Stimulation of Cadmium Uptake in Relation to the Cadmium Content of Plants

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ABSTRACT

The time course of cadmium uptake by the roots of intact tomato plants (Lycopersicon esculentum Mill.) was measured in a nutrient solution with a micromolar cadmium concentration until all cadmium in the medium was exhausted. Extraction taking a few hours, cadmium was repeatedly added to the nutrient solution. The initial rate of cadmium uptake was computed for each cadmium addition. This rate sharply increased and ultimately leveled off, the maximum value being about three times higher than the value measured after the first cadmium addition. The stimulating effect of cadmium was associated with an inhibitory effect at higher levels of cadmium concentrations. An increase in the net cadmium influx with time could not be explained by the binding of heavy metal to a fixed number of organic compounds. Conceivably, the production of binding sites could be increased and cadmium might play a part in controlling the rate of sites production.

Nonessential elements are generally present in plant tissues at very low concentrations. Up to now, no investigation seems to have been done concerning the influence of increasing the quantity of nonessential elements in plant tissues on the influx of these elements into cells. This may become of practical interest in view of the increasing level of some heavy metals, such as Cd, in the environment (3).

Cd has been observed to be largely bound nonmetabolically to organic compounds of cell walls (1). This process, including both exchange adsorption and irreversible sequestration in combination with diffusion, could largely account for the uptake of cadmium by excised root tissues (1). Cd is also known to be either an inhibitor or an activator of enzymes such as nitrate reductase or the ion-stimulated ATPase (6, 7, 17), both of which are assumed to be closely associated with ion uptake (4, 8).

The purpose of this work was to investigate the effect of Cd absorption by roots of intact plants on its subsequent rate of uptake with reference to this inhibitor-activator role.

MATERIALS AND METHODS

Tomato seeds, Lycopersicon esculentum Mill., cv. Moneymaker, were germinated for 5 days in darkness at 28 C on wet filter paper. The seedlings were then grown in a climate-controlled growth chamber on a nutrient solution which was renewed every week. The composition of the nutrient solution, prepared with deionized water and chemically pure reagents, was in mm: 6 KNO3, 5 Ca(NO3)2·4H2O, 2 MgSO4·7H2O, 1 KH2PO4, 9.1 × 10⁻³ MnSO4·H2O, 7.6 × 10⁻⁴ ZnSO4·7H2O, 3.1 × 10⁻⁴ CuSO4·5H2O, 7.0 × 10⁻⁴ MoO3·H2O, 4.6 × 10⁻² H3BO3, 8.2 × 10⁻² NaFe (EDTA). At the time of the experiment, the plants were 7 weeks old, had flower buds, and the initial growth rate had decreased considerably.

Cd uptake was measured by its depletion in the solution through the use of an apparatus especially built for kinetic studies on intact plants (18). Three plants were fixed on a perforated black Lucite disc separating the two compartments of the apparatus. The first compartment contained the aerial part of the plants and temperature, humidity, and light intensity were kept at 24 C ± 1, 50% ± 10, and 13,000 lux ± 200, respectively. The roots were in the bottom compartment which was equipped with automatic regulation and continuous monitoring apparatus. The nutrient solution, devoid of micronutrients, was kept at a constant temperature (23 C ± 0.5), pH (maintained at 5 ± 0.5 by the addition of HNO3), and volume (maintained at 4.25 liters by water addition). Water was injected into the root compartment from a graduated container from which the consumption was read. The top layer of the nutrient solution activated an electrical contact starting a peristaltic water pump. The solution was homogenized by an electromagnetic 50-Hz vibrator. Aeration of the medium was controlled, and the whole set-up made quick renewal of the solution possible.

The ¹¹⁵Cd (Radiochemical Centre of Amersham, U.K., code CAS 1) was used as Cd tracer and was measured by continuous β counting. Whenever supplementary stable Cd had to be added it was in the form of Cd(NO3)2. To reduce Cd adsorption to inert material all surfaces of the experimental set-up (except the pH electrode and the mylar membrane in front of the detector) were made of PTFE.⁴ No long term adsorption was detected at pH 5 with 0.1 μM Cd.

The plants used in the experiments were acclimatized during 24 hr to the conditions ruling within the apparatus; only then were Cd injections in the root compartment started. The sequence of Cd treatments is specified below. After each absorption period,
the old solution was completely removed and the roots were washed for 5 min with a Cd-free solution before the new solution was applied.

RESULTS AND DISCUSSION

In general, after adsorption in the apparent free space, the depletion curve is a linear function for a short period of time (2). The correlation coefficient computed from a least square linear regression during the first 3 hr was higher than 0.98 with \( P < 0.001 \). The rate of absorption at the initial concentration could be satisfactorily estimated from the slope of the straight line. Cutler and Rains (1), who used excised root tissue of barley, found an intermediary nonlinear phase for Cd uptake which lasted about 30 min, where nonmetabolic sequestering predominated. Such a nonlinear phase was not observed in the present measurements.

Plants absorbed all of the Cd in the nutrient solution at initial Cd concentrations ranging from 0.1 to 2.8 \( \mu \text{M} \). These same plants required much less time to exhaust Cd in the medium during subsequent runs of Cd absorption.

In Figure 1, at an initial Cd concentration of 2.8 \( \mu \text{M} \), the first reduction of 97% of the available Cd took about 24 hr. During a second run, 42 hr later, the same plants absorbed a similar percentage in only 8 hr. At a lower initial concentration of 0.1 \( \mu \text{M} \) Cd, Cd exhaustion took much less time, which permitted many more runs during a similar period of time. The stimulation of the absorption is obvious from Figure 2 where the Cd influx of two replicates is expressed by the initial rate of uptake. From run to run this increase followed a pattern similar to an S-curve as the increase in Cd influx leveled off after the sixth run. The existence of maximum Cd influx was clearly shown in experiments with a 1 \( \mu \text{M} \) initial Cd concentration, where the maximum was reached after only two complete runs (Fig. 3a).

Such an increase of uptake occurring within a few hr cannot be explained by concurrent growth and enlargement of the root surface. This is indirectly confirmed by the difference between the Cd uptake and the rate of water uptake as the latter did not change during the experiment (Fig. 4). Cd might be the cause of increasing the permeability of the root membranes, as root damage of Cd-treated plants has been reported (5, 15). Notwithstanding these reports, we did not observe any toxicity symptoms below 3 \( \mu \text{M} \) (10). The variation in uptake efficiency observed at 0.1 \( \mu \text{M} \) is consequently unlikely to be due to a toxic effect.

The total amount of Cd in plants might be the cause of the above variation. The initial rate of uptake for the successive runs (Fig 3a) is related to the total Cd content (Fig. 3b). This suggests that the rate of uptake depends on the amount of Cd accumulated previously until the maximum uptake level is reached. This was checked by using for each successive supply to the same plants a different Cd concentration. At the start, Cd was absorbed from a solution with a high initial concentration (2.8 \( \mu \text{M} \)) and, after full absorption thereof, subsequently from solutions with a lower Cd concentration (0.28 \( \mu \text{M} \)). Since Cd influx depends on the external Cd concentration (1, 10) the rate of Cd uptake was measured for all runs at the same external Cd concentration of 0.28 \( \mu \text{M} \).

During the first Cd uptake, the plants reduced the concentration from 2.8 \( \mu \text{M} \) to 0.28 \( \mu \text{M} \), and the total amount absorbed was 10.71 pmol. The amount of 10.71 pmol was considered to produce the
maximum rate of Cd uptake, as an absorption of 8.5 µmol was found to have produced the latter maximum in the experiment of Figure 3b. After this initial supply of 10.71 µmol (Fig. 5b), the maximum rate of uptake was not yet reached as an additional amount of only 1.19 µmol still stimulated the rate of uptake. After a total uptake of 11.9 µmol a final maximum was achieved, which was maintained for more than 30 hr (Fig. 5a). It seems that the Cd content of the root system did not by itself directly induce the stimulation of the rate of uptake. Obviously some additional factors had to be taken into account in the interval between the first and second measurements in the experiment of Figure 5. This interval included uptake from a nutrient solution in which finally Cd was reduced down to a trace level (Fig. 6). Consequently, the influence of a Cd-free intermediate phase on the increase in the influx was investigated.

In the absence of a Cd-free phase, no variation in the influx ought to be observed, whatever the initial Cd uptake might have been. Cd was supplied for short periods, each time only interrupted by the renewal of the solution. By doing so, the Cd removal from the solution was reduced and the Cd-free period, which occurred in previous experiments, did not take place. The results of two experiments, one at 0.1 µM and the other at 1 µM initial Cd concentrations, are shown in Figure 7. The time of each depletion run was limited to 5 hr for the former and 2 hr for the latter, with total experimental periods of 25 and 17 hr, respectively. The rate of uptake remained constant for both Cd concentrations. The absence of a Cd-free period apparently eliminated the increase in the uptake rate.

This was confirmed by the reappearance of the stimulation after a period of contact with a Cd-free solution. Two periods of successive short Cd supplies were alternated with a period of Cd-free supply. Two different values of influx are relevant for two periods of Cd supply, whenever the latter are separated by a period with a Cd-free solution (Fig. 8).

The increase in Cd uptake, which approached a maximum rate level after the accumulation of a certain amount of Cd, has been demonstrated. This increase in the Cd influx occurred only under the above experimental conditions, following a Cd-free period.

One of the mechanisms involved in the uptake process of heavy metals includes a large nonmetabolic binding to cell constituents.

![Fig. 4](image-url)  
**Fig. 4.** Time course of water uptake by plants during two successive Cd depletion runs from nutrient solution containing initially 2.8 µM Cd (same experiment as in Fig. 1).

![Fig. 5](image-url)  
**Fig. 5.** a: Time course of rate of Cd uptake based on successive runs of complete Cd depletion. Initial Cd concentrations of the first run and the next ones were 2.8 µM and 0.28 µM, respectively. For each run, the rate of Cd uptake was measured at 0.28 µM Cd. b: Rate of Cd uptake in relation to total amount of Cd previously absorbed by plants (experimental conditions as in Fig. 5a).

![Fig. 6](image-url)  
**Fig. 6.** Time course of several Cd depletion runs from nutrient solutions containing initially 2.8 µM Cd (first run) and 0.28 µM Cd (next runs).

![Fig. 7](image-url)  
**Fig. 7.** Time course of initial rate of Cd uptake based on successive runs of partial Cd depletion from nutrient solutions containing initially 0.28 µM Cd (○) and 0.028 µM Cd (△), respectively.
Plant involvement transcription. endodermis, supply concentrations. The occurrence in vivo of Cd in Cd-depletion has increased their amount of cystein as opposed to control residues to 82 and 175%, respectively. Such production of cystein residues, to which Cd is probably bound, is in agreement with our observations.

Fig. 8. Time course of initial rate of Cd uptake based on successive runs of partial Cd-depletion from nutrient solution containing 0.1 μM Cd. Each period of Cd supply (—) is separated by a period with a Cd-free nutrient solution (-----).

(9, 16). This binding is thought to progress at a lower rate than the adsorption of most other ions into the free space (1, 18). In itself, a binding to a fixed number of organic compounds cannot explain why the Cd influx remained constant during successive Cd supply periods but reached a higher level after a Cd-free phase. A saturation of the available sites would occur and result in a decrease in the rate of Cd uptake.

One explanation could well be the formation of new binding sites, even in older plants such as used in these experiments, with a rate of production partly under Cd control. According to this hypothesis, Cd would be acting in two manners: first by stimulating at low concentrations, and then by inhibiting at higher concentrations. Cd inhibition could be suppressed during the Cd-free phase, for instance, by exchange with the cations inside the tissue.

The occurrence of Cd uptake, as distinguished from adsorption only, is well established (1). A short period (30 min, when measured in vivo with semiconductor detectors) is enough for [(114m)Cd] to penetrate into roots, to enter the xylem after crossing the endodermis, and to move up to the first internode of 5-week-old tomato plants (11).

The hypothesis of production of additional binding sites, subsequent to Cd exposure, has some support in the literature. Cd supply specifically induces a de novo synthesis of a Cd-binding protein in animal tissue (12). In this process, the formation of a specific mRNA (14) is initiated through a cellular mechanism which involves transcription. The main characteristic of this protein is its unusual content of cysteinic residues (30%). The presence of —SH groups explains the strong affinity for heavy metals, particularly Cd (17).

Leaves of wheat seedlings (13) and roots of tomato plants (10) having been grown in a Cd medium had increased their amount of cystein as opposed to control residues to 82 and 175%, respectively. Such production of cystein residues, to which Cd is probably bound, is in agreement with our observations.

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