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Investigation of The Secondary Structure and Interaction of hPin1 with Cobalt Ion by Fourier Transform Infrared Spectroscopy

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The secondary structure and stability of Pin1 in present of Co^{2+} ion were characterized in solution using Fourier transform infrared (FTIR) spectroscopy. Spectra and its derivation, self-deconvolution and curve-fitting methods were used to analyze the secondary structure of hPin1. Composite bands of the amide I and amide III were studied by using Fourier self-deconvolution with an enhancement factor of K = 1.8 and a half width of 19.8 cm^{-1}. Quantative analyses of the amide I band (1700-1600 cm^{-1}) showed that native hPin1 contains 19 % α-helix, 33 % β-sheet, 12 % turn and 23 % disorder structures. In the presence of 1 equiv of Co^{2+}, the hPin1 contains 26 % α-helix, 60 % β-sheet, 4 % turn and 4 % disorder structures. These results indicate that conformational changes in hPin1 take place when Co^{2+} bound.

Key Words: FTIR, hPin1, Secondary structure, Cobalt ion.

INTRODUCTION

The Pro-directed phosphorylation is a major signaling mechanism in diverse cellular processes such as cell proliferation and differentiation. Pin1 is a high conservative peptidyl-prolyl *cis*/*trans* isomerase (PPIase), it isomerizes only pSer/Thr-Pro motifs[1,2]. It controls lots of protein function and structure, thus regulate many cellular processes such as cell cycle, cell signal transmit, RNA synthesization, responsion of DNA damnification, growth of stem cell[3,4]. The expression and function of Pin1 is strict controlled by many mechanisms. The abnormity of Pin1 such as exceed or lack expression may induce many human diseases, especially cancer and Alzheimeir disease[5]. The investigation of Pin1 can help to diagnose and cure some human diseases.

The human cell regulatory protein hPin1 is a 167 residue two-domain protein, composed of an N-terminal WW domain (residues 1-39) and a C-terminal *cis*/*trans*-isomerase domain (residues 45-167)[1]. NMR solution studies show that the two domains, which are connected by a flexible solvated linker, interact only weakly before ligand binding[6,7]. An X-ray structure of the full-length hPin1 *cis*/*trans*-isomerase revealed that loop 1 in the WW domain adopts an unusual conformation[8]. The special physiology activity of protein is decided by its conformation in highly degree. There are many methods to investigate protein conformation, such as circular dichroism, X-ray diffraction microscopy, infrared spectroscopy and nuclear magnetic resonance[9]. Each of them has advantage and disadvantage. Fourier transform infrared spectroscopy is a very useful method to investigate hydrogen bond[10]. It can acquire infrared spectrogram of almost all organisms in variety of environment conditions. In this article, we use FTIR to investigate the secondary structure of Pin1 and conformational changes in hPin1 upon Co^{2+}-bound.

EXPERIMENTAL

hPin1 was expressed recombinantly and purified as described in detail elsewhere using previously established protocols employing an immobilized Ni^{2+}-nitrilotriacetate-agarose column[11]. The histidine tag was removed by addition of enterokinase (10 units, Sino Biological Inc). Size exclusion chromatography experiments were performed at 4 ºC on an HR 10/30 Superpose 6 column (Parmacia) by AKTA FPLC. The purified hPin1was found to be homogenous by polyacrylamide gel electrophoresis and SDS-polyacrylamide gel electrophoresis and it was stored at -80 ºC. hPin1 protein concentration was determined using BioRad assay.

Infrared spectra were recorded using a NEXUS-670 FTIR-spectrophotometer. Only 32 scans were necessary to obtain spectra with a good signal-to-noise ratio. The protein solutions were placed in a homemade, demountable cell equipped with CaF_2 windows. The temperature of the gas-tight IR cell device

was controlled by a programmable temperature bath. FTIR spectra of the protein and the buffer solutions were recorded at a nominal resolution of 4 cm^{-1}. Fourier self-deconvolution and curve-fitting methods were used to analyze the secondary structure of hPin1[12]. Composite bands of the amide I and amide III were studied by using Fourier self-deconvolution (FSD) with an enhancement factor of K = 1.8 and a half width of 19.8 cm^{-1}.

RESULTS AND DISCUSSION

Co^{2+}-induced infrared spectral changes in hPin1: Fig. 1 shows the infrared absorption of hPin1 in the absence and present of Co^{2+}. Infrared spectroscopy reveals a small but significant increase in the ordering of the hydrocarbon chains upon interaction with Co^{2+}. Table-1 shows the peak position and their attribution. Some change around 1192 cm^{-1} is also observed suggesting Co^{2+}-induced infrared spectral changes in hPin1. Three main bands are seen in the spectral region between 1800 to 1200 cm^{-1} that can be identified as the amine I band centered at 1647 cm^{-1}, the amine II band located at 1543 cm^{-1} and the amine III band is between 1330 to 1220 cm^{-1}.

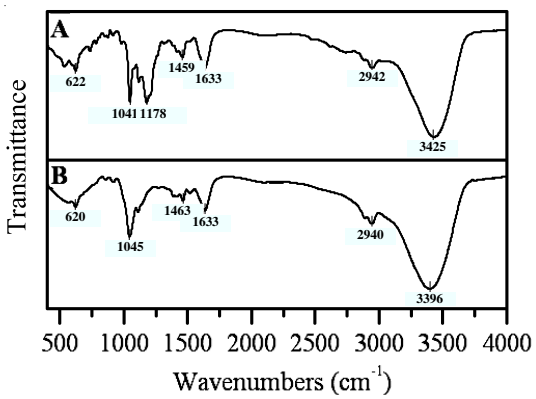

Fig. 1. Infrared spectra of hPin1 in the absence and present of $Co2^{+}$

TABLE-1
PEAK POSITION AND THEIR ATTRIBUTION FOR Pin1 IN FTIR

Frequency (cm^{-1})	Assignment
3396	ν(N-H)
2940	ν(C-H)
1633	Amide-I ν(C-O), δ(N-H)
1463	ν(C-C), δ(O-H)
1192	ν(C-O), ν(C-N)
1045	ν(C-O)

Analyses of the secondary structure of hPin1: Fig. 2 shows the infrared absorption, curve-fitted and deconvoluted spectra of hPin1 in the region of the amide I band. As seen, the broad amide I band is centered at 1647 cm^{-1}. To reveal the constituent components under the broad contour, the absorption spectrum was subject to Fourier self deconvolution and eight constituents were resolved. The peak positions and relative areas of these constituents are presented in Table-2. The largest component is the one centered at 1644 cm^{-1}, which is attributable to disorder structure[13,14]. The component at 1654 cm^{-1} most likely arises from the α-helix structure[13,14]. As observed[13,14] for most of the water-soluble proteins, extended β-sheet structure contributes between 1640 and 1610 cm^{-1}. Thus, the 1630 and 1617 cm^{-1} components observed in Fig. 2 can be attributed to the β-sheet structure. The high frequency component at 1677 and 1666 cm^{-1} indicates the existence of β-turn structure in hPin1[13,14]. Based on the above band assignments, the secondary structure of hPin1 calculated as the ratio of the area of the constituent to the overall area of all the constituents is determined to contain 19 % α-helix, 33 % β-sheet, 12 % turn and 23 % disorder structures, which is very similar to the results from X-ray crystal[1].

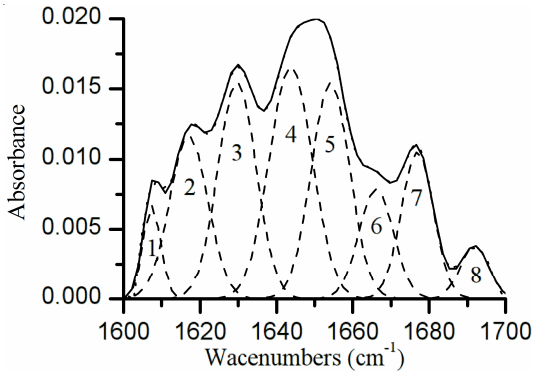

Fig. 2. Infrared curve fitted (------) and deconvoluted (——) spectra of hPin1 in the amide I band region

TABLE-2
PEAK POSITIONS AND RELATIVE AREAS OF THE CONSTITUENT COMPONENTS OF THE AMIDE I BAND AND THE QUANTITATIVE SECONDARY STRUCTURES OF hPin1

Peak position (cm^{-1})	Relative area (%)	Assignment	Secondary structure (%)
1607	3.7	Aromatic side chain	
1617	14.0	β-Sheet	α-Helix 19
1630	18.6	β-Sheet	β-Sheet 33
1644	22.5	Disorder	Turn 12
1654	18.6	α-helix	Disorder 23
1666	8.6	Turn	
1677	10.2	H-bonded side chain COOH	
1692	3.4	Turn	

Amide III band mostly represents the coincidence of the introvert libration of N-H bond and the flex libration of C-N bond in protein molecule[15]. Amide III was neglected because of its relatively weak in signals, but there is no interference from water and water vapour vibration bands and it is more sensitive to the changes of protein secondary structure. The attribution of the peaks in amide III: 1250 to 1220 cm^{-1} is β-sheet; 1270 to 1250 cm^{-1} is random coil; 1290 to 1270 cm^{-1} is β-turn and 1330 to 1290 cm^{-1} is α-helix[16]. The peak positions and relative areas of these constituents are presented in Table-3. The secondary structure of hPin1 calculated as the ratio of the area of the constituent to the overall area of all the constituents is determined to contain 22 % α-helix, 26 % β-sheet, 39 % turn and 14 % disorder structures. The results of curve fitting of amide I and amide III are comparatively coincide, that means these results are reliable.

Conformational changes of hPin1 in presence of Co^{2+}: Addition of $CoCl_2$ results in a distortion of the amide I region (Fig. 4). The Major components in the curve-fitted spectra appear at 1636 cm^{-1} (β-sheet structure) and 1658 cm^{-1} (α-helix

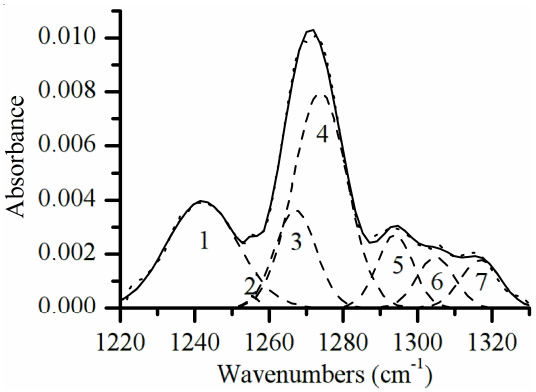

Fig. 3. Infrared curve fitted (------) and deconvoluted (——) spectra of hPin1 in the amide III band region

TABLE-3
PEAK POSITIONS AND RELATIVE AREAS OF THE CONSTITUENT COMPONENTS OF THE AMIDE III BAND AND THE QUANTITATIVE SECONDARY STRUCTURES OF hPin1

Peak position (cm^{-1})	Relative area (%)	Assignment	Secondary structure (%)
1242	25.7	β-Sheet	
1256	0.5	Disorder	α-Helix 22
1267	13.5	Disorder	β-Sheet 26
1274	38.6	β-Turn	Turn 39
1294	8.8	α-Helix	Disorder 14
1305	6.4	α-Helix	
1317	6.4	α-Helix	

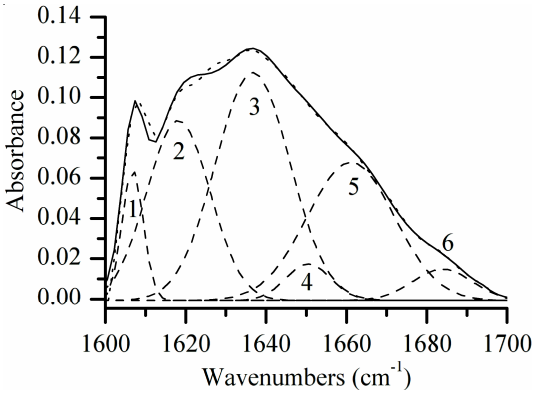

Fig. 4. Infrared curve fitted (------) and deconvoluted (——) spectra of hPin1 in the present of Co^{2+} in the amide I band regions

structure). The two low-wavenumber component bands at 1618 and 1636 cm^{-1} may be due to the β-sheet structure. The high-wavenumber component bands at 1694 and 1684 cm^{-1} can be assigned to turn-structure[13,14]. Quantitative analyses reveal that the secondary structures of hPin1 in the present Co^{2+} contains 26 % α-helix, 60 % β-sheet, 4 % turn and 4 % disorder structures (Table-4). Compared to the secondary structure at native form, both the α-helix and β-sheet structure is increased with concomitant decrease in the turn and disorder structures. Thus, it is clear that the addition of a given amount of Co^{2+} into a hPin1 solution could induce efficiently the conformational fractional change from a soluble conformation to an insoluble β-sheet conformation.

FTIR spectroscopy has been widely used to examine conformational changes of the protein because of the sensitivity

TABLE-4
PEAK POSITIONS AND RELATIVE AREAS OF THE CONSTITUENT COMPONENTS OF THE AMIDE I BAND AND THE QUANTITATIVE SECONDARY STRUCTURES OF hPin1 IN THE PRESENT Co^{2+}

Peak position (cm^{-1})	Relative area (%)	Assignment	Secondary structure (%)
1607	5.9	Aromatic side chain	
1618	24.3	β-Sheet	α-Helix 26
1636	36.3	β-Sheet	β-Sheet 60
1650	3.5	Disorder	Turn 4
1658	25.6	α-Helix	Disorder 4
1684	4.0	Turn	
1694	0.1	Turn	

of the stretching vibrations of peptide groups to their structural environment[12,13]. In the present study, the spectral alteration upon Co^{2+}-binding was not dramatic but changes in the relative contribution of individual bands were observed, suggesting a shift of the conformational equilibrium with an increase in the amount of ordered structure. The ordered structure (α+β+turn) increases from 64 to 90 % (Tables 2, 4), which indicates that the addition of Co^{2+} lead to the relative more compact structure.

In summary, protein structure is the foundation and precondition of the complex organism and biology function. The structure investigation of Pin1 affords a concernful reference to understand its biology nature. The results of curve fitting of amide I and amide III are comparatively coincide, that means these results are reliable. The analysis of the tested data that prove the secondary structure of Pin1 has been changed after reacting with metal ion.

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