Kinetics of Aluminum Uptake in *Triticum aestivum* L.

Identity of the Linear Phase of Aluminum Uptake by Excised Roots of Aluminum-Tolerant and Aluminum-Sensitive Cultivars[1]

Guichang Zhang* and Gregory J. Taylor

Department of Botany, University of Alberta, Edmonton, Alberta, T6G 2E9 Canada

ABSTRACT

The identity of a linear phase of aluminum (Al) uptake in *Triticum aestivum* was investigated by analysis of the kinetics of Al uptake by excised roots and purified cell wall fractions. Classical interpretation of kinetic data suggests that a linear phase of uptake with time reflects uptake across the plasma membrane; however, in studies with Al the possibility that the linear phase of uptake includes accumulation of Al in both the symplasm and the apoplasm has not been discounted. In our experiments, we observed a linear phase of Al uptake at both ambient and low temperatures, although the rate of uptake at 0°C was 53 to 72% less than at 23°C, depending on cultivars. This nonsaturable phase of uptake at low temperature suggests that a portion of the linear phase of Al uptake is nonmetabolic. Furthermore, analysis of Al in cell wall fractions isolated from excised roots pretreated with Al suggests that the linear phase of uptake includes a cell wall component. When excised roots were pretreated with Al, accumulation of Al in purified cell wall material included a linear phase that could not be desorbed with a 30 minute wash in citrate. The rates of linear-phase accumulation of Al by cell wall material and cell contents were similar. In contrast, the linear phase of *in vitro* uptake of Al by purified cell wall material was completely desorbed by a 30 minute wash with citrate. These results suggest that the linear phase of Al uptake observed in excised roots of *T. aestivum* included metabolism-dependent binding of Al in apoplasm.

To understand the physiological and biochemical basis of Al toxicity and tolerance in plants, information on the movement of Al into apoplasm and symplasm compartments is required. Unfortunately, the lack of a suitable isotope for monitoring short-term movement of Al into plant tissues has hampered progress in this field. Despite this shortcoming, a number of authors have used kinetic analysis of Al uptake to estimate the rate of movement of Al across the plasma membrane (4, 16, 17, 21). In *Triticum aestivum*, studies on the kinetics of Al uptake by excised roots have demonstrated a biphasic pattern of Al uptake, with a rapid phase of uptake superimposed over a linear phase of uptake (16, 24). Although

direct experimental evidence is lacking, these two phases have been interpreted as representing passive accumulation in the apoplasm (rapid phase) and transport across the plasma membrane into the symplasm (linear phase) (11, 16).

Pettersson and Strid (16), and Zhang and Taylor (24) compared uptake of Al by roots of Al-tolerant and Al-sensitive cultivars of *T. aestivum* and failed to observe differences in uptake between cultivars. While these results could suggest that Al tolerance is not linked to initial uptake of Al (16), Zhang and Taylor (24) acknowledged that the precise identity of the linear phase is still in doubt. They reported that the apparent size of the apoplasmic compartment for Al was larger when estimated by extrapolation of the linear phase of uptake to time zero than when estimated by extrapolation of the linear phase of desorption to time zero (24). If the linear phase of uptake represents accumulation of Al in both symplasmic and apoplasmic compartments (not just accumulation of Al in the symplasmic compartment), then differences between Al-tolerant and Al-sensitive cultivars in the uptake of Al across the plasma membrane might be obscured by differences in accumulation of Al in the cell wall. This would be particularly true if exclusion of Al at the plasma membrane leads to increased polymerization or precipitation of Al in the cell wall.

This study was designed to determine if the linear phase of Al uptake by excised roots of Al-tolerant and Al-sensitive cultivars of *T. aestivum* includes accumulation of Al in the cell wall. Our results support a novel view of the identity of the linear phase of Al uptake.

MATERIALS AND METHODS

Preparation of Plant Material

Seeds of two Al-tolerant cultivars (Atlas-66 and PT-741) and two Al-sensitive cultivars (Neepawa and Scout-66) of *Triticum aestivum* L. (wheat) were surface sterilized in 1.2% sodium hypochlorite for 20 min, and germinated overnight in a solution of 0.005 g L^{-1} Vitavax to prevent fungal growth. Seedlings were grown for 7 d on nylon mesh suspended over 16 L of nutrient solution containing (mM) 1.0 Ca(NO$_3$)$_2$· 4H$_2$O, 0.3 Mg(NO$_3$)$_2$·6 H$_2$O, 0.3 NH$_4$NO$_3$, 0.1 K$_2$HPO$_4$, 0.1 K$_2$SO$_4$, 0.4 KNO$_3$; and (μM) 2 MnCl$_2$·4 H$_2$O, 6 H$_3$BO$_3$, 0.5 ZnSO$_4$·7 H$_2$O, 0.15 CuSO$_4$·5 H$_2$O, 0.1 Na$_2$MoO$_4$, 10 FeCl$_3$· 6 H$_2$O, and Na$_2$EDTA (pH 4.5) in a growth chamber with 16 h of light (20°C, 68% RH) and 8 h of darkness (16°C, 85%

RH). After 5 d of growth, plants were transferred to fresh nutrient solutions.

Uptake of Al by Excised Roots

Thirty root tips (2.0 cm) were excised and placed in each of 36 to 50 'absorption tubes' as described by Zhang and Taylor (24). During excision of roots, absorption tubes containing excised roots were placed in an aerated nutrient solution. When excision was complete (within 60 min), the tubes were transferred to an aerated solution of 1.0 mM $CaSO_4$ for 30 min. Uptake experiments were initiated by transferring the absorption tubes containing roots to 80 mL glass jars containing 50 mL of an aerated solution containing 75 μM Al as $AlK(SO_4)_2 \cdot 12\ H_2O$, and 1.0 mM $CaSO_4$ (pH 4.5) in a water bath at 23°C, or in an ice-water bath at 0°C. Four or five of replicate tubes were removed from absorption solutions after 0, 15, 30, 60, 120, and 180 min of uptake, rinsed briefly with 1 mM $CaSO_4$ and deionized water (300 mL per tube), and transferred to 0.5 mM citrate (pH 4.5) at 0°C for 30 min to remove Al from the apoplasm. After 30 min of desorption, roots were removed, rinsed with deionized water, and prepared for fractionation and/or determination of Al.

The composition of our absorption solutions were designed to eliminate potential effects of phosphate on Al solubility; nonetheless, Al will not simply be present as $Al^{3+} \cdot 6\ H_2O$. Speciation calculations using the modified GEOCHEM program and log K values of −5.02, −9.30, −14.99, and −23.33 for hydrolysis of Al (14) suggest that Al will be present primarily as the $AlSO_4^+$ ion pair (22 μM), as $Al^{3+} \cdot 6\ H_2O$ (18 μM), and as a number of less abundant monomeric species. Aluminum might also be present as a polynuclear species, since the ratio of $\{Al^{3+}\}/\{H^+\}^3$ in our solutions will be approximately $10^{8.76}$, marginally lower than the $10^{8.8}$ threshold which Kinraide and Parker (9) suggest is a suitable indicator for the appearance of polynuclear or precipitated hydroxy-Al. It is important to note, however, that these speciation calculations apply only to the bulk phase of absorption solutions. Because of the unique physical and chemical properties of the apoplasm, the actual species which are in direct contact with the cell wall and plasma membrane are not known.

Crude Separation of Pellet and Supernatant

After absorption and desorption treatments as described above, roots were blotted, weighed, cut into 1 mm long segments, and stored on ice. The root segments (about 0.15 g fresh weight) were homogenized with a Brinkmann Homogenizer (PT 10/35 with a PTA 10S generator) at maximum speed for 90 s in 1.5 mL 0.1 mM tris-HCl buffer (pH 7.8) in an ice-water bath, and centrifuged for 20 min at 18,000 rpm (25,300g) at 4°C. The pellet and supernatant were collected after two washings with buffer and deionized water.

Isolation and Desorption of Purified Cell Wall Material

Purified cell wall material was isolated using a technique adapted from Tu et al. (20). After absorption and desorption treatments, roots were blotted, weighed, cut into 1 mm long segments, and stored on ice. Root segments were homogenized for 10 s in 1.5 mL 0.1 M Hepes-Mes and 0.3 M sucrose buffer (pH 7.8) using a Brinkmann Homogenizer (PT 10/35 with a PTA 10S generator), and placed in a Parr Cell Disruption Bomb (4639) under nitrogen pressure (110 kg/cm²) for 10 min. After extrusion to atmospheric pressure, the homogenate was sonicated in an ice-water bath for 7 min at 60% output control on a 25 W ultrasonic homogenizer (Cole-Parmer 4710). The homogenate was then filtered through a 20 μm nylon mesh. Cell wall material trapped on the mesh was rinsed with 50 mL cold deionized water (4°C). Sixteen of the 32 cell wall samples were desorbed in 10 mL 0.5 mM citrate (pH 4.5, 0°C) for 30 min. After desorption, the cell wall material was again trapped on the nylon mesh and rinsed with 50 mL cold deionized water (4°C). Both the cell wall
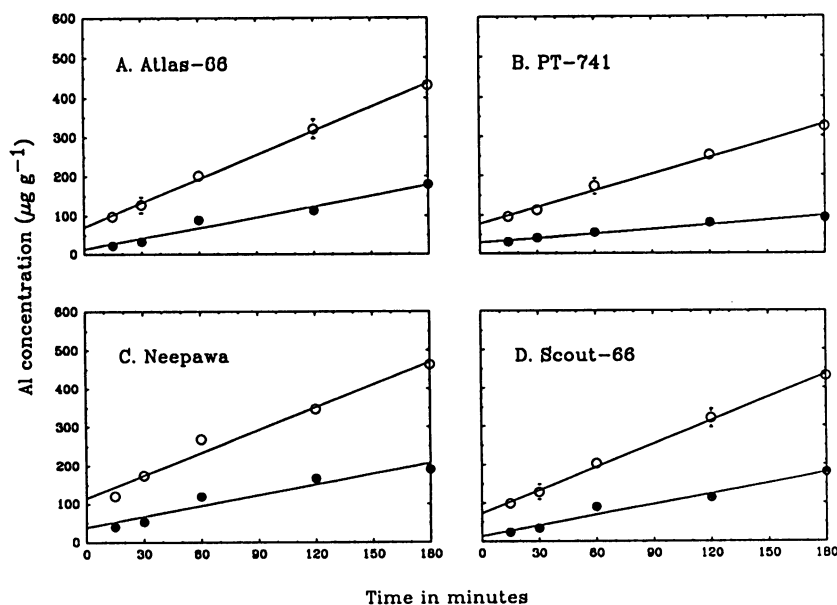

Figure 1. Uptake of Al (μg g root dry weight^{-1}) by excised roots of Al-tolerant cultivars Atlas-66 (A) and PT-741 (B), and Al-sensitive cultivars Neepawa (C) and Scout-66 (D) of T. aestivum. Excised root were treated with 75 μM Al and 1.0 mM Ca (pH 4.5) for 0, 15, 30, 60, 120, and 180 min at 0°C (●) or 23°C (○), followed by desorption in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Values represent means of five replicates.

Table I. Rate of Al Uptake and Al Concentration in the Pellet (18000 rpm) and Supernatant Fractions in Al-Tolerant and Al-Sensitive Cultivars of T. aestivum

Pellet and supernatant fractions were isolated from roots after an absorption period of 0, 15, 30, 60, 120, and 180 min in 75 μM Al and 1.0 mM Ca (pH 4.5, 23°C) followed by desorption treatment in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Concentrations were calculated from the 180 min absorption period. Values represent means of five replicates.

Cultivars	Rate of Uptake		Concentration	
	Supernatant	Pellet	Supernatant (%)	Pellet (%)
	μg g root fresh wt^{-1} min^{-1}		μg g root fresh wt^{-1}	
Atlas-66	0.06 ± 0.03	0.39 ± 0.06	1.5 ± 0.5 (15)	8.4 ± 1.2 (85)
PT-741	0.06 ± 0.01	0.39 ± 0.24	1.6 ± 0.2 (12)	11.6 ± 3.0 (88)
Neepawa	0.08 ± 0.02	0.58 ± 0.09	1.9 ± 0.1 (12)	13.9 ± 1.2 (88)
Scout-66	0.12 ± 0.02	1.01 ± 0.22	2.3 ± 0.3 (9)	23.2 ± 3.0 (91)

material and the original filtrate were collected for determination of Al.

Uptake of Al by Isolated Cell Wall Material

In several experiments, Al was also supplied to purified cell wall material isolated from Al-tolerant and Al-sensitive cultivars. In these experiments, cell wall material from excised roots with no prior exposure to Al was isolated as described above. During the fractionation procedure, the cell wall material was suspended in 15 mL centrifuge tubes containing 5 mL 1.0 mM CaSO₄ (pH 4.5) in an ice-water bath. Before the absorption treatment, the cell wall material was brought to the absorption temperature (23°C). The absorption period was initiated by adding 5 mL of a solution containing 1.0 mM CaSO₄ and 150 μM Al (pH 4.5), which brought the final concentration of Al to 75 μM. After 0, 30, 60, 120, and 180 min absorption, the cell wall material from eight replicate tubes was trapped on nylon mesh and washed with 50 mL cold deionized water (4°C). Four of the eight tubes were desorbed in an aerated 0.5 mM citrate (pH 4.5, 0°C) for 30

min as described above. Purified cell wall material both with and without desorption treatment were prepared for determination of Al.

Test of Cell Wall Purity

Microscopic examination with neutral red and Evan's blue showed complete cell breakage. The isolated cell wall material was free of cytosolic contamination, whereas cell contents showed some contamination with cell wall fragments. Total ATPase activity and Cyt c oxidase activity were used as cytosolic markers to test the purity of the isolated cell wall material. Total ATPase activity was determined by measuring liberation of inorganic phosphorous from ATP (2). Cyt c oxidase was determined spectrophotometrically by measuring the rate of oxidation of reduced Cyt c at A_{550} (10). These tests demonstrated that the purified cell wall material obtained was virtually free of cytosolic contamination. Only 0.6% of total ATPase activity and no detectable Cyt c oxidase activity were observed in the cell wall preparations.
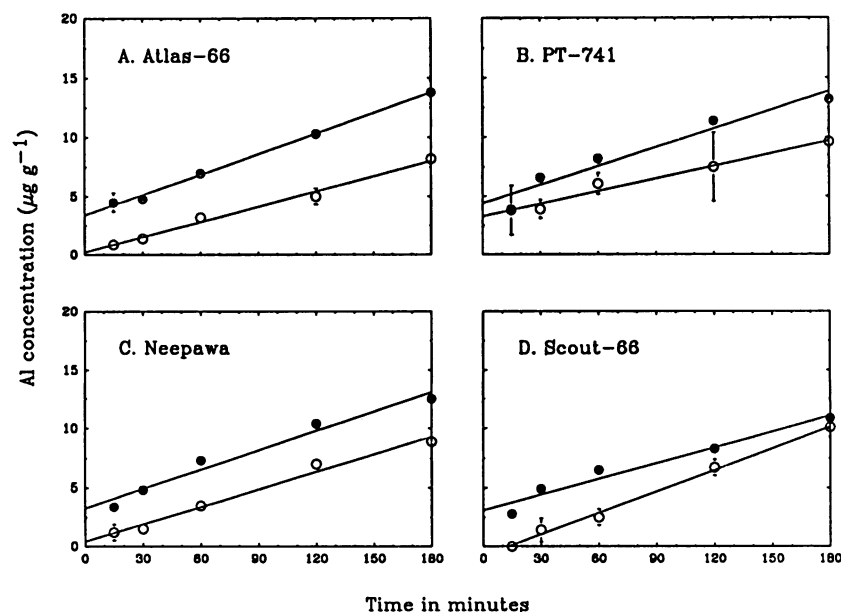
Figure 2. Uptake of Al (μg g root fresh weight^{-1}) into purified cell wall material (O) and the remaining filtrate (●) of Al-tolerant cultivars Atlas-66 (A) and PT-741 (B), and Al-sensitive cultivars Neepawa (C) and Scout-66 (D) of T. aestivum. Cell wall material was isolated from excised roots pretreated with 75 μM Al and 1.0 mM Ca (pH 4.5, 23°C) followed by desorption in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Values represent means of five replicates.

Time in minutes

Table II. *Rate of Al Uptake and Al Concentration of the Cell Wall Fraction and Remaining Filtrate from Al-Tolerant and Al-Sensitive Cultivars of T. aestivum*

Purified cell wall material was isolated from roots after an absorption period of 0, 15, 30, 60, 120, and 180 min in 75 μM Al and 1.0 mM Ca (pH 4.5, 23°C) followed by desorption treatment with 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Concentrations were calculated from the 180 min absorption period. Values represent means of five replicates.

Cultivars	Rate of Uptake		Concentration	
	Cell Wall	Filtrate	Cell Wall (%)	Filtrate (%)
	μg g root fresh wt^{-1} min^{-1}		μg g root fresh wt^{-1}	
Atlas-66	0.43 ± 0.04	0.58 ± 0.04	8.2 ± 0.6 (38)	13.8 ± 1.0 (62)
PT-741	0.36 ± 0.12	0.53 ± 0.04	9.6 ± 1.1 (42)	13.2 ± 0.5 (58)
Neepawa	0.49 ± 0.04	0.55 ± 0.03	8.9 ± 0.6 (41)	12.5 ± 0.6 (59)
Scout-66	0.61 ± 0.05	0.45 ± 0.03	10.1 ± 0.7 (48)	10.9 ± 0.5 (52)

Determination of Al

Roots and cell wall material were ashed at 500°C, dissolved with concentrated HNO_3 and oxidized with H_2O_2 as described by Zhang and Taylor (24). Filtrates were directly used for determination of Al without further processing. Aluminum concentrations in prepared samples were determined by graphite furnace atomic absorption spectrophotometry as described by Zhang and Taylor (24).

Analysis of Data

Statistical analyses of the data were performed using analysis of variance (ANOVA), simple regression, and descriptive statistics available on Statistical Graphics Corporation's statistical package, Statgraphics Version 2.6. Analyses of homogeneity of slopes were performed using ANOVA available in SAS release 5.18. Significance was defined at the 95% confidence level.

RESULTS

Uptake of Al by Al-tolerant and Al-sensitive cultivars at both 0 and 23°C showed a clear linear phase, with no sign of saturation within the experimental period (Fig. 1). Exposure to low temperature (0°C) reduced the rates of Al uptake equally in both Al-tolerant and Al-sensitive cultivars. The rate of Al uptake was reduced by 57% (from 2.10 ± 0.17 to 0.91 ± 0.07 μg g^{-1} min^{-1}) and 72% (from 1.40 ± 0.11 to 0.39 ± 0.06 μg g^{-1} min^{-1}) in the Al-tolerant cultivars Atlas-66 and PT-741, and 53% (from 1.97 ± 0.16 to 0.93 ± 0.11 μg g^{-1} min^{-1}) and 55% (from 2.03 ± 0.12 to 0.92 ± 0.11 μg g^{-1} min^{-1}) in the Al-sensitive cultivars Neepawa and Scout-66. Retention of the linear phase at 0°C and its nonremovable nature after desorption in citrate suggested that the linear phase of uptake includes a nonmetabolic component, and that this nonmetabolic component is not simply an exchange/adsorption phenomenon.

A crude fractionation technique was employed to determine if the linear phase of uptake could be completely accounted for by uptake of Al into the cytosol. Uptake of Al in the supernatant fraction isolated from roots pretreated with Al at 23°C clearly showed a linear component, but this component was small compared to the rate of uptake in the pellet. Uptake of Al in supernatant fractions accounted for
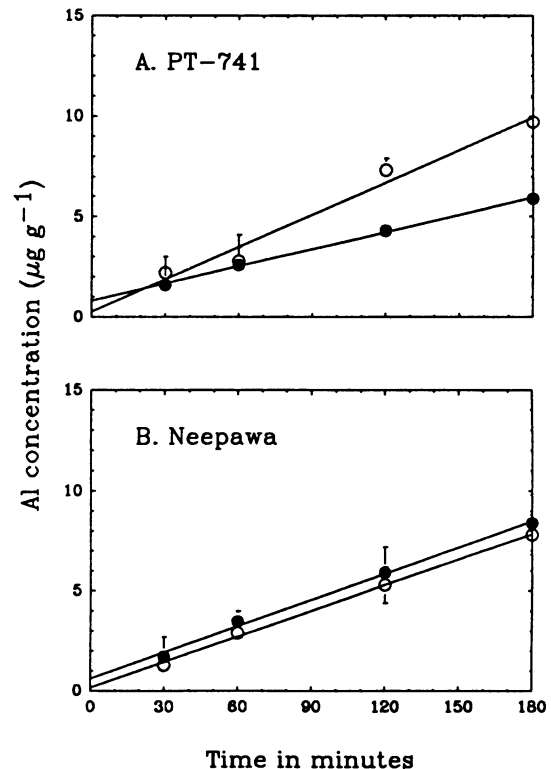
Figure 3. Uptake of Al (μg g root fresh weight^{-1}) into purified cell wall material isolated from an Al-tolerant cultivar PT-741 (A) and an Al-sensitive cultivar Neepawa (B) of *T. aestivum* with (●) or without (O) a second desorption treatment. Roots were pretreated with 75 μM Al and 1.0 mM Ca (pH 4.5, 23°C) followed by desorption in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Cell wall material was then isolated and half of the samples received a second desorption treatment with 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Values represent means of four replicates.

Table III. *Rate of Al Uptake and Al Concentration in Purified Cell Wall Material Isolated from Excised Roots of Al-Tolerant and Al-Sensitive Cultivars of T. aestivum*

Cell wall material was isolated from roots pretreated with Al for 0, 30, 60, 120, and 180 min in 75 μM Al and 1.0 mM Ca (pH 4.5, 23°C) followed by desorption treatment in 0.5 mM citrate (pH 4.5, 0°C) for 30 min, and desorbed (if indicated) with 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Concentrations were calculated from the 180 min absorption period. Values represent means of four replicates.

Cultivars	Treatments	Rate of Uptake	Concentration
		$\mu g\ g\ root\ fresh\ wt^{-1}\ min^{-1}$	$\mu g\ g\ root\ fresh\ wt^{-1}$
PT-741	Without desorption	0.53 ± 0.09	9.8 ± 1.2
	With desorption	0.28 ± 0.09	5.6 ± 1.4
Neepawa	Without desorption	0.42 ± 0.08	7.8 ± 1.0
	With desorption	0.43 ± 0.06	8.4 ± 1.4

only 9 to 15% of total uptake, with no observed differences between Al-tolerant and Al-sensitive cultivars (Table I). Clearly, uptake of Al into the soluble cytosol fraction is not sufficient to account for the linear phase of uptake. Accumulation of Al in the cell wall and/or organelles must also be postulated.

Analysis of Al from purified cell wall material isolated from excised roots pretreated with Al confirmed that the linear phase of Al uptake may include an apoplasmic component. In all cultivars, uptake of Al into purified cell wall material clearly showed a linear component (Fig. 2). Interestingly, the rate of uptake of Al into cell wall material (0.36 ± 0.12 to 0.61 ± 0.05 μg g^{-1} min^{-1}) and the remaining filtrate (0.45 ± 0.03 to 0.58 ± 0.04 μg Al g^{-1} min^{-1}) occurred at similar rates in the Al-tolerant and Al-sensitive cultivars (Table II). A linear phase of uptake in purified cell wall material would be observed if the cell wall contributed to this phase of uptake *in vitro*, or if redistribution of Al from the cytosol to the cell wall occurred during fractionation. If redistribution is important, then, a second desorption treatment of cell wall material after isolation should effectively remove loosely bound Al. In such an experiment, a second 30 min desorption with citrate following treatment of excised roots with Al, desorption with citrate, and isolation of cell wall material, did not eliminate the linear phase of uptake in the purified cell wall (Fig. 3). In the absence of this second desorption treatment, rates of Al uptake in cell wall fractions were 0.53 ± 0.09 and 0.42 ± 0.08 μg g^{-1} min^{-1} for the Al-tolerant cultivar PT-741 and the Al-sensitive cultivar Neepawa. With the second desorption treatment, rates of Al uptake in cell wall fraction were 0.28 ± 0.09 and 0.43 ± 0.06 μg g^{-1} min^{-1} for PT-741 and Neepawa respectively (Table III). While the rate of uptake in the Al-tolerant cultivar PT-741 appeared to decrease with the second desorption treatment, the difference was not statistically significant. These results indicate that the linear phase in cell wall fraction was nonremovable and, hence, we have rejected the possibility that the linear phase resulted from redistribution of Al during fractionation. These results challenge the traditional interpretation of the linear phase of Al uptake as transport across the plasma membrane, and suggest a more complex phase of uptake including Al uptake in both the apoplasm and the symplasm.

Metabolism-dependent binding of cations in the cell wall has been suggested in several studies (1, 8). It is therefore possible that the linear phase of uptake into cell wall material

may require normal functioning of the plasma membrane and continued cellular integrity. To test this hypothesis, the kinetics of Al uptake by isolated cell wall material treated with Al *in vitro* were investigated. Uptake of Al by isolated cell wall material was biphasic with a linear phase in the absence of a desorption treatment (Fig. 4). The rate of Al uptake into isolated cell wall material during the linear phase was 1.27 ± 0.23 and 1.12 ± 0.32 μg g^{-1} min^{-1} for the Al-tolerant cultivar PT-741 and the Al-sensitive cultivar Neepawa, respectively (Table IV). In contrast to the results where
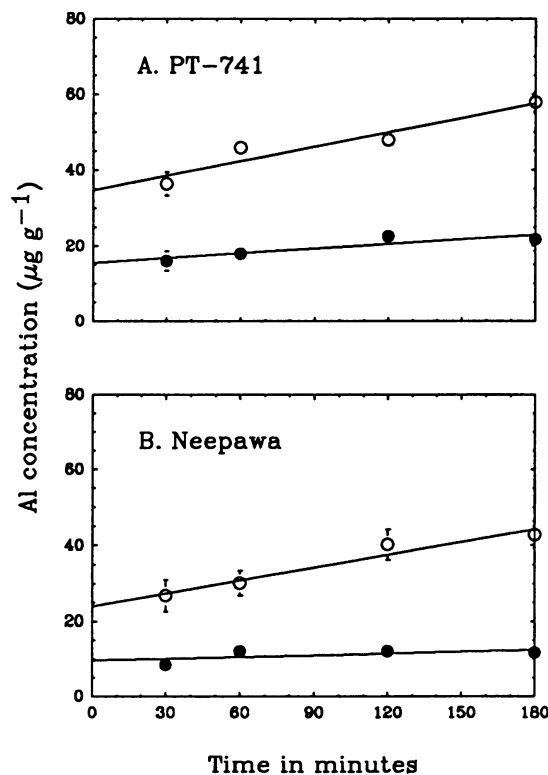
Figure 4. Uptake of Al (μg g root fresh weight^{-1}) by purified cell wall material isolated from roots of an Al-tolerant cultivar PT-741 (A) and an Al-sensitive cultivar Neepawa (B) of *T. aestivum* with (●) or without (○) desorption treatment. Purified cell wall material isolated from roots without Al pretreatment, was treated in 75 μM Al and 1 mM Ca (pH 4.5, 23°C), followed by desorption or no desorption in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Values represent means of four replicates.

Table IV. *Rate of Al Uptake and Al Concentrations in Purified Cell Wall Material Isolated from Excised Roots of Al-Tolerant and Al-Sensitive Cultivars of T. aestivum*

Cell wall material isolated from roots without Al pretreatment, was treated in 75 μM Al and 1 mM Ca (pH 4.5, 23°C) for 0, 30, 60, 120, and 180 min, followed by A desorption treatment (if indicated) in 0.5 mM citrate (pH 4.5, 0°C) for 30 min. Concentrations were calculated from the 180 min absorption period. Values represent means of four replicates.

Cultivars	Treatments	Rate of Uptake	Concentration
		μg g root fresh wt^{-1} min^{-1}	μg g root fresh wt^{-1}
PT-741	Without desorption	1.27 ± 0.23	58.0 ± 2.5
	With desorption	0.41 ± 0.19	21.8 ± 3.1
Neepawa	Without desorption	1.12 ± 0.32	42.8 ± 3.7
	With desorption	0.15 ± 0.17	11.7 ± 2.3

excised roots were treated with Al, this linear phase was completely removed by 30 min desorption with citrate. In both the Al-tolerant cultivar PT-741 and the Al-sensitive cultivar Neepawa, Al accumulated in isolated cell wall material exhibited saturated kinetics after desorption, without significant slopes (Fig. 4; Table IV). The removable nature of the linear phase of Al uptake by isolated cell wall material suggests that *in vitro* uptake into purified cell wall material reflects an exchange/adsorption process. In contrast, the nonremovable linear phase of *in vivo* uptake in cell wall fraction may represent metabolism-dependent uptake of Al into the cell wall.

DISCUSSION

Differences in the uptake of Al between 23 and 0°C by all four cultivars suggested that the linear phase of Al uptake is composed of two components, a nonmetabolic component observed at both 0 and 23°C, and a metabolic component observed only at 23°C. Because the linear phase represents Al remaining after desorption in citrate, the nonmetabolic component could represent polymerization or precipitation of Al in the cell wall, Al tightly bound to cell wall material (4, 23), or diffusion of Al across the plasma membrane with the concentration gradient and the electrical potential across the plasma membrane serving as driving forces for diffusion. The metabolic component likely represents uptake of Al across the plasma membrane and, as our results suggest, metabolism-dependent binding of Al in the cell wall. Active transport of Al has not been reported, although beneficial effects of Al on the growth of *Zea mays*, *Oryza sativa*, *Triticum aestivum*, and *Camellia sinensis* have been suggested (3, 5, 6, 12).

Pettersson *et al.* (15) and Wagatsuma (21) suggested that the metabolic component of Al uptake may represent passive diffusion of Al across plasma membrane. Elimination of the metabolic component at low temperature suggests that this component depends on the existence of a driving force associated with metabolism, the driving force is presumably a membrane potential created by proton-translocating ATPases (18). The activity of proton-translocating ATPases in the plasma membrane may be reduced or eliminated at low temperature, possibly accounting for the change in Al uptake with temperature. Furthermore, the decrease in proton concentration outside the plasma membrane, resulting from decreased activity of proton ATPases, may increase the pH of

the apoplasm. This could, in turn, affect the speciation, solubility, and mobility of Al in the apoplasm. Thus, cold temperature may affect uptake of Al in a variety of ways. These general effects may help to explain the apparently contradictory effects of low temperature and 2,4-dinitrophenol (DNP). Zhang and Taylor (24) reported that DNP stimulated uptake of Al in Al-tolerant cultivars of *T. aestivum*, opposite to the effect of cold treatment. While both low temperature and DNP provide plants with nonmetabolic conditions, the specific targets and degree of inhibition of these two agents are not the same. Dinitrophenol is a relatively specific inhibitor which uncouples oxidative phosphorylation and acts as a protonophore. Thus, different effects of low temperature and DNP on aluminum uptake are not surprising.

The effect of low temperature on uptake of Al may also vary between species. Low temperature did not affect the uptake of Al by *Brassica oleracea*, *Lactuca sativa*, *Pennisetum clandestinum*, and *Hordeum vulgare* (4, 7); however, different pH (4.0-4.2) and Al concentrations (0.2-1.1 mM) in these experiments make results difficult to compare to the results presented here. In *T. aestivum*, decreased uptake of Al at low temperature (2°C) was also observed by Pettersson and Strid (16). In contrast to the present results, however, Pettersson and Strid (16) reported a saturable phase of Al uptake at low temperature. In their experiments, roots were simply blotted dry at the end of absorption period, with no washing or desorption procedure to remove Al from cell wall exchange sites. Their saturable phase of uptake at low temperature may also have reflected a lower pH of absorption solutions (pH 4.1). Huett and Menary (7) demonstrated that a decrease in pH of the absorption solution from 4.2 to 4.0 changed the pattern of Al uptake by *B. oleracea* at low temperature (1°C) from nonsaturable to saturable.

Crude fractionation of roots into a supernatant and pellet fraction demonstrated that uptake of Al into both the supernatant and pellet was linear, although the relative size of the supernatant fraction was small. Aluminum uptake into the supernatant fraction accounted for less than 15% of the total absorbed Al (Table I). Clarkson (4) and Huett and Menary (7) also suggested a minor accumulation of Al in the cytosol of *B. oleracea*, *L. sativa*, *P. clandestinum*, and *H. vulgare*, emphasizing that most (70-90%) absorbed Al was located in cell wall. Although the supernatant fraction was not well defined here, our results are consistent with studies which

suggest that the plasma membrane and cell wall play an important role in restricting entry of Al into the cytoplasm (19)

A dual pattern of Al uptake (a rapid saturable phase superimposed over a linear phase) in excised and whole roots has been reported by several authors (7, 16, 24), and the linear phase has been suggested to represent uptake in the symplasm (11, 16). However, this interpretation of the identity of the linear phase is not consistent with our results. While we would agree that the nonmetabolic, saturable phase of uptake in excised and whole roots represents accumulation in the cell wall, the linear nature of Al uptake in purified cell wall material suggests that the linear phase is composed of both apoplasmic and symplasmic compartments. In comparison to the rate of Al uptake by the cell contents, the cell wall compartment made a substantial contribution to the linear phase (Fig. 2; Table II). Our suggestion that the linear phase of Al uptake includes accumulation of Al in the apoplasm is supported by the nonexchangeability of absorbed Al in the cell wall fraction. A second desorption treatment of the purified cell wall material after isolation did not eliminate the linear phase of absorption in cell walls isolated from roots pretreated with Al. Thus, this phase of uptake cannot reflect redistribution of Al from the cytosol during fractionation. The nature of binding in the cell wall, however, is still unclear.

Differences between *in vivo* and *in vitro* uptake of Al by isolated cell wall material also supported metabolism-dependent uptake of Al in the cell wall. In comparison to experiments in which Al was supplied to excised roots, the linear phase of uptake by isolated cell wall material exposed to Al *in vitro* was completely removed by desorption with citrate in both the Al-sensitive cultivar Neepawa and the Al-tolerant cultivar PT-741. Thus, the nonremovable nature of the linear phase of uptake in the cell wall fraction depends on the integrity of the cell and/or the plasma membrane. Once again, the precise nature of metabolism-dependent binding of Al in the cell wall fraction is not clear. It could result from formation of hydrated Al complexes associated with pectic substances (22) or free carboxyl groups (4), or polymerization of adsorbed monomeric Al (13, 23) in the cell wall. If the functional relationship between the plasma membrane and cell wall is altered during homogenization, high pressure, or sonication treatments, then the functional relationship between exclusion of Al at the plasma membrane and binding of Al by cell wall may be altered. Furthermore, loss of the integrity of the cell wall may result in changes in the physical, chemical, and biochemical properties of the surface of the cell wall, possibly causing a loss of metabolism-dependent binding.

Changes in the properties of cell wall material during isolation and purification could also account for the apparent increase in time zero values of *in vitro* uptake of Al into cell wall material (as determined by extrapolation of the linear phase of uptake). A possible reason for higher time zero estimates of *in vitro* uptake of Al (Fig. 4) compared to *in vivo* uptake (Figs. 2, 3) may be that additional binding sites were created during fractionation. These binding sites were then occupied when the isolated cell wall material was exposed to aluminum. As a result, higher time zero estimates may have been observed. These newly created binding sites would not cause further binding of aluminum in cell wall fractions isolated from intact roots pretreated with aluminum, because

roots were removed from absorption solutions, desorbed with citrate, and rinsed with deionized water prior to fractionation.

To our knowledge, this is the first report which specifically addresses the identity of the linear phase of Al uptake which has been observed in short-term kinetic studies. Our data clearly do not support the interpretation on the linear phase of Al uptake as simply representing Al uptake across the plasma membrane. We believe that the linear phase represents uptake of Al into both apoplasmic and symplasmic compartments; however, the relationship between nonmetabolic and metabolism-dependent accumulation of Al in the cell wall is not clear. Further studies are needed to investigate the mechanisms of metabolism-dependent accumulation in the cell wall, the nature of nonmetabolic and metabolic uptake across the plasma membrane, and means by which uptake of Al into these compartments might be regulated by plants growing on acid, Al-toxic soil.

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