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PHD finger of the SUMO ligase Siz/PIAS family in rice reveals specific binding for methylated histone H3 at lysine 4 and arginine 2

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ABSTRACT

We determined the three-dimensional structure of the PHD finger of the rice Siz/PIAS-type SUMO ligase, OsSiz1, by NMR spectroscopy and investigated binding ability for a variety of methylated histone H3 tails, showing that OsSiz1–PHD primarily recognizes dimethylated Arg2 of the histone H3 and that methylations at Arg2 and Lys4 reveal synergy effect on binding to OsSiz1–PHD. The K4 cage of OsSiz1–PHD for trimethylated Lys4 of H3K4me3 was similar to that of the BPTF–PHD finger, while the R2 pocket for Arg2 was different. It is intriguing that the PHD module of Siz/PIAS plays an important role, with collaboration with the DNA binding domain SAP, in gene regulation through SUMOylation of a variety of effectors associated with the methylated arginine-riched chromatin domains.

1. Introduction

Post-translational modification of proteins by a small ubiquitin-related modifier (SUMO) family has extensively been studied in the past decades to explore its implication in numerous and diverse cellular processes, including nuclear transport, gene repression and activation, chromosome segregation, DNA repair, regulation in the immune system (for reviews, references [1–8]), and especially in plants its function associated with abscisic acid signaling, flowering time, biotic and abiotic stress responses [9–11].

In recent years, the regulation of activation factors and repressors in transcription by their SUMO modification has attracted considerable attention. There are many lines of the evidence that the sumo-modification of transcription factors, histones, corepressors and deacetylases (HDACs) is associated with a variety of pathways for chromatin-mediated repression, that is, the formation of facultative heterochromatin (for reviews, see references [6,8,12,13]). The heterochromatin is a silent form of chromatin that is also promoted by a low level of histone acetylation and high levels of methylations of histone H3 at Lys9 and Lys27 ([12,13], and references herein).

SUMOylation requires the sequential action of E1-activating enzyme, E2-conjugating enzyme and often needs E3 ligases that mediate substrate specificity for SUMO conjugation [3,7,8]. Out of several classes of SUMO E3 ligases identified so far, Siz/PIAS family is the largest group characterized by the presence of an SP-RING domain which plays an essential role as a platform in positioning SUMO-conjugated E2 and target protein. Besides this domain, Siz/PIAS family conserves unique domain motifs, including SAP, PINIT, and PHD (plant homeodomain) finger present in plant Siz/PIAS proteins but absent in animals and yeast.

The primary structure of PHD finger has a typical C_4HC_3 signature (four cysteines, one histidine, three cysteines) with a characteristic spacing among cysteine residues [14,15]. Although the PHD finger was discovered over decades ago [16], its function was not known until recently. In 2006, two groups reported as to how the PHD finger proteins recognize methylated Lys4 on histone H3, and promote both gene activation and repression [17,18]. Since then, a growing number of PHD fingers have been identified to show specific interaction with methylated and unmethylated histone H3 tails. It now takes a PHD to read the histone code [19,20]. It is, therefore, interesting to see if the PHD finger of Siz1/PIAS in plants would recognize methylated lysines and/or arginine on histone H3 tails.

In this study we determined the three-dimensional structure of the PHD finger of SUMO ligase Siz1 in rice (OsSiz1–PHD) by NMR

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Table 1
Peptide ligands synthesized in this study and dissociation constants (K_d) measured in solution by NMR for ligands bound to OsSiz1–PHD.

Notation	Residue	Sequence	K_d[a]
H3K4me0	H3(1–8)	ARTKQTAR	N.D.[b]
	H3(1–15)	ARTKQTARKSTGGKA	N.D.[b]
H3K4me1	H3(1–8)	ARTK(me1)QTAR	3800 ± 350
H3K4me2	H3(1–8)	ARTK(me2)QTAR	909 ± 50
H3K4me3	H3(1–8)	ARTK(me3)QTAR	810 ± 37
H3K9me3	H3(4–13)	KQTARK(me3)STGG	N.D.[b]
H3K27me3	H3(22–31)	TKAARK(me3)SAPA	N.D.[b]
H3K36me3	H3(31–40)	ATGGVK(me3)KPHR	N.D.[b]
H4K20me3	H4(15–24)	AKRHRK(me3)VLRD	N.D.[b]
H3R2me2a	H3(1–15)	AR(me2a)TKQTARKSTGGKA	477 ± 9
H3R2me2aK4me3	H3(1–10)	AR(me2a)TK(me3)QTARKSTGGKA	209 ± 3

[a] The average of the values obtained by the least-square-fitting for the titration curves of the residues strongly perturbed upon ligand bindings.
[b] Not determined because of weak interaction of ligands with the OsSiz1–PHD finger.

spectroscopy and investigated its binding specificity for a variety of methylated histone H3 tails. We found that OsSiz1–PHD specifically recognizes both methylated Lys4 and Arg2 of histone H3. The results were discussed on structure-based binding mechanism of OsSiz1–PHD and possible biological function of the PHD finger of Siz/PIAS E3 ligases is also discussed.

2. Materials and methods

2.1. Cloning and preparation of recombinant proteins

The gene corresponding to the PHD finger (Asp107–Asp172 of OsSiz1) was amplified by PCR from cDNA (AK105290/Os05g0125000) of *Oryza sativa* (NIAS BANK in Tsukuba, Japan). Point mutants of OsSiz1–PHD were constructed by KOD-Plus-Mutagenesis (TOYOBO Co., Japan). The gene was cloned into BamH1/EcoR1 sites of vector pGEX-4T-3, with which *Escherichia coli* BL21(DE3) was transformed.

The gene expression was induced by adding 1 mM isopropyl-β-D-thiogalactopyranoside at 25 °C in L-broth, or for stable isotope-labeled proteins in the C.H.L. medium enriched with ^{15}N or $^{15}N/^{13}C$ (Chlorella Industry Co., Ltd., Tokyo, Japan). The GST-fusion proteins were purified by GSH-column, and GST-tag was removed by thrombin cleavage, followed by gel filtration chromatography. The recombinant proteins thus contain an amino-acid sequence Gly-Ser at their N-termini. For NMR measurements, the purified protein was dissolved in a buffer containing 8% ^2H_2O, 100 mM NaCl and 10 mM potassium phosphate (pH 7.0) for structural determination, and a buffer containing 10 mM potassium phosphate (pH 7.0) was used for binding experiments.

2.2. Peptide synthesis of methylated histone tails

Peptides mimicking methylated histone tails listed in Table 1 were synthesized according to the standard protocol of solid-phase synthesis. They include the peptides with methylated lysines at 4, 9, 27, 36 and arginine at 2 of histone H3 and lysine at 20 of histone H4. Peptides were dissolved in a buffer solution of 10 mM potassium phosphate and adjusted to pH 7.0. The concentrations of the peptide stock solutions were directly calculated by weight of a given peptide.

2.3. NMR measurements and structure calculations

Multi-dimensional NMR spectra were acquired at 25 °C on a Bruker Avance750 spectrometer equipped with pulse-field gradients. ^1H, ^{13}C and ^{15}N sequential resonance assignments were obtained using 2D double resonance, and 3D double and triple resonance through-bond correlation experiments [21,22]: 2D ^1H–^{15}N HSQC, 2D ^1H–^{13}C HSQC, 3D HNCO, 3D CBCA(CO)NH, 3D HNCA, 3D HCABGCO (a modified CT-HCACO experiment described in reference [23]), and 3D HCCH-TOCSY. Interproton distance restraints for structural calculations were derived from multi-dimensional NOESY spectra with mixing times of 150 ms: 3D ^{15}N-separated NOESY-HSQC, 3D $^{13}C/^{15}N$-separated NOESY-HSQC, and 4D $^{13}C/^{13}C$-separated NOESY-HSQC. The spectra were processed

Table 2
Structural statistics of the OsSiz1–PHD finger.

NOE restraints	Unique	Ambiguous[a]	Total
Intraresidue	218	221	439
Sequential ($\|i-j\|=1$)	265	249	514
Medium range ($1<\|i-j\|<5$)	154	203	357
Long range ($\|i-j\|\geqslant 5$)	291	238	529
Total	928	911(416)	1839(1344)
Hydrogen bonds		16	
Dihedral angle restraints			
φ Angles		39	
ψ Angles		43	
RMSD[b] from mean structure		Residues 109–147,155–171	
Backbone atoms		0.29 ± 0.07 Å	
All heavy atoms		0.68 ± 0.09 Å	
Ramachandran plot		All residues	
Most favored regions		72.9%	
Additional allowed regions		26.0%	
Generously allowed regions		0.8%	
Disallowed regions		0.3%	

[a] Number of ambiguous NOEs and number of well-separated peaks in parentheses.
[b] Root mean square deviation of superimposed atoms, calculated by CYANA.