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Oxidation of Elemental Sulfur and Sulfite by Wheat Chloroplasts

By

P. JOLIVET, E. BERGERON & P. KIEN[1)]

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S u m m a r y

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This study established that S^o and Na_2SO_3 are oxidized by fresh but not by boiled wheat (*Triticum aestivum* L.) chloroplasts. Since this process was observed both in the light and dark, it cannot be accounted for merely by the operation of the photosynthetic electron transport chain. Involvement of several enzymatic systems localized in the chloroplasts is hypothesized.

I n t r o d u c t i o n

About 2 % of elemental sulfur (S^o) applied on wheat leaves was absorbed and metabolized to sulfate, S-amino acids and proteins (LEGRIS-DELAPORTE & al. 1987, LANDRY & al. 1991). These authors have observed that the incorporation of S^o into SO_4^{2-} was accompanied with an extra uptake of O_2 by the leaves. The extra O_2 consumption closely correlated with that required for the oxidation of S^o to SO_4^{2-}, which occurred simultaneously. S^o oxidation has also been observed in photosynthetic (TRÜPER 1983) and non-photosynthetic (BRUNE 1989) bacteria; first S^o is oxidized to SO_3^{2-} or $S_2O_3^{2-}$ and then further to SO_4^{2-}. Several enzymes are involved in this oxidation of S^o in bacteria, however, the nature of its oxidation in higher plants is still obscure. The present paper reports data on the ability of intact wheat chloroplasts to oxidize S^o and SO_3^{2-}.

[1)] Laboratoire de Chimie Biologique, Institut National Agronomique Paris-Grignon, F-78850 Thiverval-Grignon, France.

Materials and Methods

Wheat (*Triticum aestivum* L., cv. Festival) was grown on vermiculite with nutrient solution for neutrophile plants (COÏC & LESAINT 1973). Quantum flux was 180 µmol m^{-2} s^{-1} and the photoperiod 16 h. The temperature was 23 °C in the light and 17 °C in the dark. Chloroplasts were prepared from 20 g leaves sampled from 7 or 14-day-old plants and homogenized in 100 ml isolation medium (0.1 M PO_4^{2-} buffer, 0.4 M sucrose, pH 7.8). After filtration on butter muslin, the filtrate was centrifuged (10 min, 1,500 *g*). The pellet was resuspended in the isolation medium and layered on top of a discontinuous sucrose gradient (GUILLOT-SALOMON & al. 1987). After centrifugation (50 min, 50,000 *g*), the intact chloroplast layer was recovered and diluted (1 : 1) with isolation medium containing 2 mM $MgCl_2$ and centrifuged (10 min, 6,000 *g*). The pellet containing the chloroplasts was suspended in the reaction medium (0.33 M sorbitol, 50 mM Hepes, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, pH 7.6) with 5 mM $NaHCO_3$ and S^o (65 or 360 µg ml^{-1} as a suspension in 5 % Tween 80) or Na_2SO_3 (0.25, 0.5 or 2 mM). Chlorophyll concentration estimated according to SCHMID 1971 was about 0.1 g l^{-1}. Experiments were carried out under O_2 or N_2, in the light (800 µmol m^{-2} s^{-1}) or in the dark, during 2 h at 18°C and also with boiled chloroplasts. N_2 was flushed through the reaction medium and Na_2SO_3 solution to avoid autooxidation. Experiments were stopped with the elimination of chloroplastic lamellae by centrifugation and S-compounds were analyzed in the supernatants. Turbidimetric SO_4^{2-} determination by a modification of the method of SÖRBO 1987 was reported elsewhere (JOLIVET & KIEN 1992b). Determination of $S_2O_3^{2-}$ and SO_3^{2-} was carried out by HPLC as their bimane derivatives (VETTER & al. 1989). Separation was achieved on a Ultrasphere ODS 5 µm column (4.6 mm x 25 cm, Beckman) by a gradient elution at a flow-rate of 1.2 ml (Beckman 110A pumps, Altex gradient programmer) using aqueous acetic acid (0.25 %, pH 3.5) as buffer A and acetonitrile as solvent B. Elution conditions were 15 % B for 5 min, linear increase from 15 % B to 23 % in 10 min, from 23 % to 100 % in 15 min, followed by 20 min 100 % B and then change to 15 % B in 3 min and further re-equilibration. Fluorescence detection was carried out with a Jasco fluorimeter (390 nm excitation, 480 nm emission). Experiments were also carried out after addition of $^{35}S^o$ (1.8 MBq/experiment, 5.2 MBq mg^{-1} S), with a flow-through scintillation counter (radioactivity monitor LB 506C Berthold) coupled to the fluorescence detector. Sulfate which does not react with bimane can be detected as a radioactive peak at the beginning of the chromatogram.

Results and Discussion

Chloroplasts did not contain SO_4^{2-}, $S_2O_3^{2-}$ and SO_3^{2-} before the experiment. Table 1 shows the oxidation of SO_3^{2-} to SO_4^{2-} in the presence of wheat chloroplasts maintained for 2 h in the light or the dark, and in presence or absence of O_2. A slight autooxidation of Na_2SO_3 occurred in the solution prior to use and its value corresponded with the amount of SO_4^{2-} found in the experiments carried out under N_2 with boiled chloroplasts. In the presence of O_2, SO_3^{2-} was easily oxidized to SO_4^{2-} in boiled chloroplasts via a chemical process. This spontaneous oxidation was also illustrated, both in the light and in the dark, by the difference in SO_4^{2-} formation by fresh chloroplasts between O_2 and N_2 experiments. About 32 % of Na_2SO_3 was oxidized by an aerobic chemical process. On the other hand, SO_4^{2-} formation was higher in fresh than in boiled chloroplasts both under N_2 or O_2 and light did not seem to activate systematically SO_4^{2-} production; 43 % of Na_2SO_3 was oxidized through a chloroplastic intrinsic process. In the case of fresh chloroplasts maintained under O_2, SO_3^{2-} was largely transformed. With 0.25 mM Na_2SO_3 initial

concentration, the rate of SO_3^{2-} oxidation, calculated with experiments carried out under N_2 in the light, was about 0.47 µmol h^{-1} mg^{-1} chlorophyll. With 2 mM initial concentration, the oxidation rate attained 3 µmol $h^{-1}mg^{-1}$ chlorophyll. Under N_2 in the light, photosynthetic O_2 would be involved in SO_3^{2-} oxidation. In the dark, there is probably sufficient residual O_2 in chloroplastic suspension and reaction medium to explain the observed oxidation.

Detectable amounts of SO_4^{2-} were found in chloroplastic suspension treated for 2 h with 65 µg S° but not in boiled ones (Table 2). There was no significant difference in the formation of SO_4^{2-} between light and dark treated chloroplasts or between experiments under O_2 or N_2. Amounts of $S_2O_3^{2-}$ and SO_3^{2-} were low specially under O_2, but significant. With radioactive labelling, both SO_4^{2-} and $S_2O_3^{2-}$ were labelled (Table 3). SO_3^{2-} label was often below the detection limit. The low level of SO_3^{2-} could be explained by a fast oxidation into SO_4^{2-}. Our results about SO_3^{2-} metabolization prove that this last oxidation step is probably not limiting. The total oxidation of S° to SO_4^{2-} is easier under O_2 that under N_2 since less $S_2O_3^{2-}$ and more label in SO_4^{2-} were found in the first case. It has been verified that isotopic exchange between $^{35}S°$ and pre-existent S-compounds could not explain the observed labelling.

In conclusion, our experiments have shown without ambiguousness that intact wheat chloroplasts were able to oxidize S° into SO_4^{2-} via $S_2O_3^{2-}$ and SO_3^{2-} synthesis. It has been established elsewhere (JOLIVET & KIEN 1992 a, b) that H_2O_2 could not oxidize S° and SO_3^{2-} spontaneously and that the involvement of fatty acid hydroperoxides should be discarded. Moreover, S° and SO_3^{2-} oxidation was effected in the light and in the dark as well, and should not therefore be ascribed only to a non-enzymatic oxidation initiated by the electron transport chain as reported by ASADA & KISO 1973 and DITTRICH & al. 1992. The operation of chloroplastic enzymatic mechanisms is assumed as working hypothesis and these are under current investigation.

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Table 1. SO_3^{2-} oxidation (% of initial amount : 0.25, 0.5 or 2 mM) to SO_4^{2-} in wheat chloroplasts. Data represent the mean of 7 experiments (± SD).

		Nitrogen	Oxygen
Boiled chloroplasts	light	15.1 ± 5.9	41.9 ± 5.9
Fresh chloroplasts	dark	49.8 ± 18.1	89.0 ± 6.2
	light	59.8 ± 9.8	89.5 ± 9.5

Table 2. S-compounds production (nmol ml^{-1}) from S^o (initial amount: 65 µg ml^{-1}) in wheat chloroplasts. Data represent the mean of 7 experiments (± SD).

	Oxygen		Nitrogen	
	light	dark	light	dark
SO_4^{2-}	29.8 ± 14.8	20.0 ± 20.0	21.5 ± 18.5	19.0 ± 19.0
SO_3^{2-}	0.5 ± 0.5	0	2.5 ± 2.3	0.5 ± 0.5
$S_2O_3^{2-}$	1.0 ± 0.7	0.4 ± 0.4	11.0 ± 9.7	1.2 ± 1.2

Table 3. Radioactive labelling of S-compounds (10^3 x counts) from $^{35}S^o$ (360 µg ml^{-1}, 5.2 MBq mg^{-1} S) in wheat chloroplasts.

	Oxygen		Nitrogen	
	light	dark	light	dark
SO_4^{2-}	183	75	113	-
SO_3^{2-}	-	-	19	-
$S_2O_3^{2}$	97	-	254	66