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Sulphate Uptake by Leaf Mesophyll and Bundle Sheath Cells of Maize Plants

C. PASSERA and M. FERRETTI

Agricultural Biotechnology Department,
University of Padova, 35100 Padova, Italy

Abstract. Uptake of ^{35}S-sulphate by bundle sheath strands (BSC) from leaves of maize plants (*Zea mays* L. cv. Dekalb L 72 A) was higher than that by isolated mesophyll protoplasts (MC) of maize. Ion uptake followed the Michaelis-Menten kinetic saturation curves. $SO^2{}_4{}^-$ uptake increased after addition of malate, NADPH, malate + $NADP^+$ to BSC suspensions, but not to MC suspensions.

Mesophyll protoplasts (MC) and bundle sheath strands (BSC) of C_4 plants appear to have the complete sulphate reduction mechanism from sulphate to cysteine since the activities of ATP sulphurylase (EC 2.7.7.4) and O-acetylserine sulphydrylase (EC 4.2.95.9), the first and the last enzyme of this reduction pathway, have been found in both types of cells isolated from maize leaves. In addition, BSC contribute more to the whole sulphate reduction than MC (GERWICK and BLACK 1979, PASSERA and GHISI 1982). Different responses of ATP-sulphurylase and O-acetylserine sulphydrylase activities of these cells to sulphur and nitrogen deprivation and light growth conditions have been found by PASSERA and GHISI (1982), GHISI *et al.* (1986) and GHISI and PASSERA (1987).

To obtain further information on sulphur metabolism of C_4 plants, we studied $SO_4{}^{2-}$ uptake by MC and BSC of *Zea mays* leaves. Both types of cells differ in photosynthetic CO_2 fixation and nitrogen metabolism (EDWARDS *et al.* 1970, NEYRA and HAGEMAN 1978, MOORE and BLACK 1979).

MATERIALS AND METHODS

Plants of *Zea mays L.* (cv. Dekalb 72 A) were grown in a controlled environment under 16/8 h period, 114 W m^{-2}, 25/18 °C, and 70—80 % relative humidity as reported by GHISI *et al.* (1986). The second leaf of 14 d old plants was excised. Samples of 1.0 g (fresh mass) without central veining were cut to isolate MC and BSC.

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Mesophyll Protoplast and Bundle Sheath Strand Isolation

MC and BSC were isolated enzymatically using a combination of cellulase (Onozuka R-10) and pectinase (Macerozyme R-10) obtained from Yakult Biochemical Co., (Nishinomiya, Japan) (see PASSERA and GHISI 1982 for details). The purity of cell preparations was tested according to KANAI and EDWARDS (1973). Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) and phosphoenolpyruvate carboxylase (EC 4.1.1.31) activities were assayed as reported by PASSERA and ALBUZIO (1975). ATP-sulphurylase activity was assayed *via* the molybdate dependent production of pyrophosphate from ATP (WILSON and BANDURSKI 1958). Except for the concentration of Na_2MoO_4 (30 mM instead of 20 mM) in the reaction mixture the procedure of GERWICK and BLACK (1979) was followed. Chlorophyll was determined by the method of ARNON (1949).

$^{35}SO_4^{2-}$ Uptake

The assay mixture used to measure SO_4^{2-} uptake (Carrier-free ^{35}S from the Radiochemical Centre, Amersham, Buckinghamshire, England) contained 5 mM $MgCl_2$, 500 mM sorbitol, 50 mM Tricine buffer at pH 7.5, 1 cm^3 of freshly prepared cell suspension (with 40—50 μg chlorophyll) and other experimental addenda (see figure legends) to a final volume of 1.5 cm^3 in a 10 cm^3 cuvette. The cuvettes placed in a Dubnoff bath at 25 °C were gently shaken during the experiment to keep the cells suspended and to increase the influx of $^{35}SO_4^{2-}$. The uptake measurement was initiated by illumination of cell suspension for 5 min by an incandescent lamp (Philips Comptalux 300 W). The quantum flux density at the surface of cuvettes was 440 μmol m^{-2} s^{-1} (400—700 nm). After a preillumination period, radioactive tracer (74 kBq per cuvette) and unlabelled K_2SO_4 (final concentration 0.5 mM) were added. Uptake was carried out in the presence of light. Usually, at 5, 10, 15, 25 and 30 min from addition of tracer two cuvettes were removed from the bath and the cells were rapidly separated from the medium by filtration on Millipore (pore size 0.45 μm), and rapidly washed with unlabelled icecold 500 mM sorbitol solution (50 cm^{-3}) containing 20 mM K_2SO_4. Cells with Millipore were transferred into scintillation vials, 3 cm^3 of 35 % H_2O_2 was added, and the samples were dried in an oven at 90 °C. Ashes were dissolved with water and scintillation liquid consisting of 100 g naphthalene, 7 g PPO (2,5 diphenyloxazol) and 0.3 g of dimethyl-POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene) per 1000 cm^3 dioxane, was added.

Radioactivity was determined with Packard TRI-CARB scintillation spectrometer. With the exception of time course study, uptake was determined 15 min after the addition of $^{35}SO_4^{2-}$ solution to cell suspension. All experiments were confirmed by several repetitions.

RESULTS

The activities of the marker enzymes indicated that there was little cross-contamination of the two types of cell extracts. The purity was confirmed by chlorophyll *a*/*b* ratios which were 3.1 in MC and 5.1 in BSC (Table 1).

In agreement with previous results ATP-sulphurylase activity was mainly found in BSC (Table 1).

$^{35}SO_4^{2-}$ was rapidly taken up into both kinds of cells from a medium con-

TABLE 1

Chlorophyll (Chl) *a*/*b* ratio, ATP sulphurylase [mmol ($PO^3_4^-$) kg^{-1} (chl) s^{-1}], and RuBP carboxylase and PEP carboxylase activities [mmol (CO_2) kg^{-1} (chl) s^{-1}] in mesophyll and bundle sheath cells from leaves. SD between different measurements did not exceed ± 10 %

	Mesophyll cells	Bundle sheath cells
Chl *a*/*b*	3.10	5.12
ATP sulphurylase	2.08	21.90
RuBP carboxylase	9.30	140.02
PEP carboxylase	228.00	12.53

taining 0.5 mM SO_4^{2-} (Fig. 1): on the chlorophyll content basis it was on average 50 % higher in BSC than in MC. Uptake of SO_4^{2-} by leaf cells as a function of K_2SO_4 concentration was examined over a limited range of sulphate concentration (5—140 μM) (Fig. 2). Uptake by both MC and BSC followed a Michaelis-Menten saturation kinetics. Km (SO_4^{2-}) were 43 μM and 32 μM for MC and BSC, respectively, whereas V_{max} were 11.0 and 7.5 μmol kg^{-1} (chl) s^{-1} for MC and BSC, respectively.

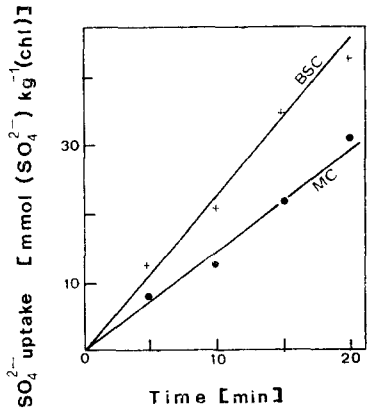

Fig. 1. Time course of SO_4^{2-} uptake by mesophyll (MC) and bundle sheath (BSC) cells from maize leaves. — K_2SO_4 = 0.5 mM; ^{35}S carrier-free 74 kBq per cuvette.

Malate or NADPH positively affected SO_4^{2-} uptake by BSC, but not by MC (Fig. 3). The addition of 10 mM malate to cell suspension induced an 80 % increment of uptake by BSC with respect to control, and an addition of 10 mM malate plus 0.5 mM $NADP^+$ increased this difference to 140 %.

DISCUSSION

Isolated protoplasts from cultured tobacco cells (METTLER and LEONARD 1979a,b) or different tissues (LIN 1980, LEURS *et al.* 1982) show active ion transport function. We found that MC and BSC from maize leaves actively

transported sulphate. A positive correlation between ATP sulphurylase and SO_4^{2-} uptake, established for tobacco cells (REUVENY 1977, REUVENY and FILNER 1977, SMITH 1975, 1980) was also found in BSC and MC of maize where both these activities were higher in BSC than in MC (Fig. 1, Table 1). The pattern obtained by plotting the transport rate of sulphate as a function of sulphate concentration fitted the classic saturation kinetics (Fig. 2).

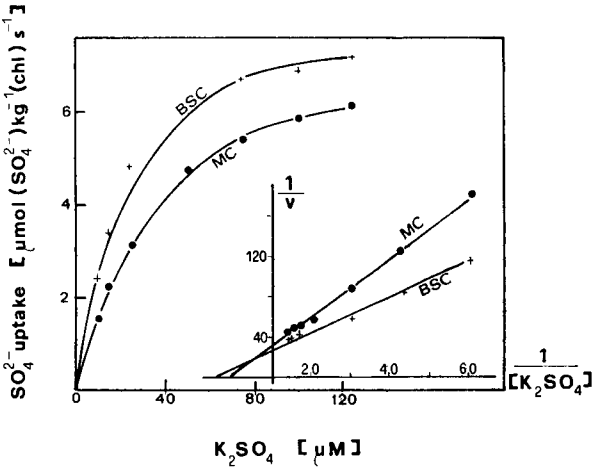

Fig. 2. Sulphate uptake by mesophyll (MC) and bundle sheath (BSC) cells from maize leaves as a function of increasing K_2SO_4 concentration.

In NADP-malic enzyme species, such as *Zea mays*, malate is the major product of CO_2 fixation. Malate formed in MC is transferred to BSC where it is decarboxylated (BLACK 1973, HATCH and KAGAWA 1976). Redox equivalents from malate decarboxylation can be used to reduce newly formed 3-phosphoglycerate (FARINEAU 1975a,b) for synthesis of saccharides.

Our results suggest that malate might also supply redox equivalents for SO_4^{2-} uptake by BSC which are lacking in the photosystem 2 activity (MOORE and BLACK 1979, CHAPMAN *et al.* 1980). SO_4^{2-} uptake by BSC from leaves of *Digitaria sanguinalis* was negatively affected by malate addition (data not reported). The above suggestion was strengthened by the increase in sulphate uptake by BSC by adding $NADP^+$ to malate. NADP does not enter chloroplasts (EDWARDS and WALKER 1983), but according to USUDA and MIYACHI

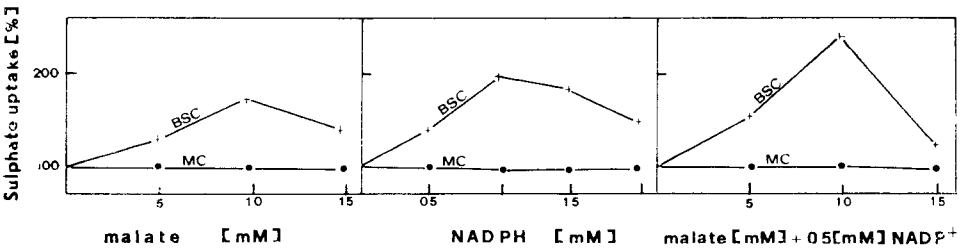

Fig. 3. Relative effects of malate (left), NADPH (middle), malate plus 0.5 mM $NADP^+$ (right) on SO_4^{2-} uptake by bundle sheath (BSC) and mesophyll (MC) cells from maize leaves.

(1977) $NADP^+$ is rapidly reduced when malate is added to BSC suspension. In addition, a transport mechanism for the net accumulation on $NADP^+$ in plant mitochondria has also been reported (TOBIN *et al.* 1980). Therefore, NADPH formed through malate decarboxylation might also be utilized for the sulphate uptake in BSC (*cf.* Fig. 3).

Reduction equivalents carried out by malate used need not be necessarily transported entirely to BSC chloroplasts: a part of malate may be subjected to oxidation by cytosolic NAD-dependent malate dehydrogenase and the redox potential is utilized for active transport of Cl^- (LÜTTGE and HIGINBOTHAM 1979, p. 257). A similar speculation can be made about the positive effect of malate on SO_4^{2-} uptake by BSC of maize leaves. The not apparent advantage in uptake by MC after addition of malate or malate plus $NADP^+$ might be attributed to a high availability of redox equivalents and ATP since these cells can perform the non-cyclic electron flow as their chloroplasts have an active photosystem 2.

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