

Structure and Function of the N-terminal Domains of SUMO Ligase Siz/PIAS Family

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Introduction

The ubiquitin (Ub)-related protein SUMO functions by becoming covalently attached to other proteins as a post-translational modification, and SUMO conjugation, so-called sumoylation, is essential for viability of eukaryotic cells. Like Ub, SUMO is attached to certain lysine residues in substrates through an amide bond between the C-terminus of SUMO and the ϵ -amino group of the lysine residue. Sumoylation is involved in many cellular processes, such as transcription, nuclear transport, signal transduction, maintenance of chromatin and so on. The effect of sumoylation could be diverse. It can affect the localization, or regulate the activity or the binding properties of the target proteins. In contrast to ubiquitination, however, sumoylation does not promote protein degradation, and in some cases, it can even antagonize ubiquitination and lead to protein stabilization. Sumoylation, like ubiquitination, is also carried out by the sequential action of three enzymes: an activation enzyme (E1), a conjugating enzyme (E2) and a ligase (E3). In all of the organisms examined so far, a single E1 and E2 but multiple E3s have been detected. Thus, E3s are likely the determinants of substrate specificity.

The Siz/PIAS family, one of the four types of SUMO ligases, is found in all eukaryotes and shares a relatively conserved ~400 amino acid N-terminal region that contains several distinct domains, including the SP-RING and SAP domains while Siz proteins in plants comprise another unique domain, PHD finger. The SP-RING is described as a recognition region for the conjugating enzyme E2, whereas the SAP and PHD are not well characterized yet. In the present studies, several independent yet related questions regarding the structure and function of the Siz/PIAS family proteins Siz1 from rice and Baker's yeast, OsSiz1 and ScSiz1, are addressed by NMR spectroscopy. First, the three-dimensional (3D) structures of the SAP domains of OsSiz1 and ScSiz1 were determined. Second, DNA binding ability of OsSiz1 and ScSiz1 was examined. Third, the 3D structure of the PHD finger of OsSiz1 and its binding ability to various methylated histone tails were investigated. The results lead to conclusion that the N-terminal

SAP and PHD-finger domains of the SUMO ligase Siz1 from rice play crucial roles in recognizing the methylated histone as well as DNA.

Results and Discussion

Solution structures of the SAP domains of OsSiz1 and ScSiz1 and Their Interactions with A/T-rich DNA: The SAP domain of rice Siz1, OsSiz1(1-107) folds into a four-helix bundle structure (α 1, residues 4-18; α 2, 22-31; α 3, 40-49; α 4, 70-84) with a right-handed twist and a topology of up-down-extended loop-down-up (Fig. 1A). Two loops connecting the two pairs of helices (α 1- α 2 and α 3- α 4) are crossed over. This bundle structure is quite similar to that of the SAP domain of human PIAS previously determined by us [1], with the exception of the relatively long loop connecting α 3 and α 4 helices. The two α -helices composed of the SAP motif, α 2 and α 3, run nearly parallel to each other. All of the hydrophobic residues conserved in the SAP motif form the hydrophobic core, which stabilizes the helix bundle.

Unlike the SAP domains of rice Siz1 and human PIAS1, the SAP domain of the yeast Siz1, ScSiz1(1-111) assumes a five-helix bundle structure (α 1, 21-34; α 2, 39-48; α 3, 57-70; α 4, 80-94; α 5, 101-110) as shown in Fig. 1C. While the N-terminal 18 residues did not converge, there was a short helix at residues 2-4. A comparison with the rice and human SAP domains revealed unique features of the yeast SAP: (1) a highly flexible N-terminal region, (2) a longer α 1-helix, (3) an extended loop between α 3- and

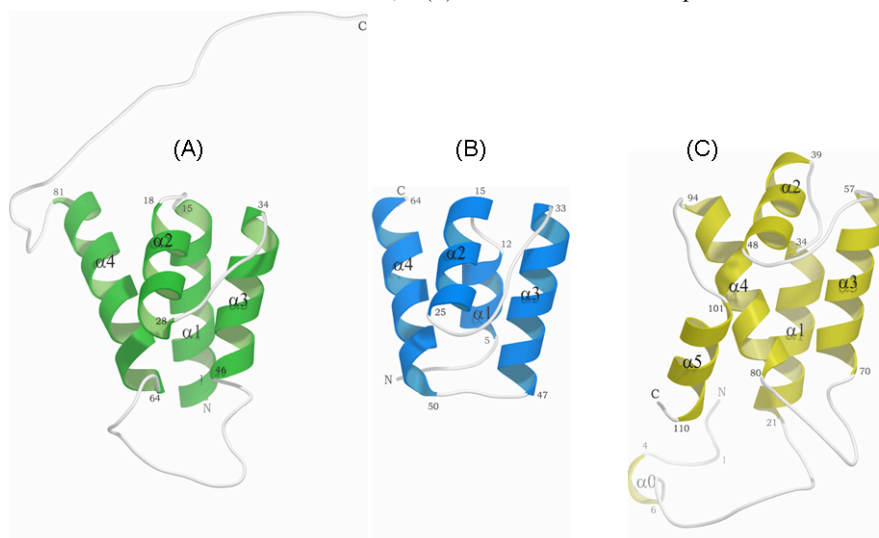


Fig. 1. NMR solution structures of (A) rice Siz1(1-107), (B) human PIAS1(1-65), and (C) yeast Siz1(1-111).

α 4-helices, and (4) an additional helix α 5 which contacts with the α 2- and α 4-helices. Such helix-helix interactions result in re-arrangement of the α 2-helix position.

Gel shift mobility assays and NMR chemical shift perturbation experiments provided evidence that both OsSiz1(1-107) and ScSiz1(1-111) bind to A/T-rich DNA. However, DNA-binding modes may be different from each other. When the DNA bound to the yeast SAP, the band of DNA was retarded in electrophoresis, suggesting a concerted manner of the binding to the protein. In contrast, the DNA-binding of the rice SAP seemed to be non-specific because the band of the bound DNA was disappeared. The observed patterns of chemical shift perturbations are slightly different between the rice and yeast SAP domains. The DNA-binding mode of OsSiz1 is essentially the same as that of the human PIAS1 [1]. In both the rice and human SAP domains, residues that undergo large chemical shift perturbations upon binding to DNA are localized around the protruding edges formed by the N-termini of the α 2- and α 3-helices. In the case of the yeast SAP, the residues located within the α 2-helix were also perturbed. Moreover, good fittings to the perturbation curves were attained with the model of 1/1 stoichiometry for the DNA/rice SAP while 1/2 for the DNA/yeast SAP. It is worthwhile mentioning that the DNA-binding affinity of the rice SAP is lower one order of magnitude than that of the yeast SAP.

Solution Structure of PHD of rice Siz1 and Its Binding to Methylated Histone Tails of H3 and H4: The PHD finger of OsSiz1 has three β -strands (β 1, 114-115; β 2, 128-130; β 3, 138-141) and two helices (h1, 142-145; h2, 164-170) as shown in Fig. 2. The antiparallel β -sheet composed of β 2- and β 3-strands and two helices form a typical PHD core fold. Two Zn²⁺ atoms, required for proper folding and located at opposite ends of the β -sheet, are separated by ca. 15 Å and are coordinated by the conserved Cys₄HisCys₃ sequence motif. Uniqueness in the PHD structure of the rice Siz1 is the existence of the extra

β 1-strand which associates with the β -sheet in a parallel fashion to β 2.

Since the first reports in 2006 that PHD fingers of BPTF [2] and ING2 [3] specifically recognize the Lys4-trimethylated histone H3, many PHD fingers from various proteins were subjected to examine the binding ability to histone tails. It is now obvious that PHD fingers, as a family, show flexibility in peptide binding. Among the various lysine-methylated and unmodified histone tails we examined, the PHD finger of the rice Siz1 specifically recognizes histone H3 with tri- and dimethylated lysine at position 4 (H3K4me3 and H3K4me2). Essentially no bindings to monomethylated H3K4me1 and unmethylated H3 were observed. The NMR chemical shift perturbation experiments demonstrated that the H3K4me3 interacts with OsSiz1 through an antiparallel β -sheet formed on the surface of the PHD finger. A recent report suggests that the SUMO ligase Siz/PIAS family is required for sumoylation of the core histones within chromatin [4]. Taken these results together, we conclude that the PHD finger of the rice Siz1 serves as the methylated histone binding domain, at least in part.

Acknowledgments

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References

1. Okubo, S., Hara, F., Tsuchida, Y., Shimotakahara, S., Suzuki, S., Hatanaka, H., Yokoyama, S., Tanaka, H., Yasuda, H., and Shindo, H. (2004) *J. Biol. Chem.*, **279**, 31455-31461.
2. Li, H., Wang, W., Duncan, E.M., Wysocka, J., Allis, C.D., and Patel, D.J. (2006) *Nature*, **442**, 91-95.
3. Peña, P.V., Davrazou, F., Shi, X., Walter, K.L., Verkhusha, V.V., Grozani, O., Zhao, R., and Kutateladze, T.G. (2006) *Nature*, **442**, 100-103.
4. Nathan, D., Ingvarsdottir, K., Sterner, D.E., Bylebyl, G.R., Dokmanovic, M., Dorsey, J.A., Whelan, K.A., Krsmanovic, M., Lane, W.S., Meluh, P.B., Johnson, E.S., and Berger, S.L. (2006) *Genes & Dev.*, **20**, 996-976.

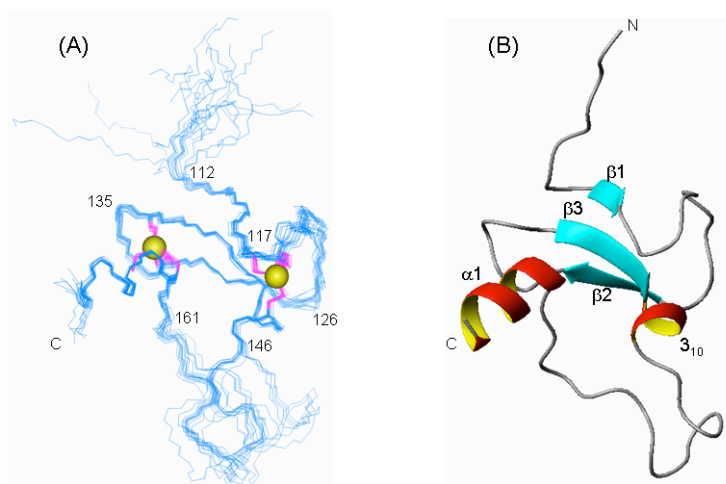


Fig. 2. NMR solution structure of rice Siz1(107-172): (A) superimposed backbone chains of 20 calculated structures and (B) ribbon diagram of the lowest energy structure.