

A Kinetic Study of Phosphate Absorption by Rice Roots

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벼에 의한 磷酸吸收의 機作에 관한 研究

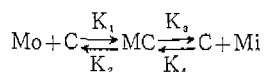
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ABSTRACT

Phosphate absorption from a $\text{Na}_2\text{H}^{21}\text{PO}_4$ solution by *Oryza sativa* L. was studied in order to elucidate kinetic mechanisms of ion transport. The rates of phosphate absorption from different concentrations indicated the presence of dual mechanisms in root tips, one in the low (1×10^{-6} to $8 \times 10^{-6}\text{M}$) and the other in the high (1×10^{-4} to $8 \times 10^{-3}\text{M}$). A phosphate compensation point of phosphate transport was revealed with a $1 \times 10^{-6}\text{M}$ solution of $\text{Na}_2\text{H}^{21}\text{PO}_4$.

The kinetic model that ion transport involves an exchange reaction of absorption and desorption is proposed as follows:



where C represents an ionic-specific organic carrier in the membrane; M, Mo and Mi are the mineral ions, M-outside and M-inside; MC is a carrier-ion complex; and the K's represent rate constants. In this model, the Mi velocity, v , is given by:

$$v = \frac{d\text{Mi}}{dt} = \frac{(\text{K}_1\text{K}_3\text{Mo} - \text{K}_2\text{K}_4\text{Mi}) \text{Ct}}{(\text{K}_2 + \text{K}_3) + \text{K}_1\text{Mo} + \text{K}_4\text{Mi}}$$

where Ct is equal to C+MC, and t is time.

INTRODUCTION

The absorption of nutrient mineral ions is metabolically energy-dependent, and is presumed to be mediated by carriers (Epstein and Hagen, 1952; Epstein and Rains, 1965). It has been further shown that the pattern of ion absorption is a dual one (Epstein 1966).

Data determining the dual absorption of phosphate by barley, corn and wheat roots have been reported by Leggett et al. (1965), Carter and Lathwell (1967), and Edwards (1970), respectively.

This dual mechanism has been interpreted to imply the parallel operation of multiple and single phases across the plasmalemma (Epstein et al. 1963; Epstein, 1972). However, concentration dependence data for phosphate absorption in plants were reanalyzed (Nissen, 1973) and shown to conform to the concept of multiphasic absorption mechanisms (Nissen, 1971). Laties (1962) has already presented the series model that both mechanisms have variously been placed in series at the plasmalemma and the tonoplast.

Kinetic studies have led to the postulation of a number of rather precise relationships with

respect to the mechanism of the phosphate absorption by excised rice roots. This paper is a report of kinetic experiments which were performed in order to interpret the dual absorption data obtained.

MATERIALS AND METHODS

Preparation of Seedlings: Rice seeds (*Oryza sativa* L, *Tongil*) provided by Crop Experimental Station, Office of Rural Development, were treated for 10 min. in a solution of 1% sodiumhypochlorite. They were then washed several times in distilled water and kept in darkness at $26 \pm 1^\circ\text{C}$ for 24 hrs. After this treatment, seeds were placed on two layers of polyethylene net and cotton gauze in 2-liter plastic box, and allowed to germinate at $22 \pm 1^\circ\text{C}$ in aerated 0.1mM CaCl_2 solution in the dark. The CaCl_2 solution was renewed every 24 hr. after germination began. This procedure has been detailed by Epstein(1961).

Experimental Procedures: Seven days later root tips of about 40mm in length were excised and, after blotting, weighed into lots of 0.5 gram for each treatment. These roots were then placed into 250ml conical flasks containing 0.1 mM CaCl_2 and aeration tubes. The flasks were placed in a temperature controlled water bath and allowed to come to equilibrium for 30 minutes. The CaCl_2 solution was removed by suction and ^{32}P labelled Na_2HPO_4 solution added. The concentrations of phosphate solutions used were $1 \times 10^{-6} \sim 8 \times 10^{-3}\text{M}$ $\text{Na}_2\text{H}^{32}\text{PO}_4$. These solutions had previously been adjusted to pH 5.0, and contained 0.1mM CaCl_2 . The period of absorption was 3 hrs. at $22 \pm 1^\circ\text{C}$ except for the time course experiment. Each set of experiments was replicated 3 times and data presented are the average of 3 replicates.

At the end of the experimental time period, the active phosphate solution was removed by suction and the roots washed six times in distilled water, transferred to a metal planchet, dried under an infra-red lamp, and counted with

an end window Geiger tube. Time curves were run in conjunction with all experiments to ensure that steady state absorption was obtained over the experimental period of 3 hr.

RESULTS

Root samples were immersed in $5 \times 10^{-4}\text{M}$ $\text{Na}_2\text{H}^{32}\text{PO}_4$ for various time periods. The time-course for absorption of phosphate ions is shown in Fig.1. The time curve indicates that steady state conditions were attained in this experiment after a 3 hr. time period.

This result suggests that 3hrs. is sufficient time for the uptake of the labelled phosphate.

Fig.2 shows the relationship between the rate of phosphate absorption into the root cells of *Oryza sativa* L. and its concentration in the external solution. The isotherm for absorption follows a dual pattern. When the external concentration is varied from $1 \times 10^{-6}\text{M}$ to $8 \times 10^{-3}\text{M}$, the rate of $\text{Na}_2\text{H}^{32}\text{PO}_4$ absorption is found to be a function of external concentration according to Michaelis-Menten equation.

A Lineweaver-Burk plot for the low concentration range (Figs.2 and 3) shows that the Michaelis constants about $K_m = 9.5 \times 10^{-7}\text{M}$ and the maximum velocity is $4.52 \mu\text{M/g}$ dry weight/hr.

In Fig.2, the experimental points for the high concentrations are connected by a smooth line. However, a closer examination of rates of

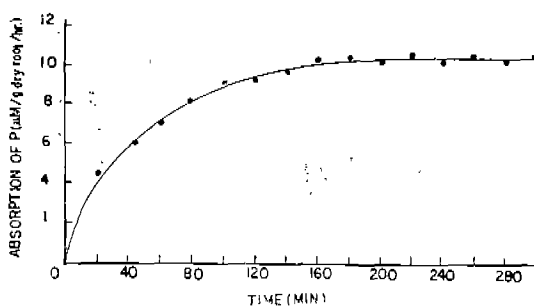


Fig. 1. Time course of $5 \times 10^{-4}\text{M}$ $\text{Na}_2\text{H}^{32}\text{PO}_4$ absorption by excised roots of *Oryza sativa* L.

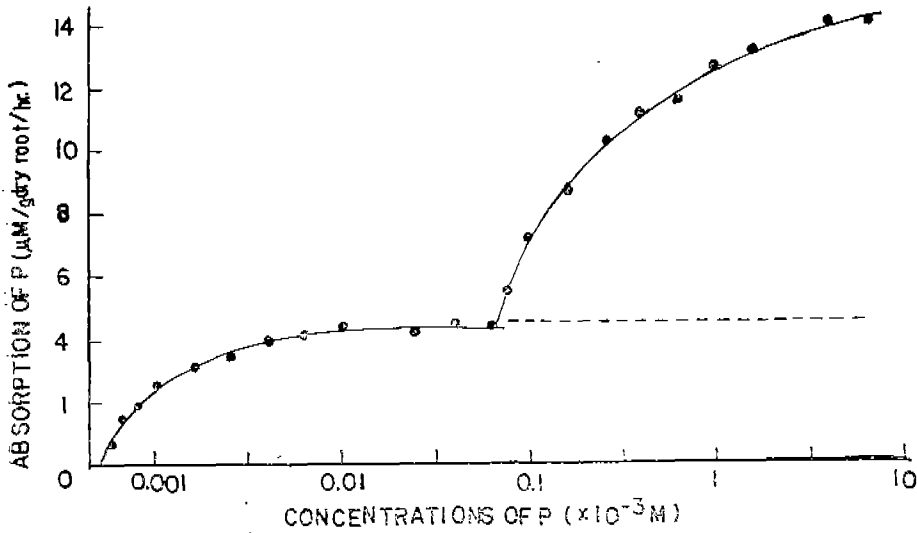


Fig. 2. Rate of phosphate absorption by excised roots of *Oryza sativa L.* as a function of the concentration of external $\text{Na}_2\text{H}^{32}\text{PO}_4$ solution. The solid line at the low concentrations, continued line, is a plot of the Michaelis-Menten equation.

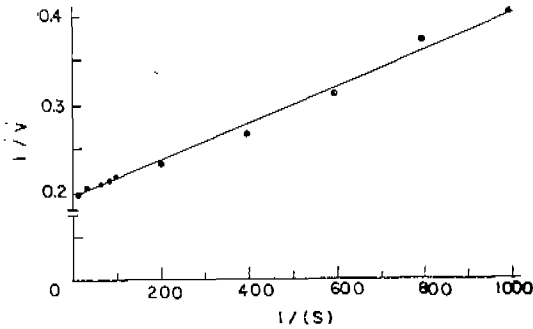


Fig. 3. Double reciprocal plot for the low concentration $\text{Na}_2\text{H}^{32}\text{PO}_4$ solutions.

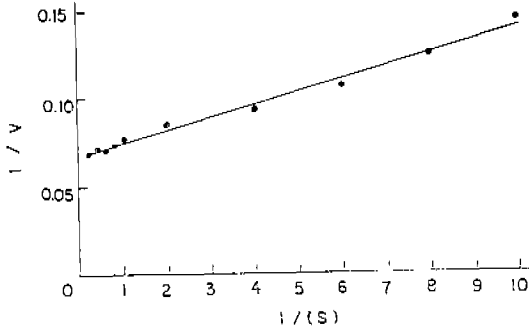


Fig. 4. Double reciprocal plot for the high concentration $\text{Na}_2\text{H}^{32}\text{PO}_4$ solutions.

$\text{Na}_2\text{H}^{32}\text{PO}_4$ absorption over the range of high concentrations revealed a more complex pattern (Fig. 5).

When the range of the lowest external concentration of $0 \sim 10^{-3}\text{M}$ is examined by difference between the external concentrations of phosphate before and after absorption by the excised rice roots, the isotherm for absorption is shown in Fig. 6. In this case, amount of phosphate was

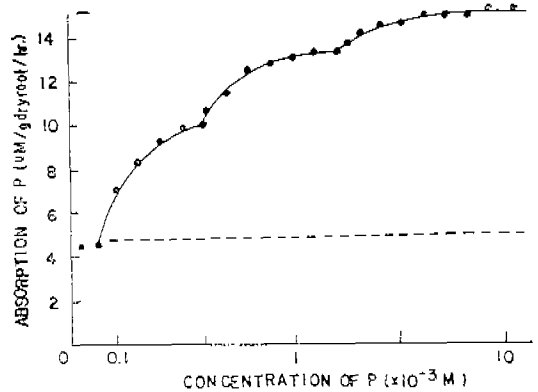


Fig. 5. Rate of absorption of phosphate labelled with ^{32}P by excised rice roots for the high concentration range. The dashed line represents the maximal rate of phosphate absorption at the low external concentration.

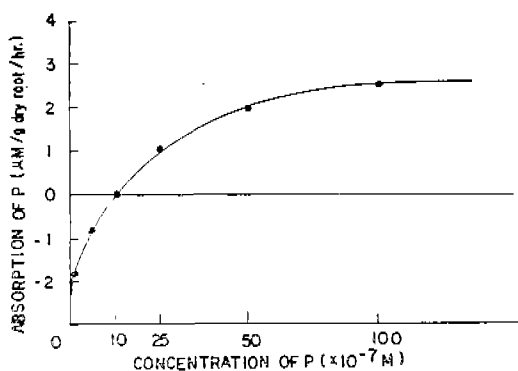


Fig. 6. Rate of absorption of unlabelled phosphate (Na_2HPO_4) by excised rice roots for the lowest concentration range. The zero point of difference between absorption and desorption represents a phosphate compensation point.

determined colorimetrically by using ammonium molybdate and stannous chloride.

As the concentration of phosphate increases, a point is reached at which, as shown in Fig. 6, phosphate absorption and desorption exactly

balance at about $1 \times 10^{-6} \text{M}$ each other. This point of zero net phosphate exchange can be called the phosphate compensation point. However, another experiment was conducted to gain information about this exchange reaction of phosphate absorption and desorption.

For this experiment, roots were immersed in a solution of $\text{Na}_2\text{H}^{32}\text{PO}_4$ labelled with ^{32}P for 3 hrs. in darkness at $22 \pm 1^\circ\text{C}$, thoroughly rinsed in 0.1mM HCl and distilled water and placed into unlabelled Na_2HPO_4 solution for the same length of time. At the end of the experimental time period, the root samples were removed and labelled phosphate (^{32}P), which was contained in this external solution, was counted with an end window Geiger tube. The result of this experiment is shown in Fig. 7.

The data in Fig. 7 indicate that according to an exchange reaction, phosphate absorption is selective and that it is dependent upon metabolism.

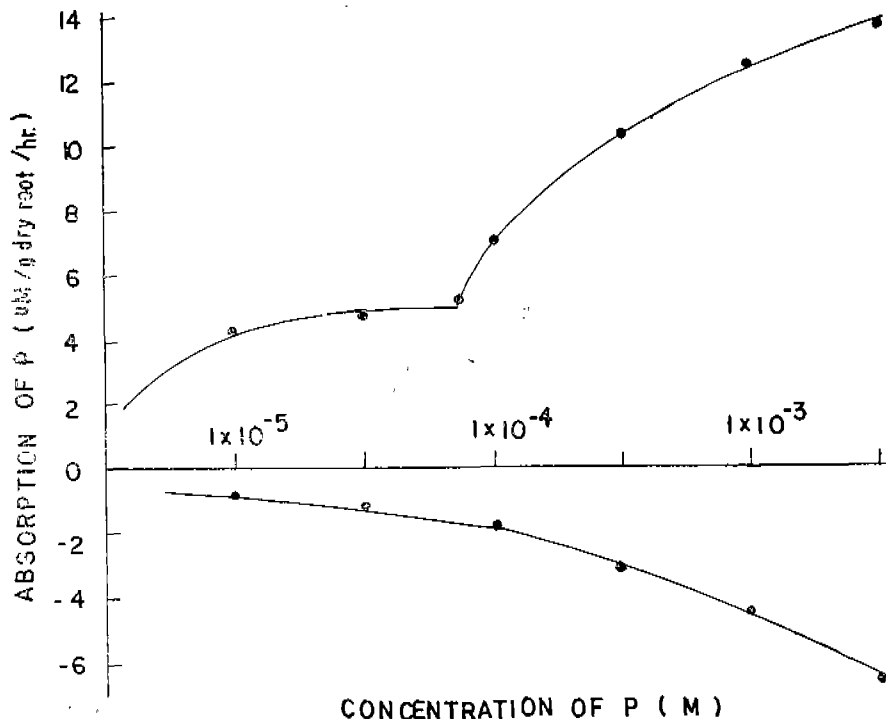


Fig. 7. Rates of phosphate absorption and desorption as a function of the concentrations of the external phosphate solution.

DISCUSSION

The concepts of enzyme kinetics were applied in absorption studies of nutrient mineral ions (Epstein and Hagen, 1952). Rates of absorption as a function of concentration show enzyme kinetics according to the Michalis-Menten equation. The rate of enzymic catalysis can be shown to be dependent upon two factors (Epstein, 1972) One is a capacity factor, denoting the maximal rate of transport that can be achieved when all available carrier sites are loaded. The other is an intensive factor reflecting the fraction of the carrier actually occupied at a given concentration of the ion. As shown in Fig. 2, the kinetic data also indicate that the absorption of phosphate by excised rice roots is mediated by a dual pattern. Delineation of the dual pattern of metabolic uptake across the membrane was possible through the use of a technique (Epstein et al., 1962) where radioactivity from the "free space" was removed, thus metabolite exchange in the cell wall did not obscure that which is related to membrane transport.

The dual pattern revealed does not seem to be a pH phenomenon since addition of $\text{Na}_2\text{H}^{32}\text{PO}_4$ to the medium resulted in no significant pH change. Also, it is important to mention that Ca^{++} was present in the media at a concentration of 5 mM, since it has been shown to be a prerequisite for unimpaired inorganic ion transport (Lauchli and Epstein, 1970).

The most significant results of these experiments have been the interpretation that two apparent sites or mechanisms of phosphate absorption are involved (Carter and Lathwell, 1967).

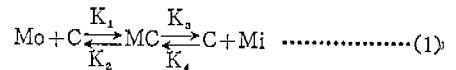
The dual mechanism data have been interpreted to imply separate sites for the absorption of the two phosphate ionic species, H_2PO_4^- and HPO_4^{--} (Hagen and Hopkins, 1955; Hagen et al., 1957). However, the experimental data presented were for the rates of absorption of the HPO_4^{--} phosphate ionic species. According to

Nissen (1974) a comprehensive reanalysis of concentration-dependence data for absorption of phosphate yielded regular and remarkably similar multiphasic patterns in a variety of plants and tissues.

For concentrations up to $8 \times 10^{-3}\text{M}$, four phases were thus evident in several experiments, but for concentrations up to 5×10^{-4} , two phases were discovered in several works. According to Epstein (1966), in Fig. 2, the experimental points for high concentrations are connected by a smooth line, but when this range of concentrations is examined more closely, the isotherm describing the relation between the external concentration of phosphate and its rate of absorption is a composite of several hyperbolic isotherms (Fig. 5). Therefore, the dual pattern as revealed by recent works may be resolved into multiphasic absorption. It seems likely that plant roots can absorb phosphate ions by means of membrane transport mechanisms that are similar to that affecting transport of other inorganic ions.

On the basis of the results shown in Fig. 6, it is suggested that rice plant roots can absorb phosphate when the concentration of phosphate in the external solution is greater than the concentration at the phosphate compensation point.

Furthermore, the results of Fig. 7 indicate that an exchange reaction between absorption and desorption appears highly dependent on phosphate absorption as compared with desorption. Therefore, another kinetic model is proposed as follows:



Where C represents an ion-specific organic carrier in the membrane; Mo and Mi are the mineral ion, M-outside and M-inside; MC is an unstable carrier+ion complex; and the K's represent rate constants.

The kinetic equations consist of

$$\frac{d\text{Mo}}{dt} = \text{K}_2\text{MC} - \text{K}_1\text{MoC} \dots\dots\dots(2)$$

$$\frac{d\text{C}}{dt} = (\text{K}_2 + \text{K}_3)\text{MC} - (\text{K}_1\text{Mo} + \text{K}_4\text{Mi})\text{C} \quad (3)$$

$$\frac{dMC}{dt} = (K_1Mo + K_1Mi)C - (K_2 + K_3)MC \dots(4)$$

$$\frac{dMi}{dt} = K_1MC - K_4MiC \dots\dots\dots(5)$$

plus a conservation equation,

$$C + MC = Ct \dots\dots\dots(6)$$

If we limit our consideration to the cases in which Ct will be sufficiently small compared with Mo and Mi, we can apply the steady state assumption that will permit us to solve for C and MC setting $dMC/dt = -dC/dt = 0$

$$\frac{dMC}{dt} = (K_1Mo + K_1Mi)C - (K_2 + K_3)MC = 0(7)$$

The algebraic results are

$$MC = \frac{(K_1Mo + K_1Mi)Ct}{K_1Mo + K_1Mi + K_2 + K_3} \dots\dots\dots(8)$$

$$C = \frac{(K_2 + K_3)Ct}{K_1Mo + K_1Mi + K_2 + K_3} \dots\dots\dots(9)$$

These values of C and MC may be substituted into the equation (5) to give

$$V = \frac{dMi}{dt} = \frac{(K_1K_3Mo - K_2K_4Mi)Ct}{(K_2 + K_3) + K_1Mo + K_1Mi} \dots\dots\dots(10)$$

Note that this equation is symmetrical in Mo and Mi, which demonstrates that both the forward and the reverse reactions are of the same type, even though the kinetic constants might differ. We shall demonstrate this statement by first setting $Mi=0$ and observing the results.

$$V = \frac{K_3CtMo}{(K_2 + K_3)/K_1 + Mo} \dots\dots\dots(11)$$

which is of the usual form, where $v = VaMo / (Ka + Mo)$. Thus $Va = K_3Ct$ and $Ka = (K_2 + K_3)/K_1$ are defined using the subscript V_1 to define a Ka for the forward reaction and the appropriate letter (a) to identify the Michaelis constant for Mo. By setting $Mo=0$ in the equation(10), we obtain

$$V = - \frac{K_4CtMi}{(K_2 + K_3)/K_4 + Mi} \dots\dots\dots(12)$$

which is the equation for the initial velocity in the reverse reaction, where $v = -VdMi / (kd + Mi)$. We may define $Vd = K_4Ct$ and $Kd = (K_2 + K_3)/K_4$ in a manner analogous to our definitions of Va and Ka .

Many experiments on the relation between the concentration of an ion, and the rates of

its absorption and of its desorption have yielded results which conform to equations (11) and (12).

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