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Study on the interaction between methyl jasmonate and the coiled-coil domain of rice blast resistance protein Pi36 by spectroscopic methods

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ABSTRACT

Interaction between the coiled-coil domain of rice blast resistance protein Pi36 and methyl-jasmonate (MeJA) was studied by fluorescence and UV–vis spectroscopic techniques. The quenching mechanism of fluorescence of MeJA by this domain was discussed to be a static quenching procedure. Fluorescence quenching was explored to measure the number of binding sites n and apparent binding constants K. The thermodynamics parameters ΔH, ΔG, ΔS were also calculated. The results indicate the binding reaction was not entropy-driven but enthalpy-driven, and hydrophobic binding played major role in the interaction. The binding sites of MeJA with the coiled-coil structural domain of rice blast resistance protein Pi36 were found to approach the microenvironment of both Tyr and Trp by the synchronous fluorescence spectrometry. The distance r between donor (the coiled-coil domain of rice blast resistance protein Pi36) and acceptor (MeJA) was obtained according to Förster theory of non-radioactive energy transfer.

1. Introduction

Methyl jasmonate (MeJA) is derivatives of jasmonic acid (JA) widely distributed in plants. As well as JA, it regulates plant development, responses to external stimulation, it is a natural plant hormone and signal transduction molecule regulating gene expression [1,2] MeJA not only involves in plant growth and regulation metabolism, but also plays an important role of regulation in mechanical damage, salinity injury, drought, low temperature and ultraviolet radiation, and other various kinds of intimidate defends. In addition, MeJA can still induce expression of plant defense genes to resist biological violations stimulated by pathogens, insects and fungi, etc. [3,4], which is an important signal molecular inducing plants defense responses [5,6]. It was documented that MeJA can induce expression of many plants' specific genes to produce specific proteins, which can be divided into vegetative storage proteins and proteins related to resistance diseases, insects and other adversity [7]. Research shows that exogenous MeJA can strongly induce the accumulation of storage protein in soybean [8]. Treated with different concentrations of exogenous MeJA, defense proteins were accumulated in all different plants, mainly alkaline defense protein, and it still can induce polyphenol oxidase, protease inhibitors, peroxidase and other defense related proteins discharge [9]. Pretreated with different levels of MeJA, resistance to plant pathogens of the potato was increased. After watermelon was treated by MeJA, the seedlings can increase chitosanase activity, which strengthens disease resistance to the stem rot disease, powdery mildew fungus and sickle wilting [10]. MeJA processing seedling can improve the ability of tobacco against anthrax, and exogenous MeJA treatment can induce an increase in ability of wild rice against blast disease [11]. In recent years, study of defense respond and its signal transduction induced by exogenous JA and MeJA become the focus, but the molecular mechanism remains unclear.

Rice blast, caused by the fungus *Magnaporthe grisea* (Hebert) Barr, is one of the most destructive diseases of the rice (*Oryza sativa* L.) worldwide. Practice proved, using resistant cultivars is the most effective and environmentally strategy against blast. So far, 16 resistance genes have been cloned, which includes 15 true resistance genes (*Pib*, *Pita*, *Pi-km*, *Pi9*, *Pizt*, *Pi-d2*, *Pi-d3*, *Pi36*, *Pi37*, *Pik*, *Pit Pi5*, *Pikh*, *Pish* and *Pia* and 2 field resistance genes (*pi21* and *Pb1*) [12–15]. With the exceptions of *pi21* and *Pid-2* [16,17], all of them are of a central nucleotide-binding domain, and C-terminal leucine-rich repeats (NBS-LRR) type. Plant NBS-LRR proteins have been classified into two broad groups, based on the presence of either a Toll-interleukin receptor domain (TIR) or (usually) a coiled-coil (CC) domain at their N terminus. The function of both these domains is believed to mediate signal transduction following sensing of the pathogen's presence [18–20]. The NBS domain binds and hydrolyzes ATP, and so acts as a molecular switch, while the LRR domain is the key determinant of recognition specificity [21]. Rice blast resistance gene *Pi36* encodes CC-NBS-LRR resistance protein [22]. Expression of the gene-related had been analyzed, and it is

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deduced that the gene may be involved in signaling pathway mediated by JA [23]. Herein, spectrometry technique is used to study the interaction between MeJA and the protein of CC domain in vitro, and obtain some parameters of their interactions.

2. Experimental

2.1. Materials

Coiled-coil domain of blast resistant protein Pi36 was expressed in *E. coli* and purified by Glutathione Sepharose 4B (Amersham). For protein expression, the Pi36-CC fragment (1–120 bp) was cloned into pGEX-6-1 vector (GE healthcare) using *Eco*RI and *Xho*I. GST and GST-Pi36-CC fusion proteins were expression in *E. coli* BL21 DE3) strain (Novagen) at 30 °C for 4 h by the addition of isopropyl b-D-a-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Proteins produced were extracted using Bug Buster HT kit (Takara Bio, Tokyo, Japan), loaded onto Glutathione-Sepharose resin GST accept (Nacalai, Japan), and eluted using glutathione-containing buffer [50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 10 mM glutathione]. Then, the proteins were separated in a 15% SDS-polyacrylamide gel.

Methyl jasmonate (MeJA) was purchased from Sigma and was prepared in absolute ethyl alcohol (4×10^{-4} mol/L). Protein solution was diluted by ddH_2O (every 3 mL phosphate buffer mixed with 150 μL purified protein), and then store in 8 °C; 0.2 mol/L phosphate buffer of pH 7.2 was prepared.

2.2. Apparatus and measurements

All fluorescence spectra were recorded on F-2500 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. A PE LAMBDA-17 ultraviolet spectrophotometer (PE, USA) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum. Because the concentration of coiled-coil domain of blast resistant protein Pi36 was unknown, the appropriate dilute degree was determined by UV absorption spectra. Purified protein (10 μL) was dissolved in 3 mL phosphate buffer (0.2 mol/L pH 7.2) in a 10 mL standard reagent bottle, then protein concentration was increased and UV absorption spectra was measured uninterruptedly until a perfect result, thus an appropriate protein concentration was determined.

MeJA and protein were mixed at an appropriate ratio in pH 7.2 phosphate buffer, and was made up to a series solution of different concentration. Then UV–vis absorption spectral was measured in the wavelength range of 200–400 nm.

For fluorescence spectrometry, the excitation wavelength was set at 280 nm to excite the protein and the emission spectra were monitored in the wavelength range of 220–790 nm. Synchronous fluorescence spectrum of Tyr and Trp were obtained by setting $\Delta\lambda$ at 20 nm and 80 nm respectively. The width of both excitation slit and emission slit were set to 10 nm. Fluorescence spectrum of 0.2 mol/L pH 7.2 phosphate buffer was used as blank control.

3. Results and discussion

3.1. Binding constant and binding number of MeJA and coiled-coil domain of the resistance protein Pi36

3.1.1. UV–vis spectra

A strong absorption of protein with a peak of 289 nm was observed (Fig. 1), while the maximum peak of MeJA was 290 nm. It was apparent, the UV absorption intensity of protein increased regularly with the increasing of MeJA concentration, and the maximum peak was red-shifted. The result showed that some interaction between protein and MeJA was observed.

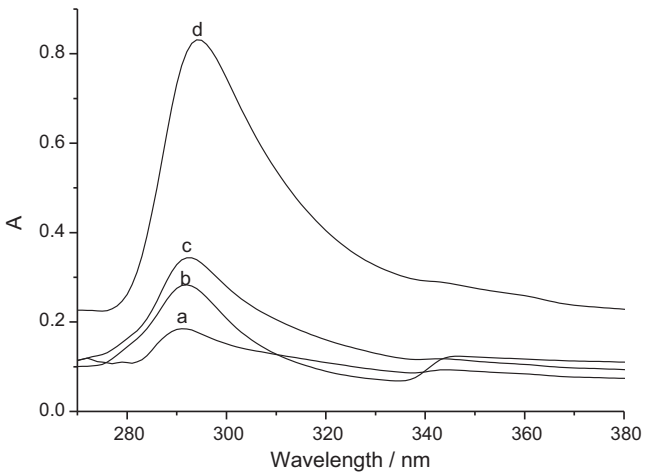

Fig. 1. Absorption spectra of MeJA bound to the coiled-coil structural domain of rice blast resistance protein Pi36 at pH 7.2. (a) $C_{pr.} = 2 \times 10^{-5}$ mol/L, $C_{MeJA} = 0$ mol/L; (b) $C_{pr.} = 2 \times 10^{-5}$ mol/L, $C_{MeJA} = 1.9 \times 10^{-5}$ mol/L; (c) $C_{pr.} = 2 \times 10^{-5}$ mol/L, $C_{MeJA} = 5.2 \times 10^{-5}$ mol/L; (d) $C_{pr.} = 2 \times 10^{-5}$ mol/L, $C_{MeJA} = 3.6 \times 10^{-5}$ mol/L.

3.1.2. Fluorescence spectra

For macromolecules, the fluorescence measurements can give some information of the binding of small substances to protein. Fluorescence spectrum of the interaction between MeJA and the coiled-coil domain of the resistance protein Pi36 was displayed from Fig. 2. Under the excitation wavelength of 280 nm, two fluorescence emission peaks of protein appeared at 208 nm and 304 nm (Fig. 2). The fluorescence intensity of protein increased with the increasing of MeJA concentration at 208 nm, and a gradually decreased peak of fluorescence emission appeared at 304 nm. These results indicated that the coiled-coil domain interacted with MeJA, and the interaction resulted in fluorescence quenching of protein.

Quenching usually classified as either static quenching or dynamic quenching. For dynamic quenching, the decrease in intensity was described by Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

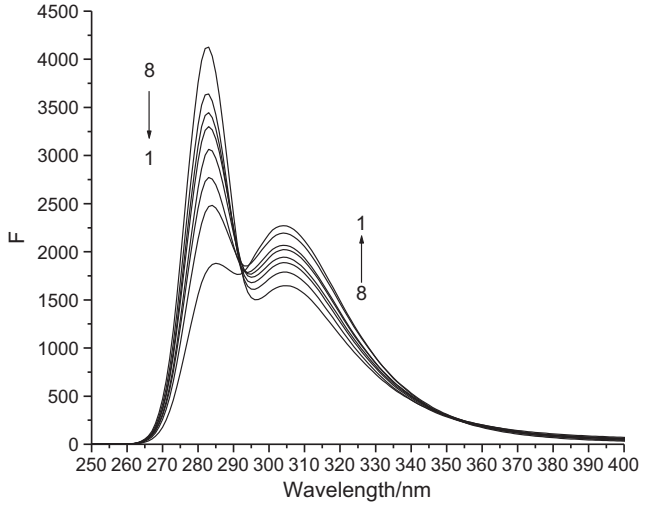

Fig. 2. Effect of MeJA on fluorescence spectra of the coiled-coil structural domain of rice blast resistance protein Pi36. C_{MeJA}: 1—0 mol/L; 2—2×10^{-6} mol/L; 3—4×10^{-6} mol/L; 4—6×10^{-6} mol/L; 5—8×10^{-6} mol/L; 6—1.0×10^{-5} mol/L; 7—1.2×10^{-5} mol/L; 8—1.4×10^{-5} mol/L. $C_{pr.} = 2 \times 10^{-8}$ mol/L, $\lambda_{ex} = 280$ nm, pH = 7.2, $T = 25$ °C.