSSeSG in the experimental systems described in this paper have not been characterized, the selenium fluxes associated with the consumption of these substrates can nevertheless be compared with those for other processes in chloroplasts (Table 2). The extremely low rate for the light-dependent incorporation of SeO_3^{2-} into selenocysteine appears inconsistent with the selenium fluxes for the various partial reactions. A likely explanation for this disparity is that whereas Se^{2-} incorporation was determined at saturating concentrations of substrate under anaerobic conditions [4], the selenium flux for $SeO_3^{2-} \rightarrow$ selenocysteine was determined under aerobic conditions. This would render the relatively low concentrations of Se^{2-}, formed under these conditions, especially prone to oxidation to Se^0 thereby underestimating the potential flux for $SeO_3^{2-} \rightarrow$ selenocysteine.

Since sonicated chloroplasts do not support SeO_4^{2-}-dependent O_2 evolution this implies that chloroplasts do not reduce SeO_4^{2-} via the mechanisms described in Scheme 1 at significant rates relative to SeO_3^{2-}. However, this does not rule out the possibility that this mechanism is not involved in SeO_4^{2-} assimilation *in vivo* since the amount of selenium detected in most plants is extremely small [16] and even very low rates of SeO_4^{2-} metabolism (far below the sensitivity of the polarographic techniques employed in this paper) could account for the selenium contents observed.

EXPERIMENTAL

Plant material and chloroplasts. Pea seedlings (*Pisum sativum* cv Massey Gem) were grown as in ref. [9]. Chloroplasts and sonicated chloroplasts were prepared as in ref. [17] except that the extracting medium also contained 0.2% D-isoascorbate and the chloroplast pellet was washed once

in washing medium prior to resuspending it in incubating medium.

Chemicals. GSSeSG was synthesized as described in ref. [11] and the product examined with ninhydrin (0.1% ninhydrin in Me_2CO) following TLC on cellulose plates with *iso*-BuOH–H_2O–aq. NH_4OH (66:33:1) as solvent; neither GSSG or GSH were detected in purified GSSeSG. The concn of GSSeSG solns was determined spectrophotometrically immediately prior to use [11]. Standard solns of SeO_3^{2-} were prepared as in ref. [18].

GSSG reductase. This was partially purified from pea seedlings as described in ref. [4]. Activity with GSSeSG as substrate was determined spectrophotometrically at 340 nm in reaction mixtures containing 0.16 mM NADPH, 60 μM GSSeSG, 100 mM KPi buffer pH 7 and enzyme at 25°. The production of Se^0 under these conditions was determined by turbidity at 400 nm [12].

O_2 evolution and SeO_3^{2-} metabolism by sonicated chloroplasts. O_2 evolution was determined polarographically using O_2 electrodes designed as in ref. [19] and supplied by Hansatech, Kings Lynn, Norfolk, England. For the determination of (SeO_3^{2-} plus glutathione)-dependent O_2 evolution, reaction mixtures contained 0.4 mM GSH or 0.2 mM GSSG, 50 μM NADPH and sonicated chloroplasts (200 μg chl) in 1 ml incubating medium (see ref. [17] for composition). Reactions were initiated with 0.2 mM Na_2SeO_3 in the light. When GSSG was used as the source of glutathione, Na_2SeO_3 was not supplied until GSSG-dependent O_2 evolution ceased (e.g. Fig. 1A). GSSeSG-dependent O_2 evolution was determined under similar conditions except that glutathione was omitted and O_2 evolution was initiated with 0.1 mM GSSeSG in place of Na_2SeO_3. GSSG-dependent O_2 evolution of sonicated chloroplasts was determined as in ref. [9]. The uncoupled rate of $Fe(CN)_6^{3-}$-dependent O_2 evolution of osmotically shocked and unshocked chloroplasts was determined as in ref. [20] except that the concns of NH_4Cl and $K_3Fe(CN)_6$ were 10 and 3 mM respectively.

The metabolism of SeO_3^{2-} by sonicated chloroplasts was determined in reaction mixtures as described for (SeO_3^{2-} plus glutathione)-dependent O_2 evolution. Samples (0.3 ml) were treated with 10 μmol *N*-ethylmaleimide (0.7 ml). After 10 min, 15% TCA (2 ml) was added and the supernatant soln analysed for SeO_3^{2-} with 3, 3′-diaminobenzidine reagent [21].

All other methods were as described in refs. [9] and [17].

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