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Measurements of aluminum transport in wheat at the cellular level

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Abstract

We have performed tracer experiments with ^{26}Al to study the transport of aluminum through the cellular structure of wheat. We conclude that there are identifiable differences between strains of wheat that are sensitive to aluminum poisoning and those that are not. Short time and long time behaviours are observed in these initial experiments.

1. Introduction

Aluminum is the most abundant metal and the third most abundant element in the Earth's crust. Generally it is present in soils in insoluble forms and is not biologically active. However solubilization of Al-containing minerals occurs in acid soils and in acid-sensitive soils and these soluble forms affect biological systems [1,2]. Many crop plants display stunted growth and reduced yield when grown on such soils. In addition to direct aluminum toxicity, plant suffer additionally from inabilities to tolerate environmental stress. The situation is exacerbated by the use of fertilizers containing ammonium and amides which acidify the soil [3].

The role of Al as a factor in yield decline in wheat and rye grown in acid soils was documented as early as 1918 [4]. Since such initial reports, aluminum toxicity problems have become increasingly evident, and today aluminum is recognized as a factor responsible for severely limiting plant productivity in many arable soils throughout the world [5]. Current estimates indicate that at least 40% and as much as 70% of the world's arable land suffers from aluminum toxicity [6]. In Canada large areas of Quebec and in B.C./Alberta, the Peace River region are so afflicted.

Despite the severity of aluminum problem, progress in arriving at an understanding of the basis of aluminum toxicity has been slow and many questions remain unresolved. For example, a primary question of paramount importance to be answered is **where the locus of the primary lesion resulting from aluminum exposure is located. Is it intracellular or is it extracellular?**

Aluminum may exert its toxic effects without traversing the root cell plasma membrane by binding to cell wall sites, displacing calcium ions or blocking calcium channels [7,8]. It is possible by using tracer techniques to measure plant compartment biological half-lives for ions in roots of plants [9]. The actual cell wall binding and cytosolic ion concentration can be manipulated to identify the kinetic roles of the cell wall and cytoplasm [10]. We are applying these techniques to the problem of aluminum toxicity in wheat.

We can anticipate biological half-lives associated with the different compartments to be on the order of those that have been measured in earlier ion-transport studies. For example, NH_4^+ has a exchange time constant of 30 s for the cell wall and between 11 and 14 min for the cytoplasm. K^+ has a cell wall time constant of about 2 min, a cytoplasm time constant of about 93 min and a vacuole time constant of 28 h [10].

Our first experiments attempted to use short lived isotopes of aluminum that could be produced at TRIUMF. By using the ^{29,30}Si(p,2p) ^{28,29}Al reaction, ^{28}Al with a half life of 2.2 min and ^{29}Al with a half life of 6.5 min can be produced with the TRIUMF TR13 cyclotron. Though adequate activity could be produced, the short half life coupled with the details of extracting aluminum from the silicon matrix made the process cumbersome. On the other hand, ^{26}Al with a half-life of 730000 years certainly removes the complexities of radioactive half-life from tracer experiments. It is such ^{26}Al tracer studies that we report here.

2. Experimental details

Two varieties of wheat were germinated and grown under identical conditions. These plants were subjected to a hydroponic growth medium that contained a 50 μMol solution of aluminum. A tracer concentration of ^{26}Al is introduced to the nutrient solution for 24 h. The plants

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were then eluted with an aluminum free nutrient solution and these elution samples were analyzed for their ^{26}Al content. These steps are described in detail below.

Two varieties of wheat, Columbus and PF7748 [11], that are respectively sensitive and tolerant to aluminum poisoning were germinated in sand and then grown in identical conditions that had no aluminum in the nutrient medium. After a week's growth, the young plants were transferred to a nutrient solution at pH5 that as well contained a 50 μMol concentration of aluminium that is similar to aluminum concentrations found in agricultural land.

A tracer amount of ^{26}Al, 0.4 ng in 4 l of nutrient solution, was also introduced at this time and the plants were exposed to the aluminum loaded nutrient solution for 24 h. The plants were removed from this solution and placed in an elution funnel. The plant roots were then eluted with the nutrient solution without the ^{26}Al tracer. Elution samples were taken at times between 15 s and 8 h. The individual elution samples thus contained the amounts of ^{26}Al released from the plants as a function of time. The individual samples varied in volume between 20 ml for the early samples to 200 ml for the later ones. The volumes were determined so that there was adequate nutrient for the plants during the elution experiment.

The elution samples were then converted to Al_2O_3. ^{27}Al carrier of 5 mg per sample was added to the elute. Then 2 ml of 5% oxine was added followed by 5 ml of 2M ammonium acetate. The solution was heated to 80°C to facilitate the precipitation of the aluminum. The precipitate was filtered onto ashless filter paper and the filtrate was ashed at 1000°C for 4 h. The recovered Al_2O_3 using this technique weighed between 6 and 10 mg and hence were adequate material to be used for Accelerator Mass Spectrometry. This procedure is a standard technique that we have used in the past for ^{26}Al experiments with biological systems [12].

The samples were transported to the Weizmann Institute Pelletron Accelerator where the AMS measurement was done. This was the inaugural experiment for the multi-sample ion-source [13,14] which facilitated a systematic changing of the samples without detuning the ion-source. Commercial Al_2O_3, process control blanks, and ^{26}Al standards were included in the sample wheel and analyzed at the same time. We observed that the output current from the ion-source was dependent on the length of time that the samples were exposed to the moisture in the air so that care was taken to drive off the moisture and install the samples immediately onto the sample wheel and into the ion-source. Analysed beam currents were typically 400 nA at the entrance to the Pelletron. The Pelletron was operated at 10.5 MV and the exit aluminum beam was in a 7 + charge state. The aluminum beam was directed onto a time-of-flight, ion chamber, and solid state detector system. Fig. 1a shows the spectrum associated with the detection system. Ion fragments give the background shown

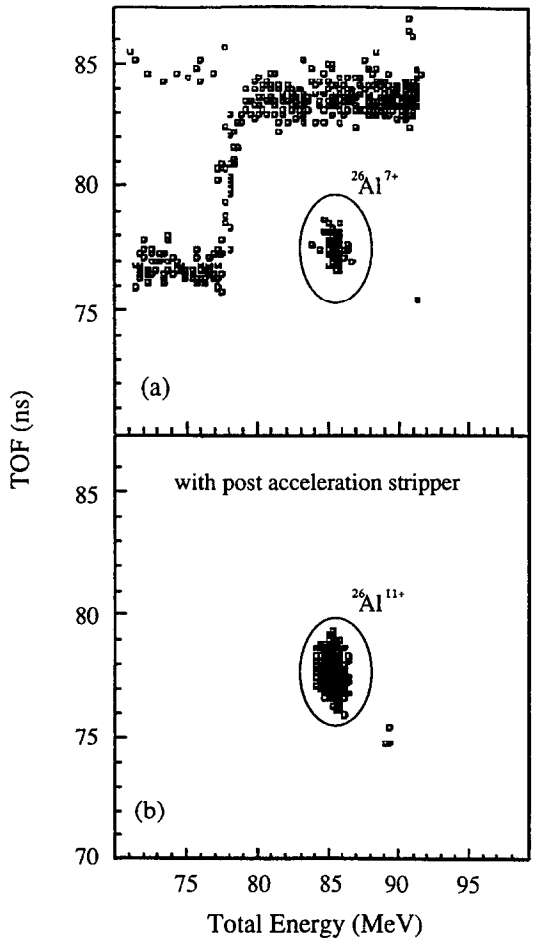

Fig. 1. Time of flight versus total energy for the ion detection system and an aluminum standard sample. (a) Spectrum without the post stripper measured with a beam attenuation of 40. (b) The beam attenuation was turned off for this measurement with the post stripper in place.

in the figure. The detector counting rates for plant samples corresponding to the background without any attenuation in the system were 24 kHz and clearly intolerable for a systematically precise experiment. A post-stripper, 10 $\mu g/cm^2$ carbon foil, was placed before a switcher magnet that was set to select $^{26}Al^{11+}$ and Fig. 1b presents the results of that insertion. Apart from a few residual counts corresponding to $^{25}Mg^{11+}$ that was injected into the system as MgH^- there are only $^{26}Al^{11+}$ counts. The experiment was performed with the post-stripper in place with a typical count rate into the detector of about 10 counts per second. The post stripper reduces the $^{26,27}Al$ transmission by a factor of 4.

3. Experimental results

Control samples were measured that indicated the backgrounds of the Sample Preparation Laboratory and the

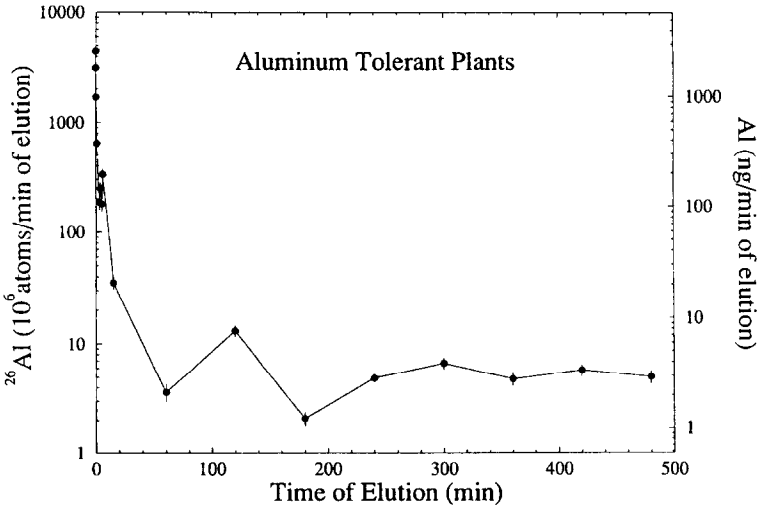

Fig. 2. The elution time profile for tolerant wheat. The $\log_{10}$ of the measured ^{26}Al release divided by the collection time of the elution sample is plotted versus the collection time. The solid line is drawn to guide the eye.

elution solutions. The plant elution samples all gave ^{26}Al/^{27}Al ratios that were greater than 1.8×10^{-12} while the Sample Preparation Lab measurement that used distilled water as the dummy elution solution while all other reagents and procedures were identical gave an ^{26}Al/^{27}Al ratio of 0.015×10^{-12}. The elution solution was also processed without being exposed to the plants. That ^{26}Al/^{27}Al ratio was measured as $0.039\ (\pm 60\%) \times 10^{-12}$. The backgrounds from these sources were measured to be well below the actual levels corresponding to the elution from the root systems. The results below are presented in terms of the number of ^{26} Al atoms eluted per minute of elution. The number of ^{26}Al atoms is obtained by multiplying the measured ^{26}Al/Al ratio by the Al carrier amount. The results can be expressed also in ng (^{27}Al)/min of elution by dividing the number of ^{26}Al atoms by the ^{26}Al/^{27}Al ratio of the labeled nutrient solution.

Fig. 2 presents the time dependent release of ^{26}Al from the tolerant Wheat strain. There is a rapid release of ^{26}Al in the first several minutes followed by a much slower release over the 8 h of the experiment. Fig. 3 shows the same release curve for ^{26}Al for the sensitive wheat strain. The behaviour is similar to the tolerant strain but for a larger release at longer times. We measured also the

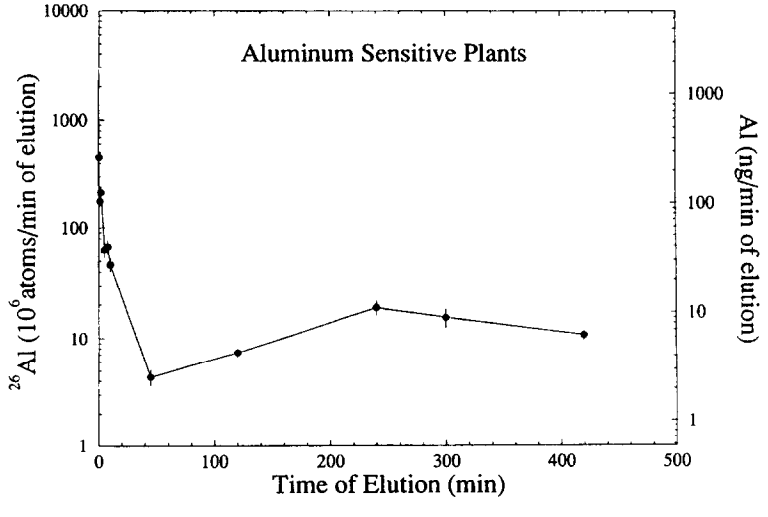

Fig. 3. The elution time profile for sensitive wheat plotted in the same manner as Fig. 2.

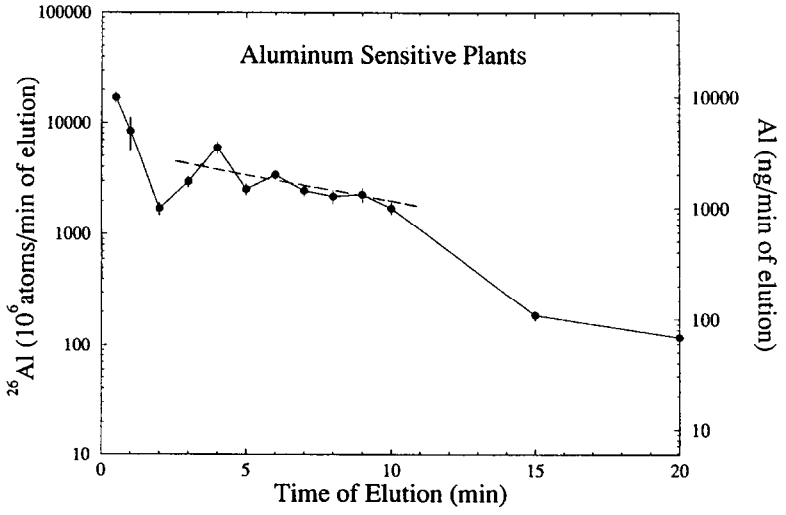

Fig. 4. The elution time profile for sensitive wheat at short times plotted in the same manner as Fig. 2. The dashed line drawn in the figure corresponds to a biological half life of 4 min.

^{26}Al/Al concentration in the roots and stem of the sensitive plants after the elution. While the stem had a ^{26}Al/al ratio comparable to that of the elution samples, the root sample showed a ratio o about 70 times higher. Fig. 4 presents the time dependent release of ^{26}Al in the sensitive variety for an experimental run that concentrated on the early elution times. There is a decrease of ^{26}Al release that could be ascribed to the cell wall or cytoplasm compartments. The line drawn on Fig. 4 corresponds to a half-life of 4 min, a figure that is in the range of other ionic transport measurements[10]. A ratio of about ten is observed between the absolute values of eluted ^{26}Al atoms in the two runs on aluminum sensitive plants (Figs. 3 and 4). The origin of the ratio is not known and could be related to experimental procedures.

4. Discussion and conclusions

These first experiments confirm that ^{26}Al tracing in plants can show the transport of aluminum across the various plant compartments. The experimental procedures are similar to earlier work with short-lived isotopes and with ^{26}Al tracing in biological systems. They are straightforward and simple. Measured backgrounds are well below the actual aluminum ratio measurements associated with the plant behaviour. The predominant concentration of the residual aluminum after the elution experiment in the roots indicate that the dominant processes occur in the root system and not higher in the plant. The behaviour exhibits both short (minutes) and long (hours) time constant processes. It will be necessary to repeat the experiment several times to estimate the intersample variability. We hope, in these experiments, to be able to identify and characterize individual compartment contributions to aluminum toxicity.

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