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Mechanism of Sulfate Uptake by Excised Maize Roots

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Abstract. The mechanism of uptake of sulfate by excised maize roots (*Zea mays* L. cv. Ganga-5) was investigated. It was found that in the concentration range 1×10^{-9} M – 5×10^{-2} M, the sulfate uptake could be represented by a single multiphasic isotherm having four phases. Each phase covered a limited concentration range and obeyed Michaelis-Menten kinetics, which indicates mediation in sulfate uptake by a structure which changes characteristics (kinetic constants) at certain discrete salts concentrations (transition points).

The complexity of ion absorption kinetics and the various ion interactions in roots are not easily interpreted, which has led to considerable debate (LATIES 1969, EPSTEIN 1973).

Sulfate was considered to be taken up by a homogeneous high-affinity mechanism and by additional uptake mechanism(s) above 5×10^{-4} M (PERSSON 1969) and above 5×10^{-5} M sulfate (LEGGET and EPSTEIN 1956). These findings were also confirmed by other workers (JESCHKE and SIMONIS 1965, LÜTTGE and BAUER 1968). On the other hand, a detailed kinetic study of uptake of sulfate by barley roots and leaf slices showed its uptake mediated by a single structure which changed characteristics at certain discrete salt concentrations (NISSEN 1971).

The present investigation on sulfate was carried out over an extended concentration range of 1×10^{-9} M – 5×10^{-2} M with a view of gaining some more detailed information on the kinetics of sulfate uptake.

MATERIAL AND METHODS

Plant Material

Primary roots of three days old etiolated maize seedlings (*Zea mays* L. cv. Ganga-5) were used throughout this study.

The seeds were germinated in plastic trays. Each tray contained a 2 cm thick layer of 0.8% agar covered by a thick cotton layer wetted with 0.2 mM $CaCl_2$ solution. Only the 2.5 cm section behind the tip of the primary root of three days old etiolated seedlings was taken for experimental purposes. Batches of 20 segments were prepared according to the procedure described

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by EPSTEIN *et al.* (1963) and tied up in "tea-bags" of cheese cloth. Roots were used in less than one hour from the time harvesting began.

Uptake Procedure

Uptake experiments were carried out by incubating 20 cheese-cloth bound root segments for one hour in 100 ml of labeled solution of sulfate, ranging in concentration from 1×10^{-9} M to 5×10^{-2} M. The uptake solutions for

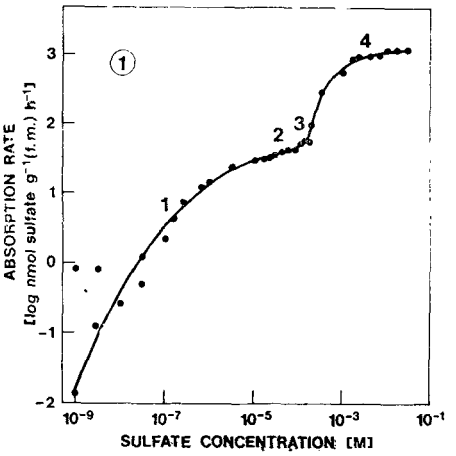

Fig. 1. Rate, V, of absorption of sulfate by excised maize roots as a function of the concentration of sulfate (1×10^{-9} M — 5×10^{-2} M). Uptake period 1 h at 30 °C. Average SE: 6%.

sulfate contained 100 ml of 0.2 mM $CaCl_2$ plus appropriate concentration of K_2SO_4 adjusted to pH 5.6 and labeled with 10 000—12 000 cpm ml^{-1} "carrier-free" ^{35}S. The temperature was held at 30 °C and moderate aeration was provided.

Experiments were terminated by rinsing the plant tissue with water for a minimum of 30 min. Desorption experiments indicate that this removes all sulfate outside the plasmalemma (LEGGET and EPSTEIN 1956). Root samples were assayed for radioactivity by counting the samples using a Packard proportional gas flow counter after the roots had been digested to dryness in 1 ml of ternary acid mixture (nitric acid: sulfuric acid: perchloric acid; 10 : 1 : 4 v/v/v). "Carrier-free" ^{35}S as $H_2{}^{35}SO_4$ was obtained from the Isotopic division of B.A.R.C. Trombay, Bombay.

TABLE 1

A summary of kinetic constants and inflection points for the uptake of 1×10^{-9} M — 5×10^{-2} M sulfate by excised maize roots

Phase number	V_{max} [nmol h^{-1} g^{-1} fr.m.]	K_m[M]	Inflection point [M]
1	37.025	1.5×10^{-6}	4×10^{-5}
2	53.60	3.649×10^{-6}	9×10^{-5}
3	61.02	3.924×10^{-6}	2×10^{-4}
4	1750.32	1.197×10^{-3}	

RESULTS

The data for uptake is expressed as velocity per gram fresh matter of tissue. The kinetic constants K_m and V_{max} were determined by using a computer program for weighted linear least squares regression analysis (ROBERTS 1977). Each point on the hyperbolic isotherm (Fig. 1) represents the mean of 3 replicates.

Sulfate uptake by excised maize roots in the concentration range 1×10^{-9} to 5×10^{-2} M could be represented by a single multiphasic isotherm (Fig. 1). Four phases, 1, 2, 3 and 4 were evident here with regularly spaced and increasing kinetic constants (Table 1). As the multiphasic isotherm (Fig. 1) was constructed solely on the basis of the kinetic constants bias can thus be introduced only in the choice of inflection points.

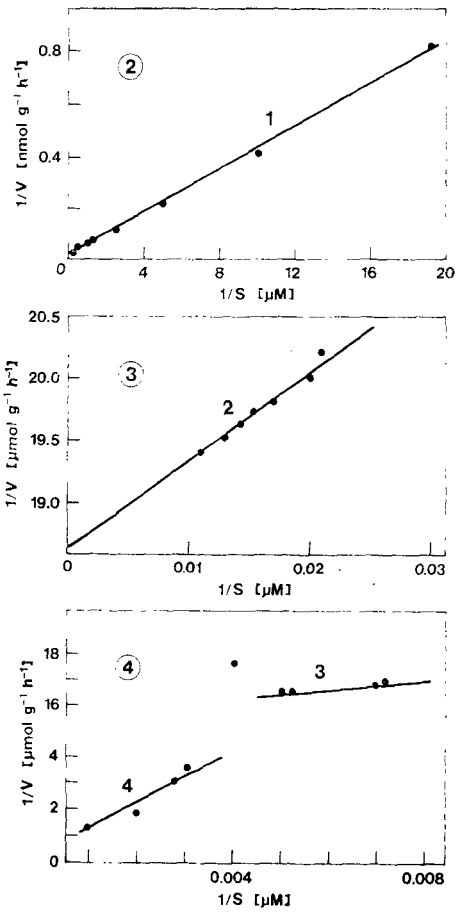

Fig. 2. Double reciprocal plot for Phase I (1×10^{-9} M – 4×10^{-5} M). $V_{max1} = 37.025$ nmol h^{-1} g^{-1} fr. m.; $K_{m1} = 1.51 \times 10^{-6}$ M.

Fig. 3. Double reciprocal plot for Phase II (4.5×10^{-5} M – 9×10^{-5} M). V_{max2} 53.6 nmol h^{-1} g^{-1} fr. m.; $K_{m2} = 3.649 \times 10^{-6}$ M.

Fig. 4. Double reciprocal plot for phases 3 and 4 (1×10^{-4} M – 5×10^{-2} M). $V_{max3} = 61.02$ nmol h^{-1} g^{-1} fr. m.; $V_{max4} = 1750.32$ nmol h^{-1} g^{-1} fr. m.; $K_{m3} = 3.924 \times 10^{-6}$ M; $K_{m4} = 1.197 \times 10^{-3}$ M.

The uptake of 1×10^{-9} M—4×10^{-5} M sulfate was mediated by phase 1 while phase 2 mediated the uptake of sulfate in the concentration range 4.5×10^{-5}—9×10^{-5} M. The uptake of 1×10^{-4} M—2×10^{-4} M sulfate was mediated by phase 3 and phase 4 mediated the uptake in concentration range 2.5×10^{-4} M—5×10^{-2} M sulfate (Fig. 1). Kinetic contants K_m and V_{max} of each phase could be calculated by obtaining double reciprocal plots of each phase (Figs. 2, 3, 4). A summary of kinetic constants and inflection points is given in Table 1.

DISCUSSION

A single multiphasic mechanism was found to be mediating the uptake of sulfate in maize roots. This is very much in tune with the findings of NISSEN (1971, 1973b) but does not support those of LEGGET and EPSTEIN (1956).

At three discrete ambient concentrations the "carrier" mediating sulfate uptake in maize roots changed kinetic constants (Table 1), the result of each such change being an inflection in the isotherm (Fig. 1) with the establishment of a new rate of uptake and also a changed affinity of the "carrier" for sulfate as shown by K_m values (Table 1). Furthermore, the sharpness by which the inflection points were defined (Table 1) showed that the "carrier" involved faithfully reflected the external concentration of the ion (NISSEN 1973a, b, c). Each phase obeyed Michaelis-Menten kinetics as shown by the regular hyperbolae (Fig. 1) and straight-line double reciprocal plots obtained from the velocity vs concentration data (Figs. 1, 2, 3). This was in line with the prediction for a single entity undergoing change in configuration and the multiphasic concept of ion uptake (Nissen 1971, 1973a).

Concluding from the results reported here and in NISSEN (1971), there is a single "carrier" involved in the uptake of sulfate in maize roots and not a dual mechanism or two "carriers" placed at different membranes (Legget and EPSTEIN 1956, JESCHKE and SIMONIS 1965). Such is the case not only for sulfate but also for phosphate and several other ions (NISSEN 1973c). We have applied the term "carrier" /entity only loosely as structural and biochemical aspects are still in short supply due to the lack of hard facts on membrane structure and function in higher plants. The isolation and characterisation of binding proteins/ "carriers" along the lines of the work done for bacteria (PARDEE 1968) would certainly lead to a better understanding of the biochemical nature of these structures.

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BOOK REVIEW

BEWLEY, J. D., BLACK, M. (ed.): PHYSIOLOGY AND BIOCHEMISTRY OF SEEDS IN RELATION TO GERMINATION. II. VIABILITY, DORMANCY, AND ENVIRONMENTAL CONTROL. — Springer Verlag, Berlin—Heidelberg—New York 1982. 375 pp., DM 128.—; approx. US $ 51.20.

Physiology and biochemistry of germinating seeds have been studied by numerous authors, but information on this topic was dispersed in various periodicals. J. D. Bewley and M. Black have prepared a concise and well written treatise on this problem called "Physiology and biochemistry of seeds in relation to germination", in two volumes. In the first one development, germination and growth of seeds are discussed from the point of view of their structure, food reserves, maturation, imbibition *etc.* It was published in 1978. The second volume issued in 1982 and its main topics are viability, dormancy and environmental control. The whole matter was divided into six chapters; there is also a glossary and indexes of English and botanical names, the author index and subject index.

In the first chapter, called "Viability and Longevity" we find a critical review of various data on the life span of seeds, on their longevity, giving a special interest to storage conditions (temperature, humidity, oxygen pressure *etc.*), which are able to change the seed classification (microbiotic, macrobiotic seeds). The authors give basic equations for defining viability and differences between some biochemical characters, distinguishing viable from non-viable seeds.

In the following chapter dormancy is defined and its biological importance discussed in context with the ecology and distribution of the species. It is shown that dormancy can be fairly polymorph not only in different individuals of the same species, but also in the same mother plant.

The third chapter is dedicated to the release from dormancy. The influence of light, phytochrome, temperature and afterripening on the interruption of dormancy, and inhibition of dormancy by substances from the group of growth regulators, respiratory inhibitors, oxidants *etc.* are described. Special interest is given to the phytochrome system and to the status and transformation of phytochrome in seeds.

In the chapter "The control of dormancy" the mechanism of dormancy and of the release from dormancy is discussed.

The quality of membrane and a higher turnover in membrane proteins in dormant seeds than in non-dormant seeds are important. The authors are critical to the supposed role of the pentosephosphate cycle in the release from dormancy,

Present state of investigation of dormancy and problems to be solved in future are discussed in the following chapter.

The volume ends with evaluating environmental control of germination, *e.g.* by temperature, light, oxygen, CO_2, water relations *etc.* Of practical value is the discussion of osmotic pretreatment and the priming of seeds. It is shown how viability of seeds can be enhanced by imbibition of various anorganic salts, sugars or polyethylene glycol.

The book brings a wealth of facts concerning physiology and biochemistry of germinating seeds and shows lines of investigation for the future.

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