

Synthesis, Characterization, and Theoretical and Experimental Investigations of Zinc(II)–Amino Acid Complexes as Ecofriendly Plant Growth Promoters and Highly Bioavailable Sources of Zinc

Somayeh Ghasemi · Amir H. Khoshgoftarmanesh · Hassan Hadadzadeh · Majid Afyuni

Received: 8 July 2012 / Accepted: 10 August 2012 / Published online: 5 October 2012
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Abstract Amino acids (AA) as metal complexing agents have the ability to form relatively stable complexes with zinc (Zn) and thereby increase its availability for plants. In this study, the complexes of Zn(II), $[Zn(L-L')_2]$ [where $L-L' =$ monoanion of arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met)], were synthesized and characterized by different analytical techniques. The results of elemental analysis support the formation of Zn(II)–AA complexes (ZnAAC) with a 2:1 ligand-to-metal molar ratio. The computational results indicated that the AA ligands coordinated to the Zn(II) ion via their nitrogen and oxygen atoms and support the coordination mode obtained from IR spectroscopy. For the first time, the semiempirical calculations were also performed to investigate the passive uptake of ZnAAC by root cells. The proposed transport pathway indicated that ZnAAC can pass via plant root cell wall pores without any strict hindrances. The efficacy of ZnAAC as a Zn source was evaluated for two lettuce cultivars (*Lactuca sativa* L., cvs. ‘Lollo Bionda’ and ‘Lollo Rossa’) grown in nutrient solution. The results confirmed the higher efficacy of ZnAAC in supplying Zn for lettuce in comparison with $ZnSO_4$. The synthesized ZnAAC also had a stimulating effect on root and shoot growth of both lettuce cultivars. According to the results, ZnAAC can be used as ecofriendly plant growth stimulators and sources of Zn to supply plants with readily available Zn.

Keywords Zinc complex · Amino acid · Bidentate ligand · Semiempirical PM6 · Lettuce growth

Introduction

Amino acids (AA) as natural chelating agents play a significant role in increasing solubility and availability of micronutrients, that is, zinc (Zn) in soil–plant systems (Aravind and Prasad 2005). These organic ligands change the dissolvability of metal nutrients in soils through chelation, oxidation/reduction, and acidification of the rhizosphere (Xu and others 2007; Oburger and others 2009). It has been shown that the exudation of chelating agents such as AA from roots is a possible mechanism for plant tolerance to Zn-deficiency conditions, particularly in calcareous soils (Kalaycia and others 1999; Rasouli-Sadaghiani and others 2011). Zinc is an important and essential micronutrient that plays a role in several crop physiological processes such as metabolism of carbohydrates, proteins, and hormones, membrane integrity, and reproduction (Broadley and others 2007). Zinc deficiency in soils and plants is a global micronutrient deficiency problem in most agricultural regions of the world (Alloway 2008). The easiest and most straightforward practice to correct micronutrient deficiency is to apply Zn fertilizers. Commercial $ZnSO_4$ and synthetic Zn-chelates (that is, Zn–EDTA and Zn–DTPA) are common sources of Zn used in agricultural lands (Alloway 2008). Applied soluble micronutrient fertilizers become ineffective rather rapidly as the dissolved metals react with soil minerals and organic matter (Khoshgoftarmanesh and others 2010). Most commercial inorganic Zn fertilizers contain Cd and other toxic heavy metals as impurities (Afyuni and others 2007). In comparison with inorganic fertilizers, synthetic and natural chelates have the advantage of keeping the applied nutrient in solution in a less reactive form

S. Ghasemi (✉) · A. H. Khoshgoftarmanesh · M. Afyuni
Department of Soil Science, College of Agriculture, Isfahan
University of Technology, 84156-83111 Isfahan, Iran
e-mail: s.ghasemi@ag.iut.ac.ir

H. Hadadzadeh
Department of Chemistry, Isfahan University of Technology,
84156-83111 Isfahan, Iran

(Khoshgofarmanesh and others 2010). Chelates are particularly appropriate for applications of Zn to alkaline and calcareous soils. In contrast with inorganic salts, synthetic Zn-chelates are effective for correction of Zn deficiency (Wallace and Wallace 1982) for a longer period. However, application of metal chelates may result in a potential leaching risk because the less biodegradable the carrier, the greater the risk for leaching (Gonzalez and others 2007). Zinc synthetic chelates, mainly Zn–EDTA and Zn–DTPA, are widely used to supply Zn in hydroponic nutrient solutions, but after Zn uptake by the plant, the concentration of free ligands is increased in the nutrient solution and as a result, the possibility of complex formation between free ligands and other micronutrients (that is, Cu and Mn) in the solution increases. Complexation with EDTA or DTPA reduces concentrations of free metal cations and thereby decreases their availability for plant uptake (Albano and Miller 2001; Vadas and others 2007). On the other hand, EDTA and DTPA are easily photodegradable compounds and their phytodegradation results in production of certain compounds such as glyoxylic acid, formaldehyde, diethylenetriaminetriacetic acid, and diethylenediaminetriacetic acid that are harmful for plant growth (Nowack and Baumann 1998; Hangarter and Stasinopoulos 1991; Metsarinne and others 2004).

Recently, we successfully synthesized Fe(II)–AA chelates and evaluated their efficiency as a Fe source for tomato plants in comparison with the Fe–EDTA chelate. Addition of Fe–AA chelates into the hydroponic nutrient solution significantly increased tissue concentrations of Fe, Zn, and N in two tomato genotypes in comparison with Fe–EDTA (Ghasemi and others 2012). The complexes of metal–AA are weaker than synthetic chelates (Alloway 2008) and their degradation in the nutrient solution is negligible (Jämtgård and others 2008). Therefore, the disadvantages of metal–AA complexes are much less than those of synthetic chelates. In addition, AA are precursors of certain plant hormones and improve plant growth via improving photosynthesis (Zeid 2009; Amin and others 2011), mRNA transcription, and sugar and protein production (Nassar and others 2003; Rashad and others 2003; Keutgen and Pawelzik 2008). The uses of AA to improve growth and yield of various crops have yielded very encouraging results with some plants. For example, exogenous application of arginine significantly increased the fresh and dry weights and concentrations of certain endogenous plant growth regulators in wheat (El-Bassiouny and others 2008), bean (Nassar and others 2003), and onion (Amin and others 2011). A significant increase in growth and yield of bean in the presence of glutamine has also been reported by Rashad and others (2003).

Considering the significant role of AA in increasing soil availability of micronutrients for plants, we hypothesized the possibility of synthesis and use of Zn(II)–AA complexes

(ZnAAC) as a plant growth stimulator and Zn source in agricultural systems. The work reported in this article describes the synthesis, characterization, and theoretical investigation of $[Zn(L-L')_2]$ (where L–L' = monoanion of arginine, glycine, glutamine, histidine, and methionine) complexes. Arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met) were chosen as ligands. These AA were used because of their significance in plant nutrition and the relatively high stability of their Zn complexes in water. The efficacy of synthesized ZnAAC as growth stimulating and Zn sources was also investigated in a nutrient solution culture with lettuce. For the first time, the semiempirical PM6 calculations for ZnAAC were used to investigate the passive uptake of ZnAAC by root cells.

Materials and Methods

Synthesis of Zinc–Amino Acid Complexes (ZnAAC)

Zinc(II)–amino acid complexes have been prepared using five AA, arginine (Arg), glycine (Gly), glutamine (Gln), histidine (His), and methionine (Met) as complexing agents. All complexes were characterized by different analytical techniques.

General Methods

A solution of Arg, Gly, Glu, His, or Met (2 mmol) in 5 ml distilled water was slowly added to a solution of $Zn(OAc)_2$ (1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. Evaporation of solvent at room temperature yielded white microcrystals of ZnAAC. The products were washed with cold ethanol followed by diethyl ether and air-dried.

Analyses

A PerkinElmer 2400 CHN elemental analyzer was used for quantitative determination of carbon (C), nitrogen (N), hydrogen (H), and oxygen (O) in various operating modes. Atomic absorption measurements of Zn were recorded with an atomic absorption spectrometer (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA). The FTIR spectra were measured with a FTIR JASCO 460 spectrophotometer over KBr pellets in the $4,000\text{--}400\text{-cm}^{-1}$ range. Electronic spectra were recorded by a JASCO-570 spectrophotometer.

Efficacy Test: Lettuce Culture in Hydroponic Nutrient Solution

Seeds of two lettuce cultivars (*Lactuca sativa* L., cvs. 'Lollo Bionda' and 'Lollo Rossa') were thoroughly rinsed with distilled water and germinated on moist filter paper in an

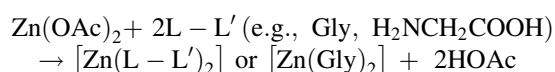
incubator at 28 °C. Uniform-sized seedlings were transferred to PVC lids that fit tightly over 2-L polyethylene containers in a greenhouse under controlled conditions with an 8-h light period at an intensity of 390 μmol m⁻² s⁻¹, 25/20 °C day/night temperature, and 65–75 % relative humidity. The pots were wrapped with black polyethylene to prevent light from reaching the roots and solution. Two plants were planted in each pot. A basic nutrient solution was prepared in double-deionized water (electrical resistivity = 18 Mohm cm⁻¹). The nutrient solution contained 1.0 mM KNO₃, 1.0 mM Ca(NO₃)₂, 1.0 mM NH₄H₂PO₄, 1.0 mM MgSO₄, 50 μM KCl, 25 μM H₃BO₃, 2.0 μM MnSO₄, 2.0 μM ZnSO₄, 0.5 μM CuSO₄, 1.0 μM NiSO₄, and 0.02 μM H₂Mo₇O₄ adjusted to pH 6 with NaOH or HCl as a buffer. Zinc was supplied from five different sources of ZnSO₄ (the most common Zn source used in nutrient solutions) and ZnAAC of Zn(Arg)₂, Zn(Gly)₂, Zn(Gln)₂, and Zn(His)₂. Zn(Met)₂ is insoluble in water so this complex was not used as a Zn source in nutrient solution culture. The Zn level in the nutrient solution was 10 μM. All solutions were renewed every day.

Plants were harvested approximately 4 weeks after seeding and divided into shoot and roots. The plant materials were dried immediately in a forced-air oven at 70 °C to a constant weight and ground to a fine powder in a Wiley mill to pass through a 20-mesh sieve. Dry samples (1 g) were placed into ceramic vessels and combusted in a muffle furnace at 550 °C for 8 h. The ashed samples were removed from the muffle furnace, cooled, and then dissolved in 2 M HCl (Chapman and Pratt 1961). The final solution was diluted to meet the range requirements of the analytical procedures. Analyses of Zn were carried out with an atomic absorption spectrophotometer (PerkinElmer model 3400).

Results and Discussion

Synthesis of ZnAAC

All [Zn(L–L')₂] complexes of this study were synthesized in good yield by reaction of Zn acetate with the AA ligands in refluxing water according to the following reaction:



The acetate anion OAc⁻ can act as a weak base and remove a proton (H⁺) from the neutral AA ligand. The [Zn(L–L')₂] complexes, except [Zn(Met)₂], are air-stable and soluble in water.

The spectral features (UV–Vis) of the ZnAAC did not change on keeping the aqueous solutions for 48 h, and no precipitation was observed, even after long storage at room temperature (at least 3 months after preparation), which indicates stability of the ZnAAC. Essential metal ions such as Zn in biology most frequently bind to donor ligands according to preferences dictated by the hard–soft theory of acids and bases (HSAB). The affinity of metal ions for ligands is controlled by size, charge, and electronegativity. This can be refined further by noting that for some metal ions, their chemistry is dominated by size and charge, whereas for others it is dominated by their electronegativity. According to Pearson’s principle of HSAB, Zn(II) is a hard acid. This ion tends to bind to hard bases such as N-chelating, O-chelating, and N,O-chelating agents such as AA.

Elemental Analysis

The analytical data for the complexes are given in Table 1. The elemental analysis of the complexes is consistent with their formulation, [Zn(L–L')₂], as are the following spectroscopic characterizations and theoretical investigations. The mole ratio of Zn(II)/L–L' is 1:2 (Table 1).

Table 2 Selected IR bands (cm⁻¹) of zinc(II)–amino acid complexes (ZnAAC) (KBr disk)

ZnAAC	ν(NH ₂)	ν(C=O)	ν(C–O)	δ(NH ₂)	δ(C=O)
[Zn(Gly) ₂]	3,306, 3,268	1,599	1,407	–	723
[Zn(Glu) ₂]	3,267, 3,210	1,645	1,409	1,686	777
[Zn(Arg) ₂]·0.5H ₂ O	3,139, 3,010	1,594	1,403	–	655
[Zn(His) ₂]·H ₂ O	3,178	1,695	1,407	–	799
[Zn(Met) ₂]	3,249	1,606	1,409	1,601	698

Table 1 Analytical data for zinc(II)–amino acid complexes (ZnAAC)

ZnAAC	Formula weight	Yield (%)	Size (nm)	% Found (calculated) ^a			
				C	H	N	Zn
[Zn(Arg) ₂]·0.5H ₂ O	420.83	83.88	1.41	34.31 (34.25)	6.36 (6.47)	26.49 (26.53)	15.32 (15.54)
[Zn(Gly) ₂]	213.53	87.11	0.55	22.54 (22.50)	3.82 (3.78)	13.06 (13.12)	30.57 (30.63)
[Zn(Gln) ₂]	355.69	80.69	1.10	33.94 (33.76)	4.98 (5.10)	15.47 (15.76)	18.12 (18.39)
[Zn(His) ₂]·H ₂ O	391.73	83.22	1.09	36.66 (36.79)	4.89 (4.63)	21.53 (21.60)	16.36 (16.70)
[Zn(Met) ₂]	361.81	87.73	0.99	33.63 (33.19)	5.60 (5.57)	7.93 (7.74)	18.02 (18.08)

^a Theoretical percentage of the elements

FTIR Spectroscopy

Amino acids exist as zwitterions in the crystalline state and predominant vibrations for the free AA ligands are associated with $\nu_a(\text{COO}^-)$, $\nu_s(\text{COO}^-)$, $\delta_d(\text{NH}_3^+)$, $\delta_s(\text{NH}_3^+)$, $\nu_a(\text{CCN})$, $\nu_s(\text{CCN})$, and $\delta(\text{COO}^-)$. In their complexes, the AA act as bidentate ligands and bind to the metal via one oxygen and

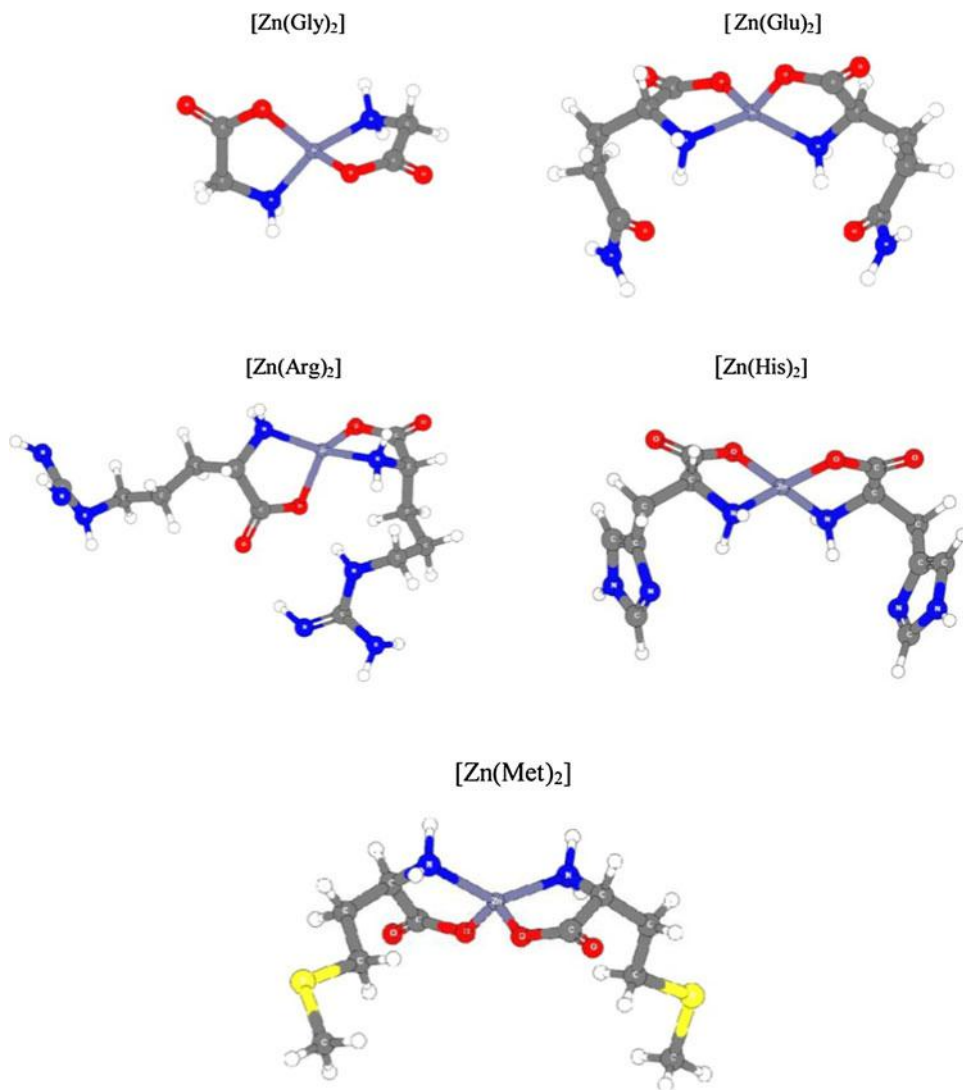
one nitrogen atom. Also, the noncoordinating C=O groups are hydrogen-bonded to the neighboring complex or lattice water, or weakly bonded to the metal of the neighboring complex. Thus, $\nu(\text{COO})$ of AA complexes are affected by coordination as well as by intermolecular interactions (Nakamoto 2009).

The effect of coordination is the major factor in determining the frequency order in AA complexes. The reported

Table 3 Calculated bond lengths (Å) and angles (°) of Zn(II)–amino acid complexes (ZnAAC)

ZnAAC	Zn–O	Zn–N	N–Zn–N	O–Zn–O	N–Zn–O	ΔH_f (kcal mol ⁻¹)
[Zn(Gly) ₂]	1.899, 1.899	2.032, 2.032	131.955	129.729	88.824, 88.822, 111.356, 111.579	–193.768
[Zn(Glu) ₂]	1.923, 1.923	2.013, 2.013	131.237	123.961	88.114, 88.074, 114.939, 114.871	–315.471
[Zn(Arg) ₂] \cdot 0.5H ₂ O	1.918, 1.907	2.029, 2.010	138.465	121.280	87.201, 87.912, 103.652, 120.801	–176.504
[Zn(His) ₂] \cdot H ₂ O	1.913, 1.913	2.018, 2.018	130.330	125.823	88.520, 88.515, 114.124, 114.081	–145.903
[Zn(Met) ₂]	1.855, 1.856	1.938, 1.938	116.783	117.078	95.981, 95.600, 115.541, 117.440	–208.822

Fig. 1 Possible structure of Zn(II)–amino acid complexes (ZnAAC)



data indicate the increasing order of the metal–oxygen interaction because the COO group becomes more asymmetrical as the metal–oxygen interaction becomes stronger. The selected vibrations and assignments of ZnAAC are given in Table 2. The FTIR spectra of ZnAAC show an absorption pattern in the 4,000–400-cm⁻¹ region, similar to AA. Predominant vibrations for the ZnAAC are associated with $\nu(\text{CO})$, $\nu(\text{C-O})$, $\nu(\text{NH}_2)$, $\delta(\text{NH}_2)$, and $\delta(\text{CO})$. The observed vibrational bands for -NH₂ groups around 3,100–3,350 cm⁻¹ are very sensitive to the effect of intermolecular interaction in the solid state and these bands sometimes appear very broad. Also, it is difficult to discuss the strength of the Zn(II)–NH₂–bond from the $\nu(\text{NH}_2)$. In comparison to free AA, the vibration of N–H bands appears to be shifted toward a higher frequency in the ZnAAC, proving the involvement of the amine group in the complex formation. The carboxylate ion of AA coordinates to Zn(II) as a unidentate mode. The C=O groups of ZnAAC have approximately the same frequency around 1,594–1,695 cm⁻¹ and the $\nu(\text{CO})$ is metal-sensitive (Nakamoto 2009).

The electronic spectra of ZnAAC were measured in aqueous solution. Because the Zn(II) ion has a d^{10} configuration and is difficult to oxidize or reduce due to charge transfer transitions, the absorption bands in the UV region are assigned to intraligand transitions (Lever 1984).

Semiempirical Calculations

There is no example of semiempirical PM6 calculations for ZnAAC. In the present study, different possible coordination modes of the AA and donor atoms and also the variable number of AA ligands were considered. Preferences between different coordination numbers and geometries tend to be controlled by steric and electronic effects. The variation found in coordination geometries for a given coordination number is consistent with the argument that spatial requirements of a ligand and coordination restrictions of multidentate ligands are controlling factors.

The bonding parameters of ZnAAC were calculated with PM6 (Table 3). The average bond lengths between the Zn(II) and each nitrogen and oxygen of the AA ligands are 1.985 and 1.902 Å, respectively. Coordination of AA to Zn(II) leads to the formation of a chelate ring (N–Zn–O) and the average bite angle of O–Zn–N is 89.943°. The computational results indicate that the AA coordinated to the Zn(II) ion via their nitrogen and oxygen atoms, which supports the coordination mode obtained from IR spectroscopy.

The AA ligand-to-Zn(II) mole ratio of 2:1 was observed after the optimization of the complexes in the solution state. The optimized structure of the complexes in

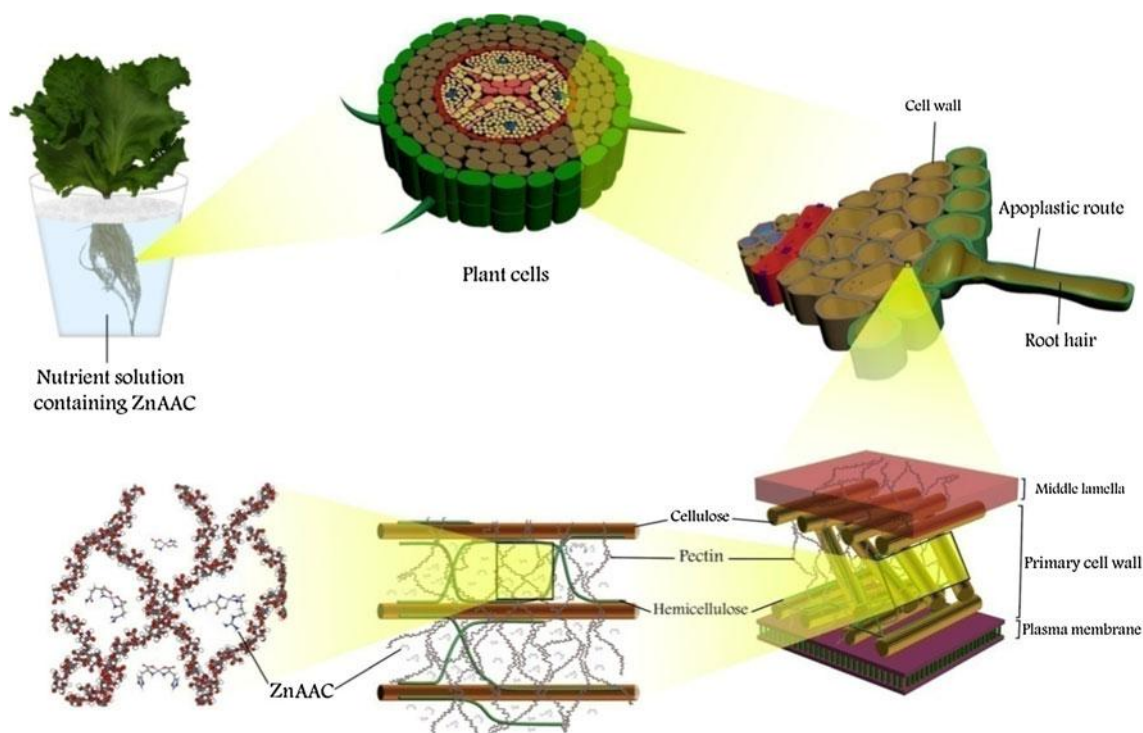


Fig. 2 A proposed transport pathway for Zn(II)–amino acid complexes (ZnAAC) uptake by lettuce roots



Fig. 3 The effect of Zn–arginine [$\text{Zn}(\text{Arg})_2$] (left) on the growth of two lettuce cultivars in comparison with ZnSO_4 (right)

the solution state is shown in Fig. 1. The calculated standard enthalpies of the complex formation of ZnAAC (Table 1) show that all complexes have negative enthalpy values and are thermodynamically favored.

Efficacy of Synthesized ZnAAC in Stimulating Growth and Supplying Zn for Lettuce in Nutrient Solution Culture

A mechanistic understanding of uptake, translocation, and utilization of nutrients in plants is a prerequisite in the production of fertilizers. There are some physiochemical properties of ions and other solutes (for example, ion diameter and valence) that determine their uptake by roots. It has been indicated that due to a larger molecular size, plant uptake of the synthesized chelate (for example, Zn–EDTA, Zn–DTPA) is much lower than the free-metal cations (Marschner 1995). In this study, five ZnAAC were synthesized and characterized by different analytical techniques to determine their physiochemical properties.

According to the semiempirical calculations, all ZnAAC have a molecular dimension of around 0.55–1.41 nm (Table 1). Because the pore diameter of plant cell walls is about 5 nm, the ZnAAC can pass through pores without any strict hindrances and enter into the free spaces of the root apoplasm. A proposed transport pathway for ZnAAC via the free spaces of the lettuce root cell wall from nutrient solution is shown in Fig. 2. The pathway indicates a passive uptake of ZnAAC by root cells. The mass transport of ZnAAC from the bulk of the nutrient solution to the primary cell wall occurs by diffusion and mass flow mechanisms.

The characteristics of ion uptake by roots are also affected by their interaction with charged groups in the cell wall of the apoplasm and membrane constituents. The strength of this interaction increases with increasing ion valence; conversely, the uptake rate often decreases (Marschner 1995). In the present study, the ZnAAC were synthesized with a 2:1 ligand-to-metal molar ratio. Coordination of AA to Zn(II) leads to the formation of uncharged molecular compounds. According to the results obtained from ZnAAC synthesis, we hypothesized that complexation of Zn with AA may improve Zn uptake by the plant. Therefore, we have investigated our hypothesis by comparing Zn uptake between ZnAAC and Zn^{2+} via lettuce roots in hydroponic culture.

The positive effect of ZnAAC on root and shoot growth (Figs. 3, 4) of lettuce plants was greater than that of ZnSO_4 , although the stimulating effect of ZnAAC on lettuce growth was dependent on the plant cultivar and AA type.

In ‘Lollo Bionda’ $\text{Zn}(\text{Gln})_2$ and in ‘Lollo Rossa’ $\text{Zn}(\text{Arg})_2$ caused the greatest increase in root growth (Fig. 4a). Shoot dry matter weight of ‘Lollo Bionda’ plants supplied with $\text{Zn}(\text{Arg})_2$ and $\text{Zn}(\text{Gln})_2$ was higher than those supplied with $\text{Zn}(\text{Gly})_2$ and $\text{Zn}(\text{His})_2$ (Fig. 4b). In, ‘Lollo Rossa’, no significant difference was found in shoot growth between ZnAAC treatments. The stimulating effect of ZnAAC on lettuce growth could be due to the role of AA in improving the plant growth rate, cell division, and/or cell development (Abdul-Qados 2009). Nassar and others (2003) found that the positive effect of Arg on the shoot and root growth of bean was associated with the elevated level of certain plant growth regulators. A significant increase in shoot growth of pak-choi by Arg application has also been reported (Wang and others 2007). In the present study, the growth-stimulating effect of $\text{Zn}(\text{Gln})_2$ and $\text{Zn}(\text{Arg})_2$ was greater than that of $\text{Zn}(\text{Gly})_2$ and $\text{Zn}(\text{His})_2$. Differential effects of various AA on plant growth have been reported by other researchers (Wang and others 2007). For example, Svennerstam and others (2007) reported that the effect of Gln on the growth of *Arabidopsis* was greater than that of other AA studied. In another experiment, Rashad and others (2003) found a greater

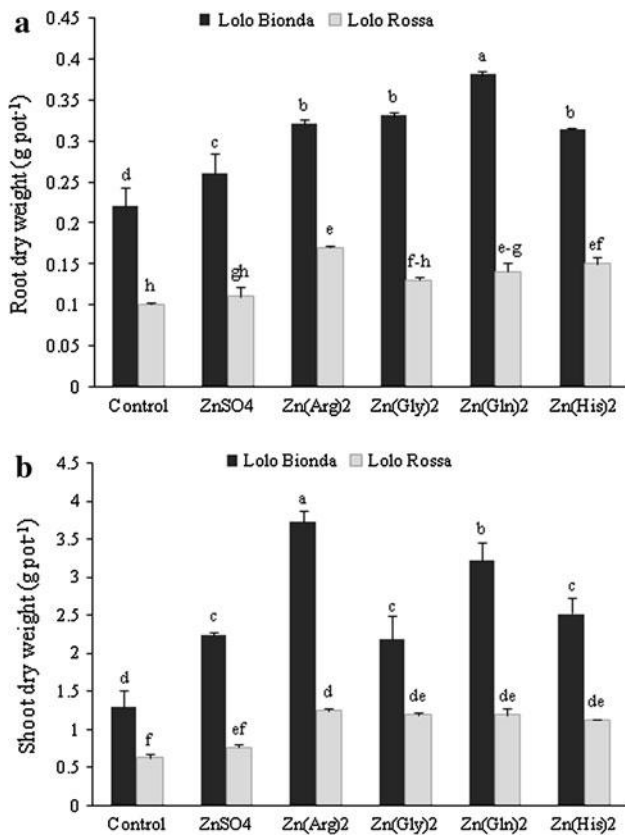


Fig. 4 Root (a) and shoot (b) dry matter weight of two lettuce cultivars grown in nutrient solution containing ZnSO₄, Zn–arginine [Zn(Arg)₂], Zn–glycine [Zn(Gly)₂], Zn–glutamine [Zn(Gln)₂], and Zn–histidine [Zn(His)₂]. Control presents Zn-free nutrient solution. Error bar represents standard error (n = 3). Bars having different letters are significantly different at the 5 % level by LSD

effect of Gln on the growth and yield of bean plants compared with that of the other AA.

The results of the present study confirmed the greater efficacy of ZnAAC in supplying Zn to lettuce plants compared with that of ZnSO₄ (Fig. 5). For both lettuce cultivars, the increase in root Zn concentration was greater in plants supplied with Zn(Gln)₂ in comparison with those supplied with the other ZnAAC. In ‘Lollo Rossa’, the effect of ZnAAC on shoot Zn concentration was in the order: Zn(Gln)₂ > Zn(Gly)₂ > Zn(His)₂ > Zn(Arg)₂. In ‘Lollo Bionda’, Zn(His)₂ and Zn(Gln)₂ had similar effects on shoot Zn concentrations. In all treatments, ‘Lollo Rossa’ accumulated higher amounts of Zn in its roots and shoots compared with ‘Lollo Bionda’. Amino acids have a great ability for forming complexes with Zn and thereby increase the bioavailability of this metal for plant uptake (Zhou and others 2007). Furthermore, stimulated plant growth by AA may result in a greater ability for Zn uptake in roots. Accordingly, Zhang and others (2009) found that addition of AA to the nutrient solutions increased uptake and root-to-shoot translocation of Zn in tomato. Eid and others

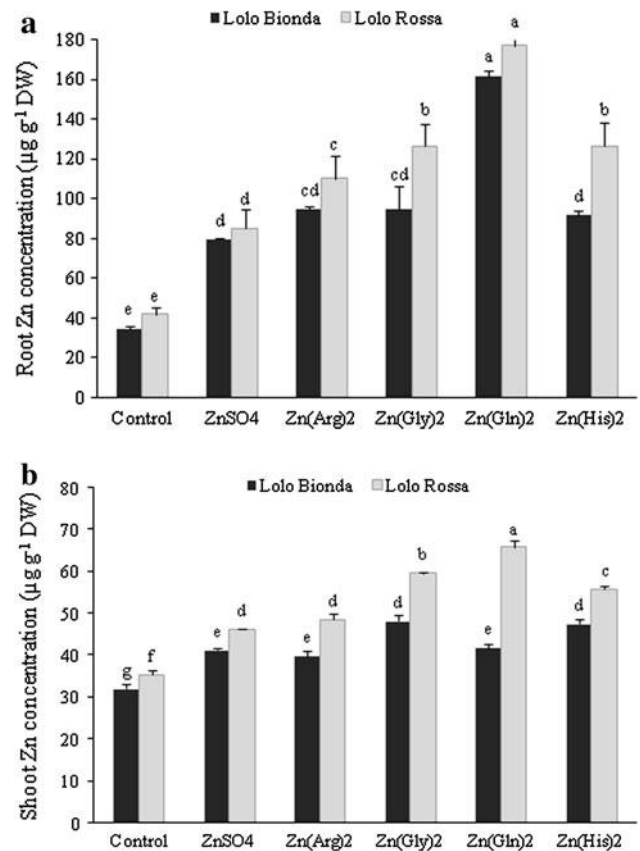


Fig. 5 Root (a) and shoot (b) Zn concentration of two lettuce cultivars grown in nutrient solution containing ZnSO₄, Zn–arginine [Zn(Arg)₂], Zn–glycine [Zn(Gly)₂], Zn–glutamine [Zn(Gln)₂], and Zn–histidine [Zn(His)₂]. Control presents Zn free nutrient solution. Error bar represent standard error (n = 3). Bars having different letters are significantly different at the 5 % level by LSD

(2011) reported the positive effect of AA on growth and nutrient uptake in marigold plants. According to the results obtained from the present study, the effect of ZnAAC on shoot and root Zn accumulation varied with AA type. In ‘Lollo Rossa’, Zn(Gln)₂ caused a higher accumulation of Zn in roots and shoots compared with the other ZnAAC. A possible reason for the different effects of ZnAAC on plant growth and Zn tissue content is variation in their uptake by the plant. The results obtained from the present study cannot show whether these complexes are absorbed directly with no dissociation or they dissociate at the root surface and then free amino acids and Zn pass through the cell membrane individually. It is also unclear whether the increased Zn uptake is due to plant growth improvement or increasing Zn transport through cell membrane. These hypotheses will be tested by isotopic experiments (labeled Zn and AA) in future studies. Based on the results of this study, ZnAAC are stable in nutrient solution and can improve the growth and the Zn nutritional status of lettuce plants in comparison with ZnSO₄. Consequently, due to

several disadvantages of synthetic chelates of Zn (for example, toxic side effects, impaired micronutrient balance), ZnAAC can be used as a suitable source of Zn in hydroponic nutrient solutions. The effectiveness of soil application of ZnAAC in plant Zn nutrition depends on the residence time of these complexes in the soil. Further research is therefore required to investigate the biodegradability of ZnAAC and to determine the role of AA in bioavailability of Zn in soil.

Conclusions

The ZnAAC in aqueous solution, Zn(Arg)₂, Zn(Gly)₂, Zn(Gln)₂, Zn(His)₂ and Zn(Met)₂, were synthesized and characterized by elemental analysis, atomic absorption, and FTIR spectroscopy. The results indicated the formation of 1:2 complexes of Zn(II) with all AA. Results also indicated that using ZnAAC in the nutrient solution could supply a sufficient amount of Zn for plant uptake and also improve root and shoot growth of lettuce plants. Although the size of Zn²⁺ would be increased due to complexation with AA, the results suggested that the movement of ZnAAC to the free space of root cells is not restricted by the pores. Further studies are required to investigate the mechanism of ZnAAC uptake using double ¹⁵N–⁶⁵Zn-labeled compounds.

Acknowledgments This research was financially supported by Support Box of Iranian Researcher (Project No. 88002077).

References

- Abdul-Qados AMS (2009) Effect of arginine on growth, yield and chemical constituents of wheat grown under salinity condition. *Acad J Plant Sci* 2:267–278
- Afyuni M, Khoshgoftarmansh AH, Dorostkar V, Moshiri R (2007) Zinc and cadmium content in fertilizers commonly used in Iran. International Conference of Zinc Crops, Istanbul
- Albano JP, Miller WB (2001) Photodegradation of FeDTPA in nutrient solutions. I. Effects of irradiance, wavelength and temperature. *HortScience* 36:313–316
- Alloway BJ (2008) Zinc in soils and crop nutrition, 2nd edn. IZA and IFA, Brussels/Paris
- Amin AA, Gharib AEF, El-Awadia M, Rashad ESM (2011) Physiological response of onion plants to foliar application of putrescine and glutamine. *Sci Hortic* 129:353–360
- Aravind P, Prasad MNV (2005) Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino and organic acids. *Chemosphere* 61:1720–1733
- Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. *New Phytol* 173:677–702
- Chapman HD, Pratt PF (1961) Methods of analysis for soils, plants, and waters. Priced Publication 4034. Division of Agriculture Sciences, University of California, Berkeley
- Eid RA, Taha LS, Ibrahiem SMM (2011) Alleviation of adverse effects of salinity on growth, and chemical constituents of marigold plants by using glutathione and ascorbate. *J Appl Sci Res* 7:714–721
- El-Bassiouny HMS, Mostafa HA, El-Khawas SA, Hassanein RA, Khalil SI, Abd El-Monem AA (2008) Physiological responses of wheat plant to foliar treatments with arginine or putrescine. *Aust J Basic Appl Sci* 2:1390–1403
- Ghasemi S, Khoshgoftarmansh AH, Hadadzadeh H, Jafari M (2012) Synthesis of iron–amino acid chelates and evaluation of their efficacy as iron source and growth stimulator for tomato in nutrient solution culture. *J Plant Growth Regul.* doi:10.1007/s00344-012-9259-7
- Gonzalez D, Obrador A, Alvarez JM (2007) Behavior of zinc from six organic fertilizers applied to a navy bean crop grown in a calcareous soil. *J Agric Food Chem* 55:7084–7092
- Hangarter RP, Stasinopoulos TC (1991) Effect of Fe-catalyzed photooxidation of EDTA on root-growth in plant culture media. *Plant Physiol* 96:843–847
- Jämtgård S, Näsholm T, Huss-Danell K (2008) Characteristics of amino acid uptake in barley. *Plant Soil* 302:221–231
- Kalaycia M, Torumb B, Ekerb S, Aydina M, Ozturkb L, Cakmak I (1999) Grain yield, zinc efficiency and zinc concentration of wheat cultivars grown in a zinc-deficient calcareous soil in field and greenhouse. *Field Crop Res* 63:87–98
- Keutgen A, Pawelzik E (2008) Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. *Food Chem* 111:642–647
- Khoshgoftarmansh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M (2010) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. *Agron Sustain Dev* 30:83–107
- Lever ABP (1984) Inorganic electronic spectroscopy. Elsevier, Amsterdam
- Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego
- Metsarinne S, Rantanen P, Aksela R, Tuhkanen T (2004) Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). *Chemosphere* 55:379–388
- Nakamoto K (2009) Infrared and Raman spectra of inorganic and coordination compounds, 6th edn. Wiley, New York
- Nassar AH, El-Tarabily KA, Sivasithamparam K (2003) Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. *Plant Growth Regul* 40:97–106
- Nowack B, Baumann U (1998) Biodegradation of the photolysis products of Fe(III)EDTA. *Acta Hydroch Hydrob* 26:104–108
- Oburger E, Kirk GJD, Wenzel WW, Puschenreiter M, Jones D (2009) Interactive effects of organic acids in the rhizosphere. *Soil Biol Biochem* 41:449–457
- Rashad ESM, El-Abagg HM, Amin AA (2003) Physiological effects of some bioregulators on growth and productivity of two broad bean cultivars. *Egypt J Appl Sci* 18:132–149
- Rasouli-Sadaghiani MH, Sadeghzadeh B, Sepehr E, Rengel Z (2011) Root exudation and zinc uptake by barley genotypes differing in Zn efficiency. *J Plant Nutr* 34:1120–1132
- Svennerstam H, Ganeteg U, Bellini C, Näsholm T (2007) Comprehensive screening of *Arabidopsis* mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. *Plant Physiol* 143:1853–1860
- Vadas TM, Zhang X, Curran AM, Ahner BA (2007) Fate of DTPA, EDTA and EDDS in hydroponic media and effects on plant mineral nutrition. *J Plant Nutr* 30:1229–1246
- Wallace GA, Wallace A (1982) Micronutrient uptake by leaves from foliar sprays of EDTA chelated metals. In: Nelson SD (ed) Iron

- nutrition and interactions in plants. Marcel Dekker, Basel, pp 975–978
- Wang HJ, Wu LH, Wang MY, Zhu YH, Tao QN, Zhang FS (2007) Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pak-choi (*Brassica chinensis* L.). *Pedosphere* 17:595–600
- Xu WH, Liu H, Ma QF, Xiong ZT (2007) Root exudates, rhizosphere Zn fractions, and Zn accumulation of ryegrass at different soil Zn levels. *Pedosphere* 17:389–396
- Zeid IM (2009) Effect of arginine and urea on polyamines content and growth of bean under salinity stress. *Acta Physiol Plant* 31:65–70
- Zhang S, Hu F, Li H, Li X (2009) Influence of earthworm mucus and amino acids on tomato seedling growth and cadmium accumulation. *Environ Pollut* 157:2737–2742
- Zhou Z, Zhou J, Li R, Wang H, Wang J (2007) Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. *Plant Soil* 292:105–117