Complexation Of Amino Acid With Cadmium And Its Application To Remove Cadmium From Contaminated Soil

Wenbin Yao

Central South University

Zhihui Yang (✉ yangzh@csu.edu.cn)

Central South University

Lei Huang

Guangzhou University

Changqing Su

Hunan University of Technology and Business

Research Article

Keywords: Amino acid, Cadmium, Complexation structure, Soil

Posted Date: April 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-415354/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Low molecular organic acids, such as amino acid, play an important role in cadmium (Cd) mobility. However, its complexation ability with Cd was not well studied. The complexation structure of amino and cadmium was investigated by theory calculation based on B3ly/SDD and detecting by FTIR spectrum. The conformers were found to be $[CO_c, CO_d]$ for fatty amino-cadmium and $PheCd^{2+}$, $[CO_c, CO_d, CO_s]$ for $GluCd^{2+}$ and $ThrCd^{2+}$, respectively. The complex energy of these conformers was calculated in water phase by SMD model and the order of chelation energy was; $PheCd^{2+} > AlaCd^{2+} > LeuCd^{2+} > GluCd^{2+} > GlyCd^{2+} > ThrCd^{2+}$. All the dissolving energy of complexes was below zero, indicating these complexes was easily dissolved in water. In aqueous solution experiment, the Cd^{2+} concentration decreased with increasing amino acid concentration. The order of $\log\beta$ (Complex stability constant) was: $PheCd^{2+} > AlaCd^{2+} > LeuCd^{2+} > GluCd^{2+} > GlyCd^{2+} > ThrCd^{2+}$, consisting with the order of calculated chelation energy. The Cd removal efficiency by Thr, Glu, Gly, Ala, Leu and Phe were 38.88%, 37.47%, 35.5%, 34.72%, 34.04% and 31.99%, respectively. From soil batch experiment, the total Cd in soil was decreased in present of amino acid with the concentration of Cd in water increased from 231.97 $\mu\text{g/L}$ to 652.94-793.51 $\mu\text{g/L}$. The results of BCR sequential extraction showed that the Cd in acid soluble and reducible fraction sharply decreased. From all the results, the amino acid has potential to be used as a chelation to remedy the Cd contaminated soil.

Introduction

The “Itai-Itai disease” happened in Japan during the 1950s was attributed to the prolonged intake of cadmium-contaminated rice and aroused worldwide concern.(Huang et al. 2009) Gummuluru et al (2004) claimed that Metal bioavailability is considerably depended on chemical speciation of metal and correlates with the activity of free Cd ion in soil solution. (Gummuluru S. R. Krishnamurti et al. 2004) Voets et al (2004) reported that the type and concentration of organic ligands play a critical role on the activity of Cd. (Voets et al. 2004) Thus, washing method with organic ligand was used to remove Cd from contaminated soil. However, the synthesized organic ligand might introducing ecological damage to environment, causing harm for plant and prevent growth of plants, so the natural organic ligand was aroused widely concentration.(Borggaard et al. 2019, Hosseini et al. 2020, Yu et al. 2019) As reported by previous study, plant root exudates have a great impact on the bio-availability and mobility of heavy metals in the soil.(Sun et al. 2020, Tao et al. 2019, Ubeynarayana et al. 2021, Vranova et al. 2013) Among these exudates, amino acid with amounts of function group like carboxyl, hydroxyl and amino group, have important effects on the mobility and bio-availability of heavy metals by forming stable complexing with heavy metal.(CHANGEr-Hua 2009, Ghasemi et al. 2013, Rogiers et al. 2016) The study of Ghasemi et al.(2013) indicated that amino acid could produce stable complex with heavy metal and affect the activity of Cd. Thus, it could be concluded that the natural amino acid might have great potential to be used to remedy the Cd contaminated soil and worth being studied.

According to Jones et al (2005), (Jones et al. 2005) the common amino acids in soil were: Glutamic (Glu), Alanine (Ala), Glycine (Gly), Threonine (Thr), Leucine (Leu) and Phenylalanine (Phe). Among them, the carboxyl group is an electron-donating group capable of complexing metal ions, and the amino group is an amphoteric group capable of donating and obtaining electrons (Bell et al. 2016). Therefore, the conformers of amino-Cd complexes are diverse and complex. According to Dudev and Lim (2009), different complexes structure caused by different side chain, might affect metal-binding affinity. (Dudev and Lim 2009) Theoretical calculation was widely used method to study the complexes' structure and affinity. (Bingyu et al. 2018, Close et al. 2018) Comparing the calculated theoretical spectrum with the experimental infrared spectrum could confirm the existence of the calculated structure and avoid computational error. (Dunbar et al. 2009) The metal-binding affinity of complexes could be studied by chelation energy. (Pearson 2005) However, the effects of amino acid complexes on the Cd removal and fraction transfer in soil are not clear.

Thus, the objectives of this study were: (1) to identify the possible conformers of those amino-cadmium; (2) to conform the complex ability of the amino-cadmium complexes; (3) to investigate the effect of ligand concentration and cation on the chelation of amino-cadmium; (4) to evaluate the remove effective and the fraction transfer of Cd in soil by amino acids.

Experimental And Computational

2.1 Calculation method

Most of amino acids would exist as zwitterions in which the N-terminus is protonated and the C-terminus is deprotonated (Jockusch et al. 1999). Manual of chemical analysis shows most pK_a of carboxyl ranging from 2.0 to 3.0 and the pK_a of $-NH_3^+$ ranging from 9.0 to 10.0, which support this point indicating the cadmium may bind with amino and form salt-bridge structure. According to the study of Armentrout, four conformers of calculated $PheCs^{2+}$ were considered as starting points for geometry and vibrational frequency calculations. (Armentrout et al. 2013, Phillips 2002) The conformers of metal-Gly complex calculated in previous work were used as starting points for geometry and vibrational frequency calculations for fatty amino acid. (Bowman et al. 2010) And the conformers $ThrCd^{2+}$ were considered according to the study of Bowman and P. B. Armentrout (Armentrout et al. 2013, Bowman et al. 2010). For $GluCd^{2+}$, the conformers of $GluBa^{2+}$ and $GluLi^+$ complexes studied by Jeremy T. O'Brien were considered (O'Brien et al. 2008). All theoretical calculations were done using Gaussian09. According to previous study, the SDD was suitable for calculate Cd complexes. (Dudev and Lim 2009, Frisch and Frisch 1999) To ensure the accuracy of SDD method, a few known compound was calculated and compared the bond length with the actual bond length in Table 1. There is only a little error from the actual M-L bond distances with calculated M-L bond distances and this result indicated the SDD method could be used in this study (Table 1). Thus, Relative energies were determined for the geometries by using single point energies calculated at B3LYP levels using SDD basis set in this study.

Table.1 Comparison between Computed and Experimental Average Metal-Ligand (M-L) Bond Distances (in Å)

Molecule	M-L	RM-L (calc)	RM-L (expt)
[Cd(H ₂ O) ₆] ²⁺	Cd-O	2.26	2.27 (± 0.04)
[Cd(NH ₃) ₆] ²⁺	Cd-N	2.35	2.37 (± 0.03)

2.2 Experiment

2.2.1 Infrared spectrum detection of complex

In order to produce enough complexes and avoid the interference of amino acids, the concentration of cadmium is much higher than that of amino acids. 10 ml of 0.01 mol/L amino acid solution was reacted with 10 ml of 0.1 mol/L Cd solution for 48 hours and the pH was adjusted to 7.0 (with 0.01 mol/L NaOH and 0.01 mol/L HCl). The reacted solution was dried in a water bath at 343K. The precipitation was centrifuged at 10000 r/min and separated. All the IR spectrum of separated precipitation was determined by FTIR.

2.2.2 Experiment in water phase

2.2.2.1 Effect of amino concentrations on chelation

The experiment in water phase was carried out in 100 ml amino acid (concentration from 0 to 0.1 mmol/L) with 0.01 mmol/L CdCl₂. The pH value was adjusted to 7.0 with 0.1 mol/L HCl and NaOH solution. The cation concentration was adjusted to 0.1 mol/L by 1 mol/L KCl solution. The equation of chelation process was shown as follow:

$$\log \beta = \log \frac{(L_pCd)}{(C_{Cd^{2+}})(L)^p} \tag{2}$$

$$\log \frac{(L_pCd)}{(C_{Cd^{2+}})} = \log \beta + p \log L \tag{3}$$

Where the L_pCd was the concentration of complexes; $C_{Cd^{2+}}$ was concentration of Cd ions; $\log \beta$ was complex stability constant; p was the numbers of amino acid complexed with Cd; L was the concentration of amino acid. When the concentration of amino acid was much more than concentration of Cd, the concentration of L_pCd is approximately equal to the concentration of total Cd in solution and the

concentration of amino acid is approximately equal to the concentration of total amino acid. The concentration of Cd^{2+} was measured by Cd ion meter (Orion 9648BNWP) at 296 K.

2.2.2.2 Effect of cation concentration on chelation

100 ml of 0.01 mmol/L CdCl_2 was mixed with a series of 0.1 mmol/L amino acid at 7.0 pH value (adjusted by 0.01 mol/L NaOH and HCl). The cation concentration was prepared by KCl solution with concentration varying from 0.05 mol/L to 0.5 mol/L. The Cd^{2+} concentration was detected by Cd ion meter at 296 K.

Table 2
Basic properties of soils

Soil components	HY soil
Organic matter	4.88%
Clay (< 0.002mm)	16.92%
Sand (2-0.02mm)	68.32%
Silt (0.02-0.002mm)	14.76%
pH	6.7
CEC (meq NH_4^+ /100 g soil)	21.41
Total Cd	20.33 mg/kg

2.2.3 Bath soil experiment

The heavily Cd-contaminated soil was collected from ChangNing city, Hunan Province, China. The soil basic properties are shown in Table 2. A serial of amino acid solution from 0.1 to 1 mmol/L (adjusted with 0.1 mol/L NaOH and 0.1 mol/L HCl to soil pH 6.5) was prepared. The amino acid solution was mixed with soil at 10:1 of liquid to soil ratio. The mixture solution was shaken for 48 hours and centrifuge separation at 8000 r/min for 10 minutes. After centrifuging, Cd concentration in the separated solution was tested by ICP-OES (Agilent 5100). The BCR^(Fajković et al. 2017) sequential extraction method was used to analyze the Cd transfer among different fractions in soil treated by amino acids. The heavy metals were was mainly distributed in four fractions: HOA_C -extractable, reducible, oxidizable, and residual fraction, which were extracted with four extractants: (1) 0.11 mol/L acetic acid (AcOH) was added into soil to extract exchangeable species and the weak acid soluble fraction (HOAC -extractable fraction); (2) 0.5 mol/L hydroxylamine hydrochloride was adjusted to pH 2 with HNO_3 to extract the reducible metal species bound to Fe–Mn oxyhydroxides (reducible fraction); (3) 8.8 mol/L hydrogen peroxide and 1 mol/L ammonium acetate (AcONH_4) was employed to extract oxidizable metal species bound to organics and sulphides (oxidizable fraction); and (4) aqua regia was used to obtain the residual fraction. The Cd concentrations in extractants were analyzed with an ICP-OES (Agilent 5100).

Species	Structure	R Cd-O _c	R Cd-X _c –N,O	R Cd-Y _s	<O _c CdX _c	<OCdY _s	<X _c CdY _s
GlyCd ²⁺	[CO _c , CO _c]	2.23	2.28		59.85		
	[CO _c]	2.18					
	[N _c , OH _c]	2.23	2.16		80.26		
	[N _c , CO _c]	2.28	2.16		80.30		
AlaCd ²⁺	[CO _c , CO _c]	2.28	2.23		59.93		
	[CO _c]	2.22					
	[N _c , OH _c]	2.21	2.17		79.82		
	[N _c , CO _c]	2.28	2.16		80.33		
LeuCd ²⁺	[CO _c , CO _c]	2.23	2.28		59.99		
	[CO _c]	2.18					
	[N _c , OH _c]	2.22	2.17		49.87		
	[N _c , CO _c]	2.25	2.17		48.60		
GluCd ²⁺	[CO _c , CO _c , CO _s]	2.21	2.31	2.28	59.42	88.50	87.34
	[CO _c , N _c , CO _s]	2.26	2.17	2.3	115.00	98.59	76.73
	[CO _c , CO _c]	2.30	2.27		59.10		
	[N _c , CO _c]	2.23	2.17		80.97		
ThrCd ²⁺	[CO _c , N _c , CO _s]	2.24	2.27	2.23	76.44	85.70	75.79
	[CO _c , CO _c]	2.23	2.27		60.01		
	[CO _c , CO _c , CO _s]	2.25	2.30	2.25	60.23	72.94	71.03
	[N, CO]	2.45	2.27		71.40		
PheCd ²⁺	[CO _c , CO _c]	2.20	2.54		48.87		
	[N, CO]	2.23	2.34		75.09		
	[N, P]		2.20	2.33	37.53		

Results And Discussions

3.1 The conformers of cadmium and amino acid

3.1.1 Conformers of Gly, Leu and Ala with Cd^{2+}

The Gly, Leu and Ala was classified as fatty amino acid for the similar side chain. The possible conformers of Gly-Cd^{2+} after calculated is shown in Fig. 1. There are series of conformers was found through calculation: [N] structure, where Cd bound with backbone amino nitrogen; [CO] structure, where Cd bound with the backbone carbonyl; [N, OH] structure, where Cd bound with amino nitrogen and hydroxyl group and [CO_c, CO_d] structure, where Cd bound with both oxygens of the carboxylic acid and ammonia forms a strong H-bond with the carboxylic group of protonated Gly.

In the spectrum of GlyCd^{2+} , the main spectral features were observed at 1630, 1590, 1480, 1450, 1410, 1325, 1110, 950, 720 cm^{-1} . For the GlyCd^{2+} , the most intense spectral features in the [CO_c, CO_d] conformer of GlyCd^{2+} were relatively well correlated with experimental spectrum. The bands observed at 1640 cm^{-1} (rotation of N-H), 1580 cm^{-1} (bending of N-H), 1500 cm^{-1} , 1410 cm^{-1} (stretching of C = O), 1325 cm^{-1} (stretching of C-O), 1100 cm^{-1} (stretching of C-N), 1040 cm^{-1} are consistent with the [CO_c, CO_d]. However, the band at 1660 cm^{-1} and 1600 cm^{-1} of [CO_c, CO_d] conformer was red shifted by 20 cm^{-1} compared with the experimental spectrum. And the bands near 1410 cm^{-1} and 1350 cm^{-1} were red shifted by 25 cm^{-1} and blue shifted by 30 cm^{-1} respectively. The detail of complexes was depicted in Table 2. The length of Cd-O of [CO_c, CO_d] were shorter than other structures with 2.18 and 2.13 Å and the $\angle \text{OCdO}$ was 59°. Due to the similar structure, the results of Ala and Leu was similar with Gly. As depicted in Fig.S1 and Fig.S2, the conformer of AlaCd^{2+} and LeuCd^{2+} also was [CO_c, CO_d]. From the detail information in Table 3, the length of O-Cd bond was 2.23 and 2.28 Å. Though, the O-Cd was longer than N-Cd, the previous study found the strength of O-Cd was much stronger than N-Cd, indicating [CO, CO] conformer was more stable than others.(Remelli et al. 2016) Thus, the GlyCd^{2+} , AlaCd^{2+} and LeuCd^{2+} were mainly exist as [CO, CO] conformer.

3.1.2 Conformers of Glu with Cd^{2+}

Glu has a carboxyl group in side chain with 4.25 dissociation PK constants, thus the dehydrogenation of side chain and the tridentate conformer should be considered. (Sarkowski 1982) After calculated, several conformers was found: [CO_c, N_c, CO_s] structure where one oxygen from each of the carboxylic acid groups and the N-terminal nitrogen all coordinate to the metal ion; [CO_c, CO_c, CO_s] structure (proton transfered from an oxygen atom of the carboxylate group to N), where metal bond to both the oxygen atom in terminal carbonyl and one of the oxygen in side chain; [CO_c, CO_d] and [N, OH] structure.(O'Brien et al. 2008) The spectral of GluCd^{2+} was shown in Fig. 2 The most intense spectral features of the [CO_c, CO_c, CO_s] conformer were fairly well correlate with those experimental IR. The bands of [CO_c, CO_c, CO_s] conformer at 1619 cm^{-1} (stretching of C = O) and 1109 cm^{-1} (bending of C-H) were correlated with the

experimental well. The bands at 1690 cm^{-1} (bending of N-H) and 1550 cm^{-1} (vibration of N-H) were red-shifted by 15 cm^{-1} and 5 cm^{-1} . The band at 1290 cm^{-1} (vibration of C = O) was blue-shifted 10 cm^{-1} . However, there was no band in the $[\text{CO}_c, \text{CO}_c, \text{CO}_s]$ spectral correlated the band at 1480 cm^{-1} of experimental IR. The $[\text{CO}_c, \text{CO}_c]$ conformer may contribute to the band at 1480 cm^{-1} (vibration of C-H) of experimental IR. The band at 1440 cm^{-1} (vibration of C-O of the carboxyl at side chain) in the spectral of $[\text{CO}_c, \text{CO}_c]$ conformer was only blue-shifted 10 cm^{-1} , comparing to the experimental. Other than the 1440 cm^{-1} , other bands of the $[\text{CO}_c, \text{CO}_c]$ conformer also were correlated the experimental IR well. The most intense bands of the $[\text{CO}_c, \text{CO}_c]$ conformer at 1615 cm^{-1} (bending of N-H) and 1580 cm^{-1} (stretching of C = O in sidechain) were blue shifted by 25 cm^{-1} and red shifted by 30 cm^{-1} from the bands at 1590 cm^{-1} and 1550 cm^{-1} , respectively. The band at 1442 cm^{-1} (stretching of C-O in sidechain) was blue-shifted about 18 cm^{-1} comparing to the band of experimental 1450 cm^{-1} . The band at 1120 cm^{-1} was red-shifted by 10 cm^{-1} comparing to the band at 1110 cm^{-1} . As shown in Table 3, the lengths of O-Cd of $[\text{CO}_c, \text{CO}_c, \text{CO}_s]$ conformer were 2.21, 2.31 and $2.28\text{ \AA}$. the lengths of $[\text{CO}_c, \text{CO}_c]$ were 2.30 and $2.27\text{ \AA}$, which was similar with that of GlyCd^{2+} . The O-Cd of $[\text{CO}_c, \text{CO}_c, \text{CO}_s]$ in terminated carboxy was shorter than that of $[\text{CO}_c, \text{CO}_c]$, indicating stronger bond of O atom and Cd^{2+} ion. Besides, according to previous studies, tridentate complexes was much more stable than bidentate complexes. (Aly et al. 2018, Beheshti et al. 2007, Fneich et al. 2018, Keskin et al. 2017, Kumarasamy et al. 2020, Zhang et al. 2014) Thus, the GluCd^{2+} was mainly exist as stable $[\text{CO}_c, \text{CO}_c, \text{CO}_s]$ conformers.

3.1.3 Conformers of Thr with Cd^{2+}

ThrCd^{2+} complexes have very similar metal-ligand bond distances for all analogous conformers and calculations identify the ground state conformer as the NZ tridentate $[\text{CO}_c, \text{CO}_s, \text{N}_d]$ for ThrCd^{2+} . The spectra of ThrCd^{2+} were shown in Fig. 2b, and the calculated spectra of $[\text{CO}_c, \text{CO}_s, \text{N}_d]$ was correlated the experimental IR. The bands at 1620 cm^{-1} , 1460 cm^{-1} , 1340 cm^{-1} were assigned to stretching of C = O, bending of C-H and bending of N-H, respectively, and fitted to the spectral feature of experimental IR. The bands at 1427 cm^{-1} for bending of O-H and 1132 cm^{-1} for vibration of C-C were red-shifted by 16 cm^{-1} and 12 cm^{-1} comparing to experimental IR. However, the $[\text{CO}_c, \text{CO}_c]$ conformer was also a reasonable structure for the agreement in the lower frequency region. The bands at 1080 cm^{-1} of C-C rotation had experimental intensities much higher than the theoretically predicted value. And the peak at 1590 cm^{-1} of stretching of C = O was blue-shifted by 30 cm^{-1} comparing to experimental IR. As reported by Cao et.al, amine of Thr was less protonated when pH up 5.6 and the group of $-\text{NH}_2$ bond with heavy metal. (Wang et al. 2016) Thus, the Cd mainly bound with NH_2 . In Table 3, the lengths of $[\text{CO}_c, \text{CO}_s, \text{N}_d]$ were 2.23 and $2.24\text{ \AA}$, respectively, much shorter than others. The N-Cd of $[\text{CO}_c, \text{CO}_s, \text{N}_d]$ and $[\text{CO}_c, \text{N}_d]$ was $2.27\text{ \AA}$. Thus, the $[\text{CO}_c, \text{CO}_s, \text{N}_d]$ of ThrCd^{2+} was relatively stable than $[\text{CO}_c, \text{N}_d]$.

3.1.4 Conformers of Phe with Cd^{2+}

For PheCd^{2+} , the benzene (P) contain electron, thus the bond of Cd and benzene was also considered. From the IR spectra of PheCd^{2+} (Fig. 4), the calculated spectra of $[\text{CO}_c, \text{CO}_d]$ was fairly well correlated the experimental IR. The main bond of the actual IR was observed at 1520, 1330. The most intense band at 1520 cm^{-1} (vibration of N-H) of $[\text{CO}_c, \text{CO}_d]$ conformers was corresponded to the experimental IR. The band at 1330 cm^{-1} (stretching of C-C) was also fit actual IR well. There are no bands of $[\text{N}_c, \text{P}]$ and $[\text{N}_c, \text{CO}_d]$ conformers fitting the IR. The length of Cd-O bond was much longer than other complexes with 2.20 and $2.54\text{ \AA}$, which was attributed to the attractivity of benzene ring (Table 3).

3.2 Theory calculation of chelation energy

From the result of FTIR spectrum, the structure of Glu and Thr was chelation tridentate, while the others were bidentate. Based on the conformers, the frees energy of dissolving process (Eq. 4) and chelation process (Eq. 5) was calculated in water phase.

As depicted in Table 4, all the dissolving energy of complexes was negative, indicating all these complexes were easily dissolved in water. All the chelation energy was also below zero, indicating the chelation process was spontaneous reaction in water phase. The order of Cd and amino acid chelation energy was: Thr < Glu < Gly < Ala < Leu < Phe. The energy of Cd and Thr chelation process was the lowest, indicating ThrCd^{2+} was the most stable complex. In addition, chelation energy of Glu and Glu was -24.33 kcal/mol and -23.87 kcal/mol in water phase, which was much higher than other amino acid. This result might be attributed to the different conformer of Thr and Glu. Compared to other amino acids, the additional -OH an -COOH in side chain of Thr and Glu might donor more electron to Cd^{2+} . Thus, the Thr and Glu is better electron-donor for heavy metal ion. Besides, the structure of Gly, Ala, Leu and Phe was bidenate conformer and the energy increased with the increasing molecule weight. In water phase, the water molecule would prevent the mobility of ligand agents. Thus, the larger molecule introduced more entropy penalty.

Table 4
the Calculated energy of amino acid and cadmium complexes (kcal/mol)

Amino acid	ThrCd ²⁺	GluCd ²⁺	GlyCd ²⁺	AlaCd ²⁺	LeuCd ²⁺	PheCd ²⁺
Chelation energy	-24.33	-23.87	-22.26	-21.58	-20.31	-18.96
Dissolving energy	-260.94	-248.42	-279.17	-272.23	-264.98	-254.29

3.3 Chelation in water phase

In fact, the forming of Cd complexes might be affected by various factors in water. As shown in Fig. 5, the Cd^{2+} increased with the increasing cation concentration, implying the decrease of ligand-Cd complexes concentration. In fact, the concentration of cation might affect the chelation by compete the function group. According to previous study, natural groundwater contained ions (such as Na^+ , and K^+), which compete with Cd^{2+} for ligand.(Xie et al. 2020)

The concentration of chelation agent also affects the concentration of Cd^{2+} . As shown in Fig. 5 the Cd^{2+} decreased with increasing amino acid concentration from 0 to 0.1 mmol/L. The increase of ligand introducing soluble complexes of Cd and decrease the Cd^{2+} without precipitation.(Zhang et al. 2014) The minimum $logCd^{2+}$ concentration was: -9.26 mmol/L, -9.06 mmol/L, -8.92 mmol/L, -8.84 mmol/L, -8.56 mmol/L and - 8.54 mmol/L for Thr, Glu, Gly, Ala, Leu and Phe. The complexation constants were calculated through Eq. (3), and the fit curve was shown in Fig. 6. The constants “p” was greater than 1, indicating the that cadmium exists in the form of multidentate complex in high concentration amino acid solution(Table 5). The constant $log\beta$ of these complexes were 7.06, 6.99, 6.69, 6.62, 6.67 and 6.30 for Thr, Glu, Gly, Leu, Ala and Phe, respectively. The order of $log\beta$ of these complexes was Thr > Glu > > Gly > Ala > Leu > > Phe, consisting with the order of chelation energy. The result implied that the Thr and Glu has greater ability to chelate Cd^{2+} to form the stable conformer of $ThrCd^{2+}$ and $GluCd^{2+}$.

Table 5
The parameter of fit curve

Amino acid	Thr	Glu	Gly	Ala	Leu	Phe
p	2.3	2.31	1.84	1.91	1.87	1.61
logβ	7.06	6.99	6.69	6.67	6.62	6.30
R ²	0.95	0.93	0.96	0.92	0.93	0.89

3.4 Removal of Cd from contaminated soil

Unlike in water phase, the condition in the soil was complicated. As shown in Fig. 7 the leachable Cd concentration increased with the increasing amino acid concentration from 0 to 1000 μmol/L. In distilled water (control treatment), the leachable Cd was only 231.97 μg/L. Maxium leachable Cd of these amino acid were 793.51 μg/L, 764.75 μg/L, 724.62 μg/L, 708.72 μg/L, 694.66 μg/L and 652.94 μg/L for 1000 μmol/L Thr, Glu, Gly, Ala, Leu and Phe, respectively. The Cd removal efficiency by Thr, Glu, Gly, Ala, Leu and Phe were 38.88%, 37.47%, 35.5%, 34.72%, 34.04% and 31.99%, respectively. Thus, it could be concluded that Cd was complex with the function groups of amino acid to formed soluble complexes, inhibiting the adsorption of Cd on soil surface. Besides, the Thr was found have much more ability to remove Cd from soil, which was consistent with the previous study. (Dolev et al. 2020)

3.6 Distribution of Cd in soil fraction

To elucidate the fraction distribution of heavy metals after treated by amino acid, the BCR extraction was implemented. These acids soluble and reducible Cd with higher bio-availability are easily taken up by

plants, while the oxidable and residual Cd are hardly taken up. In un-treated soil, distribution of Cd was mainly in acid soluble fraction.(Fernández-Ondoo et al. 2016) As shown in Fig. 8, the Cd in acid soluble fraction, reducible fraction, oxidable fraction and residual fraction were 33.81%, 21.36%, 13.73% and 31.1%, respectively. After treated by amino acid, the acid soluble and reducible Cd sharply decreased. In present of Thr, the Cd in acid soluble fraction decreased to 16.48% and the reducible fraction decreased to 14.72%. Similarly, in present of the Glu, Cd in acid soluble fraction decreased to 16.58% and the reducible fraction decreased to 15.24%. These results indicate the Cd in acid soluble fraction and reducible fraction have strong mobility and chelate with amino acid to be washed out from soil. According to previous studies, the heavy metals bound with acid soluble fraction has highly mobility and was easily extractable from soil in present of chelation agents. The Cd in residual fraction was mainly exist in crystal of mineral, which was hardly removed by chelating with organic agents.(Li et al. 2018, Xing et al. 2021) The decreased trend of Cd in acid soluble and residual fraction after treated by amino acid implied that Cd can be released into solution to be taken up by plant or washed out by.

Conclusion

In this study, the complexation effectiveness of amino acid and Cd^{2+} was investigated by theory calculate, FTIR spectrum, chelation experiment in water and soil batch experiment. The conformers of GlyCd^{2+} , LeuCd^{2+} , AlaCd^{2+} and PheCd^{2+} complexes were found to be $[\text{CO}_c, \text{CO}_d]$ where the Cd^{2+} bidentate with oxygen atom of carboxyl group; the conformers of Thr and Glu was found to be $[\text{CO}_c, \text{CO}_c, \text{CO}_s]$ where Cd^{2+} tridentate with both the oxygen atom in terminal carbonyl and one of the oxygen in side chain; the chelation energy of these conformers was: $\text{PheCd}^{2+} > \text{AlaCd}^{2+} > \text{LeuCd}^{2+} > \text{GlyCd}^{2+} > \text{GluCd}^{2+} > \text{ThrCd}^{2+}$, indicating the GluCd^{2+} and ThrCd^{2+} were more stable than the others. And the dissolving energy of these complexes was below zero, indicating these complexes were easily dissolved in water. The order of $\log\beta$ of chelation was: $\text{PheCd}^{2+} < \text{AlaCd}^{2+} < \text{LeuCd}^{2+} < \text{GlyCd}^{2+} < \text{GluCd}^{2+} < \text{ThrCd}^{2+}$, consisting with the order of chelation energy. The pH and cation concentration has negative relationship with the chelation process. The increased pH value and cation concentration prevent the chelation reaction for competing. In contaminated soil, the remove efficiency of Cd increased with the increasing amino acid concentration. At 1000 $\mu\text{mol/L}$ amino acid solution, the remove efficiency of Cd was from 31.99–38.84%. the Thr has the best ability to remove Cd from soil for the stronger affinity of ThrCd^{2+} with the lowest chelation energy and the highest $\log\beta$. With amino acid supplying, the Cd in acid soluble and reducible fraction in soil sharply decreased, indicating that Cd was transferred into more stable oxidable and residual fractions. Thus, amino acid was found having ability to be used to remedy the Cd contaminated soil.

Declarations

Funding

The study was supported by the National Key R&D Program of China (Grant number: 2020YFC1808002, 2019YFD1100500)

Loading [MathJax]/jax/output/CommonHTML/jax.js

Author contributions

Zhihui Yang designed the experiments. Wenbin Yao conducted most of the experiments and analyzed the data. Lei Huang assisted in experiments and discussed the results. Wenbin Yao drafted the manuscript. Zhihui Yang, Lei Huang and Changqing Su revised the manuscript. All authors read and approved the final manuscript.

Ethics Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Data Availability

Not applicable

References

Aly, A.A., Bräse, S. and Weis, P. (2018) Tridentate and bidentate copper complexes of [2.2]paracyclophanyl-substituted thiosemicarbazones, thiocarbazones, hydrazones and thioureas. *Journal of Molecular Structure* 1178, 311-326.

Armentrout, P.B., Yang, B. and Rodgers, M.T. (2013) Metal Cation Dependence of Interactions with Amino Acids: Bond Energies of Rb^+ and Cs^+ to Met, Phe, Tyr, and Trp. *Journal of Physical Chemistry B* 117(14), 3771-3781.

Beheshti, A., Clegg, W., Dale, S. and Hyvadi, R. (2007) Synthesis, crystal structures, and spectroscopic characterization of the neutral monomeric tetrahedral $[M(Diap)(2)(OAc)(2)] \cdot nH_2O$ complexes ($M = Zn, Cd$; Diap=1,3-diazepane-2-thione; OAc = acetate) with N-H $\cdots \cdots O$ an. *Inorganica Chimica Acta* 360(9), 2967-2972.

Bell, J., Samb, I., Toullec, P.Y., Michelet, V. and Leray, I. (2016) Synthesis and complexing properties of molecular probes linked with fluorescent phosphane oxide derivatives. *Journal of Photochemistry & Photobiology A Chemistry* 318, 25-32.

Bingyu, Nie, Ruifang, Li, Yang, Wu, Xing, Yuan, Wei and Zhang (2018) Theoretical Calculation of the Thermodynamic Properties of 20 Amino Acid Ionic Liquids. *Journal of Physical Chemistry B* 2018, 122, 46, 10548-10557.

Borggaard, O.K., Holm, P.E. and Strobel, B.W. (2019) Potential of dissolved organic matter (DOM) to extract As, Cd, Co, Cr, Cu, Ni, Pb and Zn from polluted soils: A review. *Geoderma* 343, 235-246.

Bowman, V.N., Heaton, A.L. and Armentrout, P.B. (2010) Metal cation dependence of interactions with amino acids: bond energies of Rb⁺ to Gly, Ser, Thr, and Pro. *Journal of Physical Chemistry B* 114(11), 4107-4114.

CHANGEr-Hua, Z.S.-F., WANG Zhi-Qin, WANG Xue-Ming, YANGJian-Chang (2009) Effect of Nitrogen and Phosphorus on the Amino Acids in Root Exudates and Grains of Rice During Grain Filling. *Acta Agronomica Sinica* 34(4), 612-618.

Close, David, M., Wardman and Peter (2018) Calculation of Standard Reduction Potentials of Amino Acid Radicals and the Effects of Water and Incorporation into Peptides. *The journal of physical chemistry, A. Molecules, spectroscopy, kinetics, environment, & general theory* 2018, 122, 1, 439-445.

Dolev, N., Katz, Z., Ludmer, Z., Ullmann, A. and Goikhman, R. (2020) Natural amino acids as potential chelators for soil remediation. *Environmental Research* 183, 109140-.

Dudev, T. and Lim, C. (2009) Metal-binding affinity and selectivity of nonstandard natural amino acid residues from DFT/CDM calculations. *Journal of Physical Chemistry B* 113(34), 11754-11764.

Dunbar, R.C., Hopkinson, A.C., Oomens, J., Siu, C.K., Siu, K.W.M., Steill, J.D., Verkerk, U.H. and Zhao, J. (2009) Conformation Switching in Gas-Phase Complexes of Histidine with Alkaline Earth Ions. *Journal of Physical Chemistry B* 113(30), 10403-10408.

Fajković, H., Rončević, S., Nemet, I., Prohić, E. and Leontićvazdar, D. (2017) Fractionation of metals by sequential extraction procedures (BCR and Tessier) in soil exposed to fire of wide temperature range. 19. 454-458

Fernández-Ondoo, E., Bacchetta, G., Lallena, A.M., Navarro, F.B. and Jiménez, M. (2016) Use of BCR sequential extraction procedures for soils and plant metal transfer predictions in contaminated mine tailings in Sardinia. *Journal of Geochemical Exploration* 172, 133-141.

Fneich, B.N., Das, A., Kirschbaum, K. and Mason, M.R. (2018) Bidentate and tridentate coordination modes of bis(3-methylindolyl)-2-pyridylmethane in complexes of aluminum and gallium: Structural characterization of bridging N-indolide in a dialuminum complex - ScienceDirect. *Journal of Organometallic Chemistry* 872, 12-23.

Frisch, Æ. and Frisch, M.J. (1999) Gaussian 98 user's reference, Gaussian 98 user's reference.

Ghasemi, S., Khoshgoftarmanesh, A.H., Afyuni, M., Hadadzadeh, H. and Schulin, R. (2013) Zinc–amino acid complexes are more stable than free amino acids in saline and washed soils. *Soil Biology & Biochemistry* 63, 73-79.

Gummuluru S. R. Krishnamurti, †, Mallavarapu Megharaj, † and Naidu†, R. (2004) Bioavailability of Cadmium–Organic Complexes to Soil Alga An Exception to the Free Ion Model. *Journal of Agricultural & Food Chemistry* 52(12), 3894-3899.

Hosseini, S.S., Lakzian, A., Halajnia, A. and Razavi, B.S. (2020) Optimization of EDTA and citric acid for risk assessment in the remediation of lead contaminated soil. *Rhizosphere* 17.

Huang, Y., Ying, H. and Liu, Y. (2009) Heavy metal accumulation in iron plaque and growth of rice plants upon exposure to single and combined contamination by copper, cadmium and lead. *Acta Ecologica Sinica* 29(6), 320-326.

Jockusch, R.A., Price, W.D. and Williams, E.R. (1999) Structure of Cationized Arginine ($\text{Arg} \cdot \text{M}^+$, $\text{M} = \text{H, Li, Na, K, Rb, and Cs}$) in the Gas Phase: Further Evidence for Zwitterionic Arginine. *Journal of Physical Chemistry A* 103(46), 9266-9274.

Jones, D.L., Shannon, D., Junvee-Fortune, T. and Farrar, J.F. (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. *Soil Biology & Biochemistry* 37(1), 179-181.

Keskin, S.G., Stanley, J.M. and Cowley, A.H. (2017) Synthesis, characterization and theoretical investigations of molybdenum carbonyl complexes with phosphorus/nitrogen/phosphorus ligand as bidentate and tridentate modes. *Polyhedron* 138, 78-85

Kumarasamy, K., Devendhiran, T., Lin, M.C. and Chiu, C.W. (2020) Synthesis and physical property studies of cyclometalated Pt(II) and Pd(II) complexes with tridentate ligands containing pyrazole and pyridine groups. *Polyhedron* 191, 114799.

Li, J.S., Chen, Z., Wang, Q.M., Fang, L., Xue, Q., Cheeseman, C.R., Donatello, S., Liu, L. and Poon, C.S. (2018) Change in re-use value of incinerated sewage sludge ash due to chemical extraction of phosphorus. *Waste Manag* 74(APR.), 404-412.

O'Brien, J.T., Prell, J.S., Steill, J.D., Oomens, J. and Williams, E.R. (2008) Interactions of mono- and divalent metal ions with aspartic and glutamic acid investigated with IR photodissociation spectroscopy and theory. *Journal of Physical Chemistry A* 112(43), 10823-10830.

O'Brien, J.T., Prell, J.S., Steill, J.D., Oomens, J. and Williams, E.R. (2008) Interactions of Mono- and Divalent Metal Ions with Aspartic and Glutamic Acid Investigated with IR Photodissociation Spectroscopy and Theory. *Journal of Physical Chemistry A* 112(43), 10823.

Pearson, R.G. (2005) Chemical hardness and density functional theory. *Journal of Chemical Sciences*

Phillips, F.C. (2002) MANUAL OF QUALITATIVE CHEMICAL ANALYSIS. Journal of the American Chemical Society 20(2),

142

–144.

Remelli, M., Nurchi, V.M., Lachowicz, J.I., Medici, S., Zoroddu, M.A. and Peana, M. (2016) Competition between Cd(II) and other divalent transition metal ions during complex formation with amino acids, peptides, and chelating agents. Coordination Chemistry Reviews 327, 55-69.

Rogiers, Suzy, Y., Holzapfel, Bruno, P., Scollary, Geoffrey, R., Canny, Martin, J., Gourieroux and Aude, M. (2016) The amino acid distribution in rachis xylem sap and phloem exudate of Vitis vinifera 'Cabernet Sauvignon' bunches. Plant Physiology and Biochemistry 105, 45-54.

Sarkowski, H. (1982) Beilsteins, Handbuch der organischen Chemie', Springer Berlin Heidelberg.

Sun, L., Cao, X., Tan, C., Deng, Y. and Bai, J. (2020) Analysis of the effect of cadmium stress on root exudates of Sedum plumbizincicola based on metabolomics. Ecotoxicology and Environmental Safety 205, 111152.

Tao, Q., Zhao, J., Li, J., Liu, Y. and Wang, C. (2019) Unique root exudate tartaric acid enhanced cadmium mobilization and uptake in Cd-hyperaccumulator Sedum alfredii. Journal of Hazardous Materials 383, 121177.

Ubeynarayana, N., Jeyakumar, P., Bishop, P., Pereira, R.C. and Anderson, C. (2021) Effect of soil cadmium on root organic acid secretion by forage crops *. Environmental Pollution 268(A).

Voets, J., Bervoets, L. and Blust, R. (2004) Cadmium bioavailability and accumulation in the presence of humic acid to the zebra mussel, Dreissena polymorpha. Environmental Science & Technology 38(4), 1003.

Vranova, Rejsek, Skene, KR, Janous and Formanek (2013) Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. J PLANT NUTR SOIL SC 2013,176(2)(-), 175-199.

Wang, Cheng, Cao, Jia-Shun, Fang, Lin and Jun-Xiong (2016) Removal of heavy metal Cu(II) in simulated aquaculture wastewater by modified palygorskite. Environmental Pollution 219, 924-931.

Xie, X., Yan, L., Li, J., Guan, L. and Chi, Z. (2020) Cadmium isotope fractionation during Cd-calcite coprecipitation: Insight from batch experiment. Science of the Total Environment 760, 143330.

Xing, L., Wen, J., Yan, C., Wang, Q. and Xue, Z. (2021) Improving the microenvironment of Cd-contaminated river sediments through humic substances washing and zeolite immobilization. Process Safety and Environmental Protection 146(10), 779-788.

Loading [MathJax]/jax/output/CommonHTML/jax.js

Yu, F., Li, Y., Li, F., Li, C. and Liu, K. (2019) The effects of EDTA on plant growth and manganese (Mn) accumulation in *Polygonum pubescens* Blume cultured in unexplored soil, mining soil and tailing soil from the Pingle Mn mine, China. *Ecotoxicology and Environmental Safety* 173(MAY), 235-242.

Zhang, S., Yu, T., Sun, M., Yu, H., Zhang, Z., Wang, S. and Jiang, H. (2014) Highly sensitive and selective fluorescence detection of copper (II) ion based on multi-ligand metal chelation. *Talanta* 126, 185-190.

Figures

Figure 1

IR spectrum of GlyCd²⁺

Figure 2

IR spectrum of GluCd2+

Figure 3

IR spectrum of ThrCd²⁺

Figure 4

IR spectrum of PheCd₂

Figure 5

Loading [MathJax]/jax/output/CommonHTML/jax.js

Concentration of Cd²⁺ in different (a) amino acid concentration and (b) cation concentration

Figure 6

The fit curve of (a) Thr, (b) Glu, (c) Gly, (d) Leu, (e) Ala, (f) Phe with Cd²⁺

Figure 7

The (a) leachable Cd concentration and (b) remove efficiency of Cd in contaminated soil

Figure 8

Loading [MathJax]/jax/output/CommonHTML/jax.js

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement.docx](#)