Vibrational Spectroscopy 92 (2017) 215–219

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Fourier-transform spectra of metal salts of phytic acid in the mid- to far-infrared spectral range

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ARTICLE INFO

Article history:
Received 27 March 2017
Received in revised form 13 June 2017
Accepted 11 July 2017
Available online 14 July 2017

Keywords:
Chemical bond
FT-IR
Mineral
Phosphorus storage compound
Phytic acid
Metal valency

ABSTRACT

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate; IP_6) is the storage substance for phosphorus and many metals in plant seeds. Based on Synchrotron-based Fourier-transform infrared (FT-IR) spectra in the mid-infrared range from 1300 to 700 cm^{-1}, nine synthesized metal-IP_6 salts, with monovalent to trivalent coordination, were classified into four groups: trivalent metal-IP_6 salts, second-group divalent metal-IP_6 salts, d-block and p-block divalent metal-IP_6 salts, and monovalent metal-IP_6 salts. Synchrotron FT-IR analysis in the far-infrared range, from 650 to 100 cm^{-1}, contained clear, broad bands specific to each metal-IP_6 salt, including monovalent and divalent metal-IP_6 salts. These broad bands are likely derived from vibrations of bonds between the metal and the oxygen of IP_6. These unique far-infrared spectra may provide useful information for identifying metal-IP_6 salts in biochemical fine-image analyses of seed tissues using synchrotron-based FT-IR microspectroscopy.

1. Introduction

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate; IP_6) is a storage substance for phosphorus (P) in plant seeds. IP_6 binds tightly to nutritionally important metal elements, such as magnesium (Mg), potassium (K), iron (Fe), and zinc (Zn), forming metal-IP_6 salts (Fig. 1) [1,2]. Thus, IP_6 serves as a storage substance for metals, as well as P. Because humans and non-ruminants have no digestive enzyme for IP_6 in their intestinal tracts, they excrete large amounts of metal-IP_6 salts. This leads to mineral nutrition deficiencies and P pollution in water. To address these nutritional and environmental problems, breeding for low-phytic-acid (lpa) seeds has been pursued in many crops [3].

Several lpa mutants have been isolated in rice, and causal genes have been identified [4–6]. In rice grains, IP_6 is synthesized and accumulated exclusively in embryos and aleurone layers, not in the starchy endosperm [7]. Metals are also accumulated preferentially in embryos and aleurone layers [8]. Identification of the distribution of minerals in lpa seeds is particularly important in rice. Polished white rice, commonly consumed in many countries, is obtained by removing the bran (i.e., the embryos and aleurone layers, and thus, the storage sites of IP_6-metal salts). Even if metals become available as IP_6 decreases, metals cannot be obtained from white rice when they remain in the bran.

Recently, using synchrotron-based micro X-ray fluorescence (μ-XRF) imaging analyses, we found that a reduced IP_6 level of rice lpa seeds caused large changes in storage sites for minerals, such as P, K, Zn, and copper (Cu) [9]. Unlike wild-type seeds, P and K diffused from the aleurone layer into the inner endosperm and the distribution of Zn and Cu narrowed around the aleurone layer in the seeds of lpa mutants. Because the nutritional availability of metal elements is affected greatly by the molecules that coordinate the metals, it is also important to identify the chemical structure of mineral compounds as well as the mineral distribution. Although μ-XRF analysis can provide a fine elemental mapping image, it is unclear whether the elements in lpa seeds are present as metal-IP_6 salts, which are hard to digest, or in other forms.

Infrared spectroscopy is an established analytical technique that is used to obtain detailed structural information on molecules and molecular groups. The mid-infrared region (4000–650 cm^{-1}) includes rich spectra of absorptions corresponding to biological molecular vibrations. He et al. [10,11] investigated the mid-infrared spectra of several metal-IP_6 salts using Fourier-transform infrared spectroscopy (FT-IR); they revealed that metal-IP_6 salts showed characteristic spectra depending on the metal binding to IP_6. However, differences in the mid-infrared spectra between metal-IP_6 salts were relatively small. It is possible that the infrared

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http://dx.doi.org/10.1016/j.vibspec.2017.07.003
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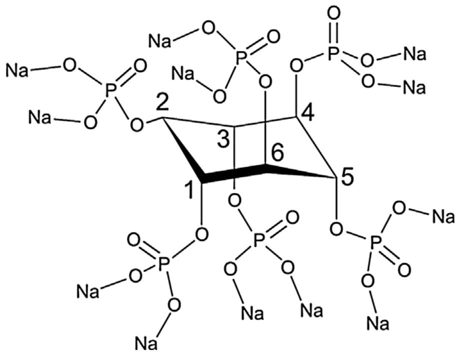

Fig. 1. Possible molecular structure of Na-IP_6 salt.

absorption bands derived from vibrations of chemical bonds between a metal and oxygen in IP_6 may provide a distinct and characteristic differences between metal-IP_6 salts. The infrared absorption bands based on bonds between a metal and oxygen, unlike nitrogen–oxygen bonds and carbon–oxygen bonds, are frequently detected in the far-infrared region (650–50 cm^{-1}), because metals have heavier atomic weights than do carbon and nitrogen [12]. To identify the unique FT-IR spectrum of each metal-IP_6 salt depending on the bond between the metal and the oxygen of IP_6, we synthesized nine metal-IP_6 salts and examined both their far-infrared and mid-infrared spectra. This may provide reference spectral data for biochemical imaging of individual metal-IP_6 salts in rice lpa seeds.

2. Materials and methods

2.1. Preparation of metal-IP_6 salts

Phytic acid dodecasodium salt (Na_{12}-IP_6, Sigma-Aldrich) was used for FT-IR measurements without further purification. Metal-IP_6 salts other than Na-IP_6, specifically Mg, calcium (Ca), manganese (Mn), aluminum (Al), Fe(III), Cu, and Zn, were synthesized according to the methods of He et al. [10], Evans and Pierce [13], and Evans et al. [14]. A mixed solution of 0.05 M Na-IP_6 and 0.2 M HCl (3:10, v/v) was used for the syntheses. A sufficient quantity of each metal chloride or Zn sulfate was dissolved in the mixture solution to obtain a P to metal ratio of 1:1. The pH of the solution was adjusted by slowly adding 1 M NaOH with stirring in time intervals of >40 min to precipitate metal-IP_6 compounds. The final pH of the solutions was adjusted to 2.0 for Fe, 5.9 for Zn, 6.0 for Mg, Mn, and Cu, and 8.5 for Ca. To precipitate Al-IP_6, 10 mL of 0.3 M $AlCl_3$ was added slowly drop-by-drop to 10 mL of 0.05 M Na-IP_6, with stirring at >40-min intervals. To determine whether the preparation method affects the spectra of metal-IP_6, we also prepared Fe-IP_6 using the same method as for Al-IP_6. No differences in the mid- and far-infrared spectra were observed. Therefore, we used Fe-IP_6 that had been prepared using the former method.

The precipitates of Mg-IP_6, Ca-IP_6, Mn-IP_6, and Zn-IP_6 were filtered and washed with boiling sterilized deionized water three times. Al-IP_6 and Fe-IP_6 precipitates were collected by centrifugation (7300 × g; 8–10 min), and the supernatants were then removed. The precipitates were resuspended in ethanol, and the supernatants were removed once again. This procedure was repeated three times to remove water and metal chlorides. Finally, all of the precipitates other than Fe-IP_6 and Mn-IP_6 were air-dried for more than 8 h at room temperature and subsequently dried for 1–2 h at 105 °C. The precipitates of Fe-IP_6 and Mn-IP_6 were immediately dried for 1–2 h at 105 °C.

Water molecules remaining in prepared IP_6 salts may affect their FT-IR spectra. To determine the degree of spectral influence of water, Ca-IP_6 was dried using two different methods and the spectra were compared. In one method, precipitates of Ca-IP_6 were washed three times with deionized water and air-dried for 1 h at 105 °C. In the second method, the precipitates were washed three times with ethanol to remove water. The ethanol was immediately substituted with diethyl ether to promote drying. The precipitates were air-dried for 1 h at room temperature. No differences were observed in the mid-infrared spectra of two Ca-IP_6 salts dried using the different methods. For the remainder of the study, Ca-IP_6 salts were dried using the former method.

Phytic acid dipotassium salt (K_2H_{10}-IP_6, Sigma-Aldrich) was dissolved in 2 M KOH to create a molar ratio of K to IP_6 of 12:1. To precipitate K-IP_6, excess ethanol was added to the solution and mixed well. The precipitate was rinsed with ethanol at least three times. During this procedure, the hard mass formation of K-IP_6 was crushed into powder using a glass rod. The final precipitate of K-IP_6 was air-dried at room temperature.

2.2. Measurements of infrared spectra

Synchrotron FT-IR spectra were obtained at the infrared beamline BL43IR at the Super Photon Ring-8 (SPring-8) synchrotron radiation facility in Hyogo, Japan, which features high brightness. A FT-IR spectrophotometer (IFS120HR; Bruker Corp., Billerica, MA, USA), combined with a long-working-distance (LWD) microscope (Bunkoukeiki, Tokyo, Japan) with good extendability into the far-infrared range, was used for measurements. To obtain precise spectra in the far-infrared region of 650–100 cm^{-1}, a Si bolometer was used as the detector and samples were scanned 100 times at a resolution of 3 cm^{-1} [15]. Mid-infrared spectra, in the wavenumber range from 4000 to 650 cm^{-1}, were obtained using a HgCdTe (MCT) detector and by scanning each sample 500–700 times at a resolution of 3 cm^{-1}. A small amount of each sample was thinly and uniformly spread using a Diamond EX'Press compression cell (S.T. Japan Inc., Tokyo, Japan). A benefit of measuring crushed powder samples on a diamond substrate is that the method is suitable for spectral measurements in the far-infrared, as well as the mid-infrared region, because mid-to-far infrared radiation can be transmitted through the diamond substrate.

3. Results and discussion

3.1. Mid-infrared spectra of synthetic metal-IP_6 salts

To reveal how the valency of the metal elements affects the FT-IR spectra, we analyzed two monovalent, five divalent, and two trivalent IP_6-salts. We examined mid-infrared (4000–650 cm^{-1}) FT-IR spectra of the nine synthetic metal-IP_6 salts using synchrotron radiation (Fig. 2). A broad peak from 3400 to 3200 cm^{-1}, derived from stretching vibrations of O—H bonds and a peak around 1670–1640 cm^{-1}, due to bending vibrations of O—H bonds were detected (Fig. 2A) [10,16,17]. However, the spectrum of K-IP_6 showed no clear peak from 1670 to 1640 cm^{-1}. It has been suggested that these peaks are due to the presence of water molecules complexed with the metal-IP_6 salts (Table 1). However, the peaks maintained the same shape regardless of differences in sample preparation or drying method. The spectra between 2000 and 2500 cm^{-1} are not reliable because of a diamond vacuum window that was in use further upstream on the beamline.

Several peaks from 1300 to 730 cm^{-1}, derived from the vibrations of $COPO_3$, were also detected (Fig. 2A, Table 1) [10]. Peaks from 1300 to 730 cm^{-1} varied depending on valency (Fig. 2B–E). Trivalent compounds, Al-IP_6 and Fe-IP_6, showed twin peaks around 880–730 cm^{-1} and one broad band with a shoulder