

Development of Sulfate Uptake Capacity and ATP-Sulfurylase Activity during Root Elongation in Maize¹

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ABSTRACT

Sulfate uptake capacity and ATP-sulfurylase activity were determined in maize roots (*Zea mays* L. var. XL 363 and mutant XL 363 o2) at increasing root length. The pattern of uptake showed a close similarity to that of ATP-sulfurylase, both activities reaching the maximum level at 9 and 10 cm root length in the XL 363 and XL 363 o2 hybrids, respectively. In addition to the shift of the maximum, *opaque-2* mutation caused an enhancement of the two activities at root length below and above the activity peak. The kinetic parameter of uptake, K_m , showed a maximum at 3 to 4 and a minimum at 7 to 8 cm. The isoenzyme pattern of ATP-sulfurylase was the same in the two hybrids and did not change with root elongation. A common regulatory mechanism is postulated for uptake and activation of sulfate. The kinetic behavior is interpreted as an index of flexibility of the transport system toward different nutrient status of the environment.

The physiological events considered in this study occur just after the germination process, defined as the forcing of the radicle through the seed (4). They pertain to that phase of plant differentiation characterized by the development of a fully operating root system. During this period roots display the most important effort of adaptation to the soil environment: the metabolic factory undergoes a deep modification from a substantially self-supporting matter and energy provision, to a condition requiring an intense exchange with the external world. The two main features of this new metabolic state are: (a) the appearance of transport systems enabling roots to pump nutrients selectively against an electrochemical gradient; (b) the establishment of new metabolic pathways allowing the utilization of low energy materials, such as inorganic ions, for the *de novo* synthesis of organic constituents of the living system. Ion uptake has long been underestimated as a factor of plant productivity with respect to the proper metabolic steps. In recent years, rapidly expanding work on the subject has confirmed the true metabolic character of the uptake step, showing also its suitability to a genetic and environment regulation (10). Furthermore, the close connection between uptake and successive metabolic events has supported the hypothesis of a common regulation mechanism, as in the classic lactose operon (6).

In the present work SO_4^{2-} ion was chosen as the substrate of both a transport and a metabolic step, the latter consisting in the synthesis of the energy-rich compound adenosine 5'-phosphosulfate from ATP and SO_4^{2-} (8). The first step is mediated by proteins making up the SO_4^{2-} transport system (9), the second by the enzyme-protein ATP-sulfurylase (ATP sulfate adenylyl transferase EC 2.7.7.4). A connection between the

two catalytic systems at the level of genetic control was the main assumption of this work, whose objectives were: to verify whether a parallelism exists between the development of SO_4^{2-} uptake capacity and that of ATP-sulfurylase activity during root elongation; to observe the kinetics of SO_4^{2-} uptake, as expressed by the apparent K_m value, at increasing root length.

In order to verify the effect of a gene modification on the expression of SO_4^{2-} uptake capacity and ATP-sulfurylase activity, the study was extended to the hybrid obtained from the same parental lines carrying the *opaque-2* mutation (XL 363 o2).

Responses to the above inquiries were expected to increase knowledge of the level of integration of SO_4^{2-} uptake into the sulfur metabolic pathways in higher plants, and to point out the absorption characteristics of segments obtained from roots of different length.

MATERIALS AND METHODS

Seeds of *Zea mays* L. (var. XL 363 and XL 363 o2) were surface-sterilized with 0.1 M $\text{Ca}(\text{ClO})_2$ and germinated at 25 C in the apparatus described by Hodges (5). The seedlings were grown in order to obtain roots of different lengths, varying from 2 to 14 cm. Equal length roots were selected and 1-cm random segments were used for the SO_4^{2-} uptake experiments and for the ATP-sulfurylase determination. The uptake capacity was evaluated by 10-min incubation of 1-g root samples in 500 ml of 0.5 mM CaCl_2 solution containing ^{35}S -labeled K_2SO_4 (5 mCi/mmol) at concentrations in accordance with the experimental schedule.

The incubation mixture was maintained at 27 C in a Dubnoff shaker and oxygenated by continuous bubbling of sterile air. The incubated roots were rinsed with 500 ml of 0.1 N nonradioactive K_2SO_4 for 5 min at 0 C. Root homogenate was obtained with an Ultraturrax apparatus and radioactivity evaluated by counting aliquots of the homogenate in a Packard Tri-Carb spectrometer with Instagel as scintillation mixture. The kinetic parameter K_m was derived from the double reciprocal plot (Lineweaver-Burk) of the uptake rate *viz.* the SO_4^{2-} concentration in the range 0.05 to 0.5 mM.

ATP-sulfurylase activity was assayed using the procedure described by Wilson and Bandurski (14). Three-tenths ml of crude extract (100-250 μg protein) was present in 1 ml of assay mixture containing in μmol : 200 tris-HCl (pH 7.5), 10 ATP, 10 MgCl_2 , 50 Na_2MoO_4 , and 2 μg pyrophosphatase. Protein was measured by the method of Lowry *et al.* (7).

The isoenzyme pattern of ATP-sulfurylase was determined by electrofocusing of 50- μl aliquots of the dialyzed homogenate obtained from 1 g root in 3 ml 0.1 tris-HCl (pH 8), 1 mM Na EDTA buffer. The apparatus was that described by Righetti and Drysdale (12). After the run (300 v, 20 mamp, 14 hr) gels were washed three times with 200 mM tris-HCl (pH 7.5), 10 mM MgCl_2 , 50 mM Na_2MoO_4 solution containing 2 $\mu\text{g}/\text{ml}$ pyrophosphatase. The same solution, added with 10 mM ATP, was used for 30-min incubation at 40 C of the gels. The

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inorganic phosphorus released in the gel was revealed using 1:1.5 diluted Fiske-Subbarow reagent (3).

RESULTS

Root elongation was characterized by changes in dry matter and protein content according to the following patterns. In both XL 363 and XL 363 o2 hybrids, dry matter percentage decreased from 9.3 ± 0.34 at 2 cm, to 7.5 ± 0.31 at 5 cm root length (means of three replications \pm SE). The latter value was kept constant during further root elongation. At 2-cm length protein content (per cent dry matter) was 16.9 ± 0.32 in the XL 363, and 12.5 ± 0.29 in the XL 363 o2 hybrid. It decreased to 8.6 ± 0.23 and 7.8 ± 0.21 , respectively, at 7 cm, keeping these values constant with further increases of root length.

The efficiency of SO_4^{2-} uptake was evaluated by measuring the uptake rate per unit root protein in the presence of a definite and constant concentration of the ion (11) (0.1 mM in our experiments). Root sections showed very low SO_4^{2-} transport capacity at root length below 4 cm. This was particularly valid for the XL 363, while the roots of XL 363 o2 hybrid exhibited an increased rate of SO_4^{2-} uptake even below 4 cm length (Fig. 1). A sharp increase of the uptake rate appeared in roots of both hybrids above 4 cm length, with maxima at 9 and 10 cm for XL 363 and XL 363 o2, respectively. At further increasing length a common pattern characterized the two hybrids, showing a decrease of the uptake efficiency. This was probably due to the synthesis of new protein deprived of transport capacity for SO_4^{2-} . A definitive level of uptake activity could be the consequence of the attainment of a constant proportion of total protein by the SO_4^{2-} transport protein.

ATP-sulfurylase activity mimicked SO_4^{2-} uptake rate with regard to the general pattern during root elongation and to the differences between the normal hybrid and that carrying the *opaque-2* mutation. This mutation caused the shift of the maximum activity from 9- to 10-cm root length for both SO_4^{2-} uptake and ATP-sulfurylase. Another effect of the mutation was the enhanced activity of both transport system and ATP-sulfurylase at root length below and above the activity peak.

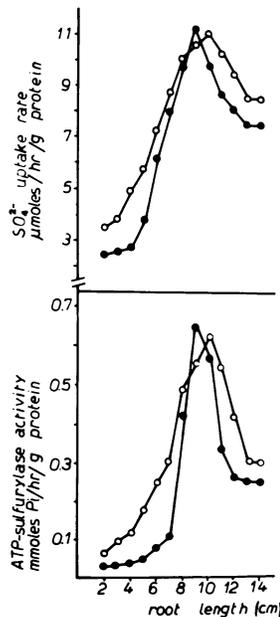


FIG. 1. Variation with root length of ATP-sulfurylase activity and SO_4^{2-} uptake rate in maize roots. (●—●): XL 363; (○—○): XL 363 o2. Each experimental point represents the mean of triplicate determinations. Standard errors of mean ranged from 2 to 8% of values reported.

The apparent K_m for SO_4^{2-} uptake showed a wavy pattern during root elongation, with a maximum at 3 to 4, and a minimum at 7 cm (Fig. 2). Above 7 to 8 cm K_m increased again up to a constant value at 12-cm root length.

The isoenzyme pattern of ATP-sulfurylase (Fig. 3) did not change with root elongation and was also the same for the two hybrids.

DISCUSSION

The overlapping patterns (Fig. 1) of SO_4^{2-} uptake rate and ATP-sulfurylase activity must be evaluated in the context of the sequential development of different enzyme and transport activities during root elongation. If the appearance of the bulk of these activities occurred within a narrow period of root development, the observed overlapping could be deprived of meaning. On the contrary, the metabolic events occurring in elongating roots at different physiological ages of cells have been demonstrated to cover a broad interval from both temporal and topographical standpoints (13). The close similarity between SO_4^{2-} uptake and ATP-sulfurylase patterns supports the view of a common regulatory mechanism for the two activities.

Analogy in the response to time between uptake and reduction of nitrate in barley seedlings was also considered to indicate a meaningful correlation at the regulatory level between the two stages (1). The change with root length of ATP-sulfurylase cannot be attributed to modifications of the enzyme multiplicity, as shown by the electrofocusing patterns. When a different genetic condition was considered, as in the case of the introduction of mutation, the timing of both uptake and activation into adenosine 5'-phosphosulfate of SO_4^{2-} continued to coincide.

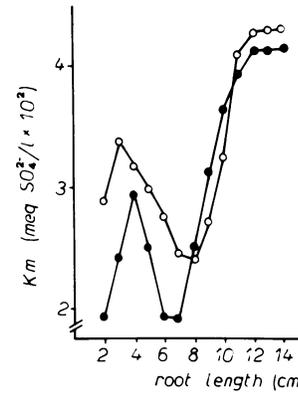


FIG. 2. Changes of apparent K_m for SO_4^{2-} uptake at increasing root length. (●—●): XL 363; (○—○): XL 363 o2.

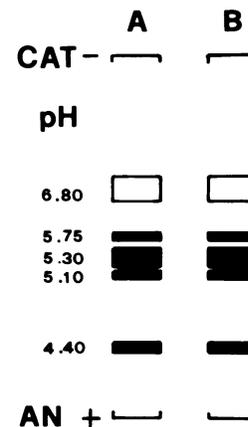


FIG. 3. Electrofocusing in polyacrylamide gel of ATP-sulfurylase extracted from 2- (A) and 12- (B) cm maize roots.

The behavior of the kinetic parameter K_m for SO_4^{2-} transport can be evaluated in the light of the strategy displayed by roots toward the nutrient status of the environment (2). A "velocity strategy" concentrates the effort of adaptation on the maximum increase of the V_{\max} , while an "affinity strategy" looks at the maximum decrease of the K_m value. The suitability of either strategy depends on the nutrient concentration in the soil solution. The behavior of the SO_4^{2-} transport system appears to meet the requirement of the highest flexibility toward the environment, through changes in the specific activity and in the apparent K_m values during root elongation.

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