ANALYSIS OF IRON-NICOTIANAMINE COMPLEXES BY ELECTROSPRAY-MASS SPECTROMETRY

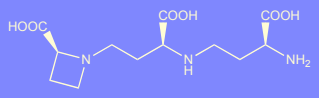
R. Rellán-Álvarez, A. Álvarez-Fernández and J. Abadía.

Plant Stress Physiology Group, Plant Nutrition Dpt., Aula Dei Experimental Station, Zaragoza, Spain.
E-mail: jabadia@eead.csic.es visit us at: www.eead.csic.es/stressphysiology

Background

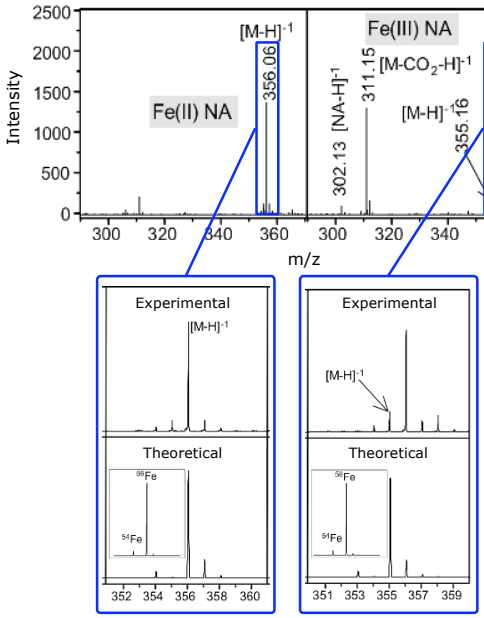
The non-proteinogenic amino acid nicotianamine (NA) is ubiquitous among plants and it is supposed to be involved in the transport of metals, including micronutrients such as Fe. The distances between the groups of the NA molecule are optimal for the formation of chelate rings, where six functional groups allow coordination with different metal ions such as Cu, Fe, Zn and Ni. Support for the occurrence of metal-NA complexes in plant fluids, however, has been mainly gained so far from indirect measurements and *in silico* studies. Investigation in this area should include the direct determination of the possible metal-NA complexes (Hider et al., 2004). Recent examples of this approach are the determination of the Ni-NA complex in a Ni-hyperaccumulator species by mass spectrometry (Vacchina et al., 2003) and the studies on several metal-NA complexes carried out by liquid chromatography coupled to mass spectrometry (Xuan et al., 2006).

The aim of our work was to apply electrospray-mass spectrometry (ESI-MS(TOF)) to study i) the pH effects on the formation of Fe-NA complexes, ii) the metal exchange reactions between Fe(II)-NA and free Cu and Zn ions, and iii) the ligand exchange reactions between Fe-NA and citrate, in order to understand the role that NA may be playing in micronutrient speciation in plant fluids.



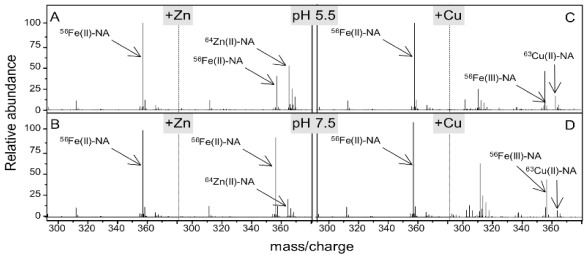
Chemical structure of nicotianamine (NA)

Figure 1. Negative ESI-MS(TOF) mass spectra of Fe-NA complexes



Fe(II)-NA showed a good ionization for the $[M-H]^{-1}$ molecular ion at pH 7.0. However, Fe(III)-NA, a neutral molecule, did not show a good ionization of the ion $[M-H]^{-1}$, showing instead two major peaks, attributable to the mono-decarboxylated molecular ion $[M-CO_2-H]^{-1}$ of Fe(III)-NA and the $[M-H]^{-1}$ ion of Fe(II)-NA. Zoomed mass spectra for the $[M-H]^{-1}$ molecular ions are shown below, along with the theoretical mass spectra. Insets in the theoretical mass spectra show the isotopic distribution of Fe.

Figure 3. Metal exchange reactions between Fe(II)-NA, Zn and Cu



Zn(II) and Cu(II) were able to displace Fe(II) from the Fe(II)-NA complex, specially at the pH values typical of the xylem (5.5).

References

Benes I *et al.* (1983) Experientia 39:261–262; Hider RC *et al.* (2004) New Phytol. 164: 204; Vacchina V *et al.* (2003) Anal. Chem. 75: 2740; von Wirén N *et al.* (1999) Plant Physiol. 119: 1107; Xuan Y *et al.* (2006) J. Chrom. A. 1136: 73.

Acknowledgments

This work was funded by project AGL2004-00194 from the Spanish Ministry of Education and Science (MEC) (co-financed with FEDER) to J.A. R.R-A. and A. A-F. were supported by a FPI fellowship and a "Ramón y Cajal" research contract from the Spanish MEC, respectively. Acquisition of the MS(TOF) apparatus was co-financed with FEDER.

Experimental

Preparation of metal-NA complexes

NA complexes with Mn(II), Fe(II), Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) were prepared by mixing equimolar amounts of NA and metal chloride solutions in 100 mM ammonium acetate (for solutions with a pH value ≤7.0) or ammonium bicarbonate (for solutions with pH values higher than 7.0). Immediately before analysis, pH was measured and solutions were diluted with acetonitrile.

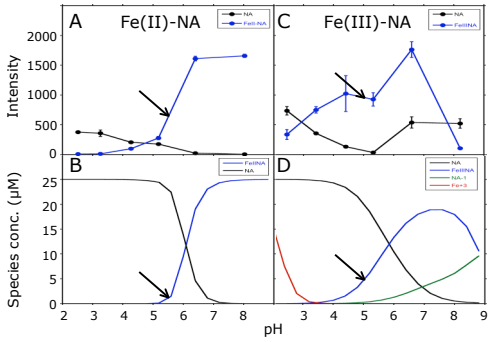
Direct determinations of NA and metal-NA complexes by ESI-MS

Analyses of NA and metal complexes were carried out with a BioTOF II (Bruker Daltonics, Billerica, MA, USA) coaxial multipass time of flight mass spectrometer (MS) equipped with an Apollo electrospray ionization (ESI) source operating in negative ion mode. For the pH effect study, samples were injected directly with a syringe pump in the ESI-MS, whereas two syringe pumps connected with a tee to the ESI chamber were used in metal exchange and ligand exchange studies. All the exchange experiments were done using 50 µM concentrations at the same flow rate in both syringes. Concentrations in plant xylem are approximately 20, 40, 3, 5 and 150 µM for NA, Fe, Cu, Zn and citrate, respectively, whereas concentrations in plant phloem are approximately 100, 130, 15, 80 and 1500 µM for NA, Fe, Cu, Zn and citrate, respectively.

In silico estimations of NA and metal-NA complex concentrations

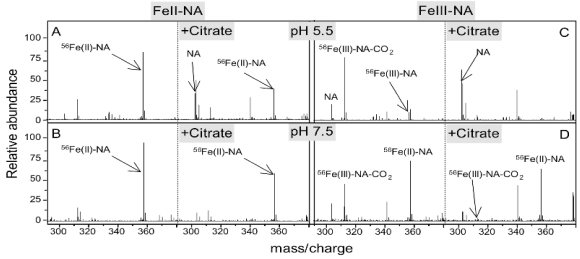
MINTEQA2 for Windows (Version 1.50, Allison Geoscience Consultants and HydroGeoLogic, Inc.) was used to model NA and metal speciation in solution. Input parameters were metal and NA concentrations and the measured pH values. NA protonation constants and stability constants for metal-NA complexes were taken from Benes et al. (1983) and von Wirén et al. (1999).

Figure 2. pH dependence of the Fe(II)-NA and Fe(III)-NA complexes



The experimental pH dependence of Fe-NA complexes (A and D) was not identical to the *in silico* predictions (B and D). At pH values typical of the xylem (5.5) NA reached a significant Fe(II) complexation, conversely to the *in silico* prediction (see arrows). The complex of Fe(III) with NA, assessed from the mono-decarboxylated ion, occurred even at low pH values (3.0-5.0), whereas *in silico* simulations indicate that this would occur only to a limited extent even at pH values of 5.0 (see arrows). At a pH value of 8.3 NA did not appear to chelate significantly Fe(III), whereas *in silico* simulations indicate the opposite.

Figure 4. Ligand exchange reactions between citrate and Fe-NA complexes



At the xylem pH of 5.5, citrate was able to break the Fe-NA complexes (A and C), as indicated by the increase of the free NA signal, with decreases in the Fe(II)-NA and the mono-decarboxylated Fe(III)-NA complex. At the phloem pH of 7.5 (B and D), citrate caused a significant reduction in the Fe(II)-NA complex and a large decrease in the mono-decarboxylated Fe(III)-NA complex.

Conclusions

- The Fe(II)-NA peak is detected in ESI-MS(TOF) spectra, with good results in terms of intensity, mass accuracy (data not shown) and isotopic distribution. However, Fe(III)-NA did not ionize well due to its neutral charge, showing instead a major peak attributable to a mono-decarboxylated molecular ion $[M-CO_2-H]^{-1}$ (Fig. 1).
- The experimental pH dependence of Fe-NA complexes were not identical to those obtained with *in silico* estimations (Fig. 2). For Fe(II)-NA, both approaches support the idea that the role that NA could play in Fe(II) chelation must be more important at the neutral or slightly basic pH values usually found in cytoplasm and phloem than at the acidic pH values typical of the xylem (around 5.5). However, experimental data indicated that NA could chelate more Fe(II) in the xylem than that expected from *in silico* simulations. For Fe(III)-NA, experimental data do not fully support that NA could play a more important role in Fe(III) chelation in the phloem than in the xylem. In fact, experimental data indicated that NA could chelate less Fe(III) in the phloem than that expected from *in silico* studies.
- Exchange reaction data indicated that at the pH values of the xylem Cu and Zn would be able to displace Fe from Fe(II)-NA (Fig. 3). Moreover, at these pH values citrate facilitated rupture of both Fe-NA complexes (Fig. 4).
- In conclusion, further studies would be needed to ascertain the role of NA in Fe complexation. Also, ESI-MS(TOF) methodologies appear to be be good tools to study this subject.