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Characterization of binding behaviors of Cd^{2+} to rice proteins

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ABSTRACT

In this paper, for the first time the binding behavior of cadmium (Cd^{2+}) to rice proteins (RPs) was studied. The results showed that the equilibrium of binding was reached within 30 min at 303 K with a maximum binding amount (*q*) of 15.26 mg/g, and the pH was an important factor positively influencing *q*. At both 308 K and 313 K, the binding of Cd^{2+} to RPs belonged to spontaneous, endothermic interactions with high-affinity, assigned to a multidentate coordination. Except for acetate, all the investigated competing coordination agents, such as edetate, pyrophosphate and citrate, showed inhibitory effects on RPs-Cd^{2+} binding, and edetate seemed to be the most effective one. At pH 6.5, calcium, copper and zinc began to restrict RPs-Cd^{2+} binding when the metal ion concentration reached 500 mg/kg, and the decreasing of pH would strengthen the inhibitory effects of the investigated metal ions including ferric ions.

1. Introduction

With rapid modernization and industrialization, the pollution of water, air, and soil by heavy metals is becoming a global concern (Behbahani et al., 2013; Fouladian & Behbahani, 2015). Ingestion of heavy metals such as cadmium (Cd^{2+}), lead (Pb^{2+}), copper (Cu^{2+}) is detrimental to human bodies (Behbahani, Abolhasani, et al., 2015; Behbahani et al., 2013; Behbahani, Hassanlou, et al., 2015; Ghorbani-Kalhor, Behbahani, Abolhasani, & Hosseinzadeh Khanmiri, 2014; Parvizi, Behbahani, zeraatpisheh, & Esrafili, 2018). The sources of heavy metal pollution are mainly around the industries where metal cleaning, mining activities, and metal finishing are frequently practiced (Behbahani et al., 2014; Kakavandi, Behbahani, Omidi, & Hesam, 2017).

In Southern China, large areas of cultivated land are polluted by Cd^{2+} (Hu et al., 2013; Jin et al., 2002), which accumulated in the seeds of crops, especially in rice (Grant, Clarke, Duguid, & Chaney, 2008). Despite the fact that phosphorus (P^{5+}), potassium (K^{+}), manganese (Mn^{3+}), calcium (Ca^{2+}) and the like elements can be removed during washing and cooking, Cd^{2+} remains stable in rice (Shindoh & Yasui, 2003). As a result, rice imposes as the main source of dietary Cd^{2+} for residents around Cd-polluted regions (Meharg et al., 2013; Yuan, Wang, Shang, & Sun, 2014). Development of Cd-free soil and Cd-resistant rice varieties is the most effective, yet time-consuming strategy for Cd^{2+} removal (Hu, Cheng, & Tao, 2016). Thus, it is of practical importance to remove Cd^{2+} during the processing of rice products.

It has been reported that Cd^{2+} mostly binds with proteins in rice (Huo, Du, Xue, Niu, & Zhao, 2016). While several studies investigated the Cd-protein composites and their distribution throughout rice endosperm (He, Wong, & Yang, 2002; M. He, Yang, & Cha, 2000), there is a lack of full understanding of mechanism underlying binding properties between Cd^{2+} and rice proteins (RPs). Therefore, few reports highlight the principle of Cd^{2+} removal from a theoretical standpoint of view.

By characterizing a large number of proteins from Protein Data Bank (PDB), pioneering studies indicated that those proteins are frequently bond with metal ions via carboxyls in Glu and Asp, imidazolyls in His and sulfydryls in Cys (Dokmanic, Sikic, & Tomic, 2008; Gamble & Peacock, 2014). There are considerable amounts of Glu and Aps and quite a few Cys and His in RPs (Wang, Ye, Hui, & Lin, 2016), creating abundant binding sites for metals. In addition, RPs obtain complicated structures due to hydrogen bonding, disulfide bridges, hydrophobic reactions, etc., thus can be excellent ligands for metal ions through multidentate coordination.

Because complexation usually tends to form coordinate compounds with greater stability, the complexes can be destructed by introducing

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competitors like coordination agents and metal ions. Sodium citrate, sodium acetate, sodium pyrophosphate and disodium edentate dihydrate are common coordination agents of food grade and can compete with ligands to bind metal ions (Evangelou, Ebel, & Schaeffer, 2007). On the other hand, Ca^{2+}, Cu^{2+}, zinc (Zn^{2+}) and iron (Fe^{3+}) are indispensable metals to human health and can compete with Cd^{2+} to bind ligands (Astolfi et al., 2012; Meda et al., 2007). To the best of our knowledge, few studies report the stability of Cd-protein complexes as influenced by coordination agents and metal ions. In this study, we aim to unveil the thermodynamic mechanism substantiating the binding between Cd^{2+} and rice proteins, and characterize the stability of RPs-Cd^{2+} complexes affected by coordination competition.

2. Materials and methods

2.1. Materials

RPs were purchased from Jinnong Biological Technology Co., Ltd. (Wuxi, China) with 3.5% (w/w), 81.7% (w/w) and 0.07 mg/kg of moisture, protein, and Cd^{2+}, respectively. Cadmium chloride, trisodium citrate dihydrate, sodium pyrophosphate decahydrate, sodium acetate, disodium edetatedihydrate, copper sulfate pentahydrate, zinc sulfate heptahydrate, calcium chloride, ferric chloride hexahydrate, and other general chemicals used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and used as received without further purification.

2.2. The binding reaction of Cd^{2+} to rice protein

For each experimental run, 1.0 g of RPs was well dispersed in 180 mL deionized water by ultrasonic vibration for 2 min. The initial pH of the dispersion was adjusted as required with 0.1 M HCl or 0.1 M NaOH. Then, a certain volume aqueous solution of a known concentration of Cd^{2+}, prepared by $CdCl_2$, was added and the final volume of the reaction mixture was adjusted to 200 mL with deionized water. After sealed with a cap, the 500 mL centrifugal cup containing the reaction mixture was immediately kept in a constant shaking (180 rpm) water bath (BS-21, Ronghua science equipment co. LTD, Nanjing, China) which was maintained at a desired temperature in advance. After reaction, the supernatants were collected by centrifugation at 4000 rpm for 10 min (M. Behbahani et al., 2013), and then filtrated through quantitative paper. The filtrates were used for the measurements of Cd^{2+}.

2.3. Effects of reaction time and pH on RPs-Cd^{2+} binding

To investigate the effect of reaction time on RPs-Cd^{2+} binding, the reaction was carried out at 303 K, pH 7.5 with an initial Cd^{2+} concentration of 100 mg/L, and the reaction time was varied from 5 min to 2 h. To study the pH dependency of the amount of Cd^{2+} binding to RPs, the reaction was conducted at 303 K for 30 min with an initial Cd^{2+} concentration of 10 mg/L, and the initial pH of the reaction mixture ranges from 3.5 to 7.5.

2.4. Effects of coordination agents and metal ions on RPs-Cd^{2+} binding

To investigate the effects of coordination agents on the binding behavior of Cd^{2+} to RPs, acetates, citrates, pyrophosphates and edetates were added to the reaction mixture, respectively, as competitive coordination agents for RPs, and the reaction was carried out at pH 7.5 with three different coordination agent concentrations (50, 500, and 5000 mg/L). With a certain coordination agent concentration of 500 mg/L, the binding behavior of Cd^{2+} to RPs was also studied at three different pH levels (6.5, 7.5, and 8.5). In a similar way, the effects of metal ions, i.e., Na^{+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{3+}, on RPs-Cd^{2+} binding were also evaluated.

For RPs-Cd^{2+} complexes prepared at an initial Cd^{2+} concentration of 10 mg/L, supplemented with Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} of an identical concentration of 500 mg/L, the resultant precipitates were washed by water once and analyzed for metal contents.

2.5. Determination of Cd^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}

Quantitative measurements of Cd^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} were performed by a flame atomic absorption spectrophotometer (FAAS) (AAnalyst600, PerkinElmer Inc., MA, USA) equipped with a deuterium background corrector in an air-acetylene flame, according to the user's manual provided by the manufacturer. Calcium, zinc, iron, copper, and cadmium hollow cathode lamps were used as the radiation source with wavelength of 422.6, 213.8, 248.3, 324.8, and 228.8 nm, respectively.

The amounts of Cd^{2+} binding to RPs (q, mg/g) were expressed using the following equation:

$$q = (c_0 - c_1) \times \frac{v}{m} \tag{1}$$

where c_0 (mg/L) and c_1 (mg/L) represent the concentrations of Cd^{2+} in the initial solution and equilibrium solution, respectively; m represents the reacted RPs (g), and v represents the solution volume (L).

2.6. Morphological characterization of RPs

The RPs was coated with gold for scanning electron microscopy (SEM, Hitachi S-3600N VP SEM, Hitachi, Japan) observation at 10 kV (Unursaikhan et al., 2012).

2.7. Fourier transformed infrared (FTIR)

FTIR spectrum was acquired by a Nicolet iS10 FTIR spectrometer (ThermoFisher Co., OH, USA). About 2–3 mg of freeze-dried protein powder was mixed with KBr. The mixture was then grounded, and pressed into a pellet. The transmittance spectrum in the wavenumber range of 4000–400 cm^{-1} was obtained at 2 cm^{-1} resolution. At least 32 scans were collected and the resultant data were averaged, prior to subtraction of the KBr background.

2.8. Establishment of isothermal adsorption model

By variating the initial concentration of Cd^{2+}, the isothermal adsorption test was carried out at 303 K and 318 K, respectively, to characterize the Cd^{2+} binding capacity of RPs. Two major isotherm equations, i.e. the Langmuir and Freundlich isotherms, are employed to fit the experimental data and describe the isotherm constants of Cd^{2+} uptake by RPs. The isothermal equations of the Langmuir and Freundlich adsorption models are respectively shown by Eqs. (2) and (3) (Pedro Silva et al., 2004):

$$q = \frac{abc_1}{(1 + bc_1)} \tag{2}$$

$$q = kc_1^{1/n} \tag{3}$$

where q is the adsorption amount of Cd^{2+} at equilibrium (mg/g), a is the constant of the maximum adsorbing capacity (mg/g), b is the Langmuir constant related to the affinity of binding sites (L/mg or L/mol), c_1 is the equilibrium concentrations of Cd^{2+} (mg/L), k is the Freundlich constant related to adsorption capacity, n is the Freundlich constant related to the affinity of binding sites.

2.9. Determination of thermodynamic parameters

Thermodynamic parameters were calculated according to the study performed by Bulut and Aydın (2006). The free energy of adsorption