NMR Structure of the N-terminal Domain of SUMO Ligase PIAS1 and Its Interaction with Tumor Supressor p53 and A/T-rich DNA Oligomers*

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A member of the PIAS (protein inhibitor of activated STAT) family of proteins, PIAS1, have been reported to serve as an E3-type SUMO ligase for tumor suppressor p53 and its own. It was also proposed that the N-terminal domain of PIAS1 interacts with DNA as well as p53. Extensive biochemical studies have been devoted recently to understand sumoylations and its biological implications, whereas the structural aspects of the PIAS family and the mechanism of its interactions with various factors are less well known to date. In this study, the three-dimensional structure of the N-terminal domain (residues 1–65) of SUMO ligase PIAS1 was determined by NMR spectroscopy. The structure revealed a unique four-helix bundle with a topology of an up-down-ex- tended loop-down-up, a part of which the helix-extended loop-helix represented the SAP (SAF-A/B, Acinus, and PIAS) motif. Thus, this N-terminal domain may be referred to as a four-helix SAP domain. The glutathione S-transferase pull-down assay demonstrated that this domain possesses a binding ability to tumor suppressor p53, a target protein for sumoylation by PIAS1, whereas gel mobility assays showed that it has a strong affinity toward A/T-rich DNA. An NMR analysis of the four-helix SAP domain complexed with the 16-bp-long DNA demonstrated that one end of the four-helix bundle is the binding site and may fit into the minor groove of DNA. The three-dimensional structure and its binding duality are discussed in conjunction with the biological functions of PIAS1 as a SUMO ligase.

The PIAS1 (protein inhibitor of activated STAT) family, which consists of at least five members of proteins (PIAS1, PIAS3, PIASxa, PIAS xb, and PIASy), has been reported to function as an E3-type SUMO (for reviews, see Refs. 1–5). SUMO, which is a small protein with a 32% sequence identity to ubiquitin, is attached covalently to its target proteins via a chain reaction catalyzed by E1 (activating enzyme), E2 (conjugating enzyme), and E3 (SUMO ligase). Although the pathways for such ubiquitination and sumoylation are very similar, the biological consequences are quite distinct (1, 4, 5). Ubiquitination commonly leads to specific protein degradation by the 26 S proteasome. In contrast, sumoylation has been reported to stabilize the protein by blocking ubiquitination at a given sumoylation site (6, 7) and alter the localization of the proteins in the cells (8–10).

Most of the target proteins for sumoylation are transcription factors and proteins involved in signal transductions. For examples, sumoylation of the promyelocytic leukemia (PML) protein has been reported to be required for its localization to PML bodies (10, 11) and is thus a critical step in enabling transcription. In another example, it has been reported that tumor suppressor factor p53 is sumoylated by members of the PIAS family with the conjugating enzyme Ubc9 but that the effects of sumoylation on p53 activity are still controversial (11, 12). Many other transcription factors such as c-Jun (13) and Lef-1 (14) and androgen receptor (15–17) as well as signal transduction factors such as NFκB (6, 18), which are almost exclusively nuclear proteins, have been reported to interact with and be modulated by SUMO ligases of the PIAS family. However, the functional significance of sumoylation is less well understood in these instances, and the detailed interactions of PIAS with such target proteins have not been elucidated yet.

Members of the PIAS protein family with a high sequence identity of over 60% contain several conserved domains (19). These domains include the N-terminal SAP motif (SAF-A/B, Acinus, and PIAS), residues 11–45), the PINIT domain (residues 149–223), the zinc finger-like RING domain (residues 335–377), and the C-terminal acidic domain consisting of six consecutive residues (positions 469–477). All of the residue numbers used

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The atomic coordinates and structure factors (code 1V66) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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1 The abbreviations used are: PIAS, protein inhibitor of activated STAT; STAT, signal transducers and activators of transcription; SAP, SAF-A/B, Acinus, and PIAS; SUMO, small ubiquitin-like modifier; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein; E3, ubiquitin-protein isopeptide ligase; PML, promyelocytic leukemia; GST, glutathione S-transferase; P/D, protein/DNA.
here correspond to positions in PIAS1. The SAP motif or SAF-box has been proposed to function as a DNA-binding domain (20, 21). The RING domain is considered essential for SUMO ligase activity (1, 2), and the PINIT domain is thought to be central in nuclear retention (20). To date, however, there is no structural basis for these attributed functions.

PIAS1 is localized in the nucleus with a speckled pattern and has several of the characteristics observed in scaffold attachment proteins (2–4, 14, 22). Consistent with this nuclear localization, PIAS1 was shown to bind A/R-rich DNA fragments, presumably through the SAP motif in the N terminus (22). Moreover, the conserved 35-amino acid SAP motif found in members of the PIAS family also is found in a certain scaffold attachment protein family such as SAF-A and SAF-B (21). However, their sequence identities are fairly poor, ranging up to no more than ~30% between the proteins of different families.

The SAP motif is predicted to have a helix bundle with two amphipathic helices that are thought to play a role in DNA binding. Indeed, this has been proven to be at least partly true for a SAP-like motif in the C terminus of the double-stranded break repair protein, Ku70 (23, 24). The SAP domain exhibited DNA binding ability and was composed of a three-helix bundle as observed in the x-ray (24) and NMR structures (23) of the SAP-like domain in Ku70. Because the sequence identity is fairly poor between the SAP motif of Ku70 and the PIAS family (~20%), it is interesting to see whether the putative SAP motif (residues 11–45) of PIAS1 has a similar helical structure and DNA binding behavior. Certainly, the results should provide new insight into the functions of the PIAS family.

In the work presented here, three independent yet related questions regarding the structure and function of PIAS1 are addressed. First, the relative binding affinities in a series of truncated PIAS1 mutants to p53 were considered in order to identify which regions of the protein are responsible for its interactions with p53. Our results indicated that PIAS1-p53 binding is specific to the SAP motif. Second, the three-dimensional structure of the PIAS1 N-terminal SAP motif (residues 1–65) was determined using heteronuclear multi-dimensional NMR spectroscopy. The structural analysis revealed a unique four-helix bundle with the first three helices exhibiting very similar topology to that of the SAP motif found in Ku70. Third, DNA binding ability of the four-helix SAP domain of PIAS1 was investigated showing that this domain is capable of binding to A/R-rich DNA fragments. Thus, it is concluded that the SAP motif in the PIAS family plays a crucial role in recognizing both transcription factors as well as DNA.

MATERIALS AND METHODS

Preparation of Recombinant PIAS1 and p53—The PIAS1 gene and its deletion mutants were obtained from human PIAS1 cDNA by PCR and subcloned into expression vector PET28b (Novagen). The expressions of His-tagged PIAS1 (His-PIAS1) and its deletion mutants (residues 1–65, 1–110, 1–156, 1–200, 1–318, 306–651, and 65–260) were constructed. Escherichia coli BL21(DE3) cells carrying the His-PIAS1 construct were grown at 37 °C, and the expression of the construct was induced by the addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside. After further growing for 2 h at 27 °C, the cells were collected by centrifugation, resuspended in buffer A (50 mM potassium phosphate, pH 7.0, 0.15 mM NaCl, 0.1% Nonidet P-40) and lysed by sonication. The resulting supernatant was used for the subsequent pull-down assay. Glutathione S-transferase-p53 (GST-p53) fusion protein was expressed in s9 cells by using the baculovirus protein expression system (Invitrogen), and GST-p53 fusion protein was purified by glutathione-Sepharose 4B beads (Amersham Biosciences) according to Kyahyo et al. (12).

For NMR measurements, the N-terminal domain of human PIAS1, denoted PIAS1(1–65), was prepared. For uniform 15N or 13C labeling, the M9 medium containing 15NH4Cl (1 g/liter) and/or 13C-glucose (2 g/liter) was used for expression with E. coli strain BL21(DE3) carrying the plasmid.(1–65) expression vector. The protein was purified by a nickel-affinity column chromatography followed by gel filtration using a Superdex-75 column (Amersham Biosciences). The protein without tag was purified by a gel filtration after cleaving His6-tag by proteolysis with thrombin protease. The NMR sample consisted of 0.25–0.5 mM protein in 10 mM potassium phosphate, pH 6.0, 300 mM NaCl, 2 mM dithiothreitol-d3, and 10% D2O, 90% H2O.

Pull-down Assay for Protein Interaction with p53—E. coli cell extract expressing His-tagged PIAS1 fusion protein was mixed together with recombinant GST-tagged p53 in buffer A (50 mM potassium phosphate, pH 7.0, 0.15 mM NaCl, 0.1% Nonidet P-40) and incubated at 25 °C for 30 min. GST-p53 was trapped by glutathione-Sepharose 4B resin. After the resin was washed thoroughly with a washing buffer, the protein bound to the resin was subjected to SDS-PAGE followed by Western blotting with a monoclonal anti-penta-His antibody as the primary antibody and an horseradish peroxidase-conjugated anti-mouse IgG antibody as the secondary antibody. The proteins, which interacted with the antibodies, were detected by the ECL method (Amersham Biosciences) using x-ray film.

Gel Mobility Shift Assay for Protein Interaction with DNA—The scaffold attachment region-like A/R-rich oligonucleotide 45-mers, 5′-ATACAGAAAATAAATAAATAAATAGTTTATATTTTTTTCT-3′ and its complementary nucleotide (21), were purchased from NIPPON EGT Inc., and no further purification was made. Equivalent amounts of two complementary 45-mers were mixed in Tris-EDTA buffer containing 50 mM NaCl, annealed by heating at 70 °C for 5 min, and cooled slowly to room temperature. A/R-rich self-complementary 16-mer (5′-CATATATTGTG-3′) and complementary 16-mer (5′-ATATTTTGTG-3′) also was purchased and annealed in the same way. The former 45-bp DNA duplex and the latter 16-bp DNA are referred to as DNA45 and DNA16, respectively.

Increasing amounts of His6-PIAS1(1–65), the PIAS1 N-terminal domain, were added to a given amount of DNA in the reaction buffer containing 100 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 6% glycerol (final concentration of 0.35 and 0.70 µmol/liter for DNA45 and DNA16, respectively, in total volume of 30 µl). After incubation at 4 °C for 1 h, the sample was subjected to a polyacrylamide gel electrophoresis in running buffer (Tris borate-EDTA). The gel was stained for 15 min in 2.0 µg/ml ethidium bromide solution and photographed.

NMR Measurements and Structure Calculations—All of the NMR spectra were acquired at 25 °C on a Varian Inova-600 or a Varian Inova-800 spectrometer. The spectra were processed with NMRPipe software system (25) and analyzed by the program SPARKY (26) and NMRView (27) with the macroprogram suite Kuji. All of the backbone atoms were assigned using CBCA(CO)NH, HNCACB, and HNCO experiments. Nearly complete assignments of the side-chain resonances were carried out based on data sets of C CO/NH, H BBNH, HCC/COH, 1H 13C HSSQC, 1H TOCSY, and HCHC/TOCSY experiments (Ref. 28 and references therein). Distance restraints for structural calculations were obtained from 13C-edited NOESY-HSQC and 15N-edited NOEY-HSQC spectra.

Structural calculation was carried out using program CYANA/CANDID, version 1.0.6 (29, 30) with its standard protocol using the automated assignment tool for NOEY spectra. The program UNAJO (un-symmetrical NOE assignment verification utility) in the JUBAKO suite (a program for verification of unsupported peaks based on back-calculated NOE) also was applied during structural calculations in order to remove peaks or assignments that showed no intensity at the transposed position of three-dimensional 1H-15N NOEY spectra. In each cycle, 100 starting structures were generated randomly and the best 20 conformers were selected. Dihedral angles derived from the program TA-L (31) were also used for generating torsion angle restraints. Resulting structures were analyzed using MOLMOL (32) and PROCHECK NMR software (33).

Interaction of PIAS1(1–65) with DNA16 was observed by the 1H 13N HSSQC experiment at 25 °C in buffer containing 300 mM NaCl, 10 mM sodium phosphate, pH 6.0. A protein solution of 0.25 mM 15N-labeled PIAS1(1–65) titrated with unlabeled self-complementary DNA duplex (DNA16) to a protein/DNA PDI molar ratio of 3:1. The titration was analyzed using 1H and 13N chemical shift perturbations of PIAS1(1–65) upon DNA.

2. T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco, www.cgl.ucsf.edu/home/sparky. 3. J. Iwahara and N. Kobayashi, unpublished data. 4. H. Hatana, unpublished result.
RESULTS

Mapping of p53-Binding Site on PIAS1—To define the p53-binding sites on PIAS1, a series of His$_6$-tagged PIAS1 deletion mutants, the N-terminal half PIA-(1-318) of protein, the C-terminal half PIA-(306–651) of protein, PIA-(1–200), PIA-(1–156), PIA-(1–110), PIA-(1–65), and PIA-(65–200), were constructed. The GST-p53 pull-down assay with Western blotting was carried out on all of these deletion mutants to ascertain the relative affinities.

In Fig. 1, a set of three lanes for each mutant displays Western blotting with anti-His antibodies. The first two lanes are used for control and contain only the mutant protein (lane 1) and the mutant protein mixed with GST alone (lane 2). The third lane contains the GST-p53 fusion protein bound to the mutant protein when such binding occurs. As can be seen by comparing lanes 1 and 3, the N-terminal half of protein PIA-(1–318) interacts significantly with p53. In contrast, the C-terminal half of the protein PIA-(306–651), as represented by lanes 4 and 6, shows no such interaction, consistent with the results obtained previously by Kahyo et al. (12).

The remaining series of truncated N-terminal mutants were tested to more precisely define the p53-binding sites in the N-terminal half of PIAS1. His$_6$-PIA-(1–200) was found to have a nearly full binding affinity (lanes 7 and 9), whereas His$_6$-PIA-(1–156) exhibited a relatively weak affinity (lanes 10 and 12). Interestingly, the shortest mutant, His$_6$-PIA-(1–65), and its complement mutant, His$_6$-PIA-(65–200), were both found to bind p53, albeit relatively weakly, thus suggesting that p53 either has at least two binding sites within the region (residues 1–200) or one binding site spanning across PIA-(1–65) and PIA-(65–200).

Interaction of N-terminal Domain PIA-(1–65) with DNA—Tan et al. (22) first showed in an affinity matrix assay for DNA binding that PIAS1 is capable of binding to A/T-rich double-stranded DNA and proposed that the putative SAP motif (11–45) in the N terminus of PIAS1 is responsible for DNA binding. To test this possibility, a gel mobility assay for His$_6$-PIA-(1–65) binding to two kinds of A/T-rich DNA oligomers, DNA45 and DNA16, was carried out.

As can be seen in Fig. 2, the gel bands of DNA45 (upper panel) weaken with an increasing P/D molar ratio and become undetectable at a P/D ratio of 8 (lane 7) with a concentration of His$_6$-PIA-(1–65) at 2.8 × 10$^{-6}$ M. Similarly, the gel bands of DNA16 (lower panel) begin to disappear at the higher P/D ratio of 16 (lane 8). These results imply that His$_6$-PIA-(1–65) has a strong binding affinity for DNA45 with a dissociation constant of a few micromolar orders, although the affinity for DNA16 is a little weaker. Moreover, the disappearance of DNA band with an increasing P/D ratio suggests that nonspecific binding of multiple molecules of His$_6$-PIA-(1–65) to a DNA molecule takes place.

Structure of the N-terminal Domain of PIAS1—The results of both the gel mobility and GST pull-down assays indicated that the N-terminal domain, PIA-(1–65), plays a critical role in PIAS1 binding to both p53 and DNA. This hypothesis was supported further by a chemical shift analysis of the backbone Ca and Cβ carbon atoms of a truncated protein, PIA-(1–110), which demonstrated that residues 66–110 define a relatively unstructured region, whereas residues 1–65 exhibited a well-defined secondary structure (data not shown). Accordingly, one of our goals was to determine the solution structure of the minimal binding domain PIA-(1–65).

All of the backbone atoms and nearly all of the side-chain resonances of PIA-(1–65) were assigned based on the datasets of a series of multi-dimensional NMR experiments as described under “Materials and Methods.” The obtained 1$^H$, 13$^C$, and 15$^N$ chemical shifts have been deposited in BioMagResBank (accession number 6072).

The structure calculations for PIA-(1–65) were carried out using program CYANA/CANDID, version 1.0.6 (29, 30) powered by UNAJU. In each cycle, 100 starting structures were generated randomly and the best 20 conformers were selected to ensure NOE assignments. In the final cycle, a total of 925 NOEs were used for structure calculations. Of these NOEs, 344 were intraresidues, 184 were sequential (|i – j| = 1), 222 were...
medium-range (1 < |i − j| < 5), and 177 were long-range (|i − j| = ≥5). At the final cycle, the structure calculations were performed based on the 925 NOE-driven distance restraints together with 80 dihedral angle restraints (Table I). The 20 conformers with the lowest energy showed no NOE distance violations greater than 0.5 Å and a good covalent geometry with no bond or angle violations. The analysis of the dihedral angles using PROCHECK (33) revealed that 99.0% of all of the residues were within either the most favorable region or the additionally allowed region. His31 and Cys49 were in a disallowed region in eight and three conformers of 20 calculated structures, respectively. These two residues are located in the loop regions between α2- and α3-helices or α3- and α4-helices, respectively. The structural statistics in the calculations are listed in Table I.

A best-fit superposition of the backbone traces of the 20 lowest energy conformations is shown in Fig. 3a, and the lowest energy structure is shown in ribbon diagram of the side view and the top view (Fig. 3, b and c). As it is clear from Fig. 3a, the NMR structure determined here is well defined with an root mean square deviation of 0.41 Å for the backbone heavy atoms and 0.94 Å for all of the heavy atoms in residues 4–65 (Table I). The atomic coordinates have been deposited in the Protein Data Bank (code 1Y66).

As shown in the ribbon diagram (Fig. 3b), PIA-(1–65) consists of four helices comprised of the following residues: 5–11(a1); 15–22(a2); 33–46(a3); and 51–63(a4). The structure is unique in the sense that it is a four-helix bundle with a right-handed twist and a topology of up-down-up loop-down-up and that two loops connecting the two pairs of helices (α1-α2 and α3-α4) are crossed over as seen in Fig. 3c, top view. The N-terminal domain of PIAS1 is comprised of a compact four-helix bundle and includes the putative SAP motif corresponding to the region of the α2- and α3-helices. These two α-helices in the SAP motif run nearly parallel to each other, and all of the hydrophobic residues conserved in the SAP motif form a hydrophobic core, which stabilizes the helix bundle. Specifically, the core includes Val16 and Leu19 in the α2-helix and Leu27, Leu42, and Leu45 in the α3-helix.

**NMR Analysis of the Complex of PIA-(1–65) with DNA**—To obtain more direct evidence of DNA binding to PIA-(1–65), 0.25 mM 15N-labeled PIA-(1–65) was titrated with DNA16 and the chemical shift perturbations were observed using 1H,15N HSQC spectra upon DNA binding at various P/D ratios. All of the NMR measurements were acquired at an ionic strength of 300 mM NaCl as high as that adopted in the structure determination of PIA-(1–65) to avoid self-aggregation of the protein.

In Fig. 4, the overlaid HSQC spectra of PIA-(1–65) in the absence and presence of DNA at a P/D molar ratio of 3.0 is displayed. As shown in Fig. 4, several cross-peaks such as those of Lys20 and His25 were perturbed strongly upon DNA binding and residues such as Arg15 in the N terminus of helix α2 also were perturbed. In contrast, the cross-peaks of residues 5–11 and 51–63 on the α1- and α4-helices were unchanged. The results lead us to conclude that PIA-(1–65) has a fairly strong binding ability to A/T-rich DNA fragments, even at relatively low ionic strength.

The 1H,15N HSQC spectrum of the PIA-(1–65) complex with DNA16 could be assigned easily because of the fast exchange rate in the complex formation. In Fig. 5, the indexes of the chemical shift perturbations as a function of the residue number are shown where each index is calculated as \[ \Delta = (\Delta^H)^2 + (\Delta^N)^2 \] in parts/million. Here, \( \Delta^H \) and \( \Delta^N \) are the chemical shift perturbations because of the DNA binding of hydrogen and nitrogen atoms, respectively. In Fig. 5, the two regions comprising Arg15-Gln20 and Lys20-His25 were most perturbed, suggesting that these regions are the most probable contact sites for DNA.

Fig. 6 represents the ribbon model of PIA-(1–65) with those residues having a chemical shift perturbation of \( \Delta > 0.3 \) ppm highlighted in red and with those residues having \( \Delta \) in the range of 0.3–0.1 ppm in pink. The observed pattern of the chemical shift perturbations indicates that the protruding edge formed by the N terminus of the α2- and α3-helices binds to DNA but rules out the possible existence of a recognition helix such as that found in the helix-turn-helix DNA binding motif.

**DISCUSSION**

**Structural Uniqueness of the Four-helix Bundle**—The structure of the N-terminal domain (residues 1–65) of PIAS1 was determined and found to be a four-helix bundle, i.e. residues 5–11(a1), 15–22(a2), 33–46(a3), and 51–63(a4). The four-helix bundle adopts a unique topology of up-down-extended loop-down-up with two cross-over loops connecting the two pairs of helices (α1-α2 and α3-α4) as depicted in Fig. 3c. The α2-ex-
tended loop-α3 represents the putative SAP motif (20); thus, the N-terminal domain of PIAS1 may be referred to as a four-helix SAP domain. This domain exhibits strong DNA binding ability (Figs. 2 and 4) but does not resemble any of the known motifs of DNA-binding domains such as the helix-turn-helix or helix-loop-helix DNA binding motif and the leucine zipper or the zinc finger motif.

Using the DALI program (34) to search for structures similar to the four-helix SAP domain of PIAS1, only four structures with z-scores higher than 4.4 were obtained. Those include the C-terminal domain (residues 106–154) of T4 endonuclease VII (35), the C-terminal domain (residues 559–609) of ATP-dependent DNA helicase (Ku70) (24), the N-terminal domain (residues 2–90) of protease inhibitor Iceberg (36), and the N-terminal domain (residues 1–45) of transcription terminator Rho (37, 38).

In Fig. 7, a–c, stereoviews of three of these structures (in blue) are superimposed onto the structure of the four-helix SAP domain of PIAS1 (in red). The structure of protease inhibitor Iceberg (Fig. 7a) is very similar to the four-helix SAP domain in PIAS1 with the exception that the extended loop between α2- and α3-helices in PIAS1 is substituted by the α-helix in Iceberg. As shown in Fig. 7, b and c, the so-called SAP domains of T4 endonuclease VII and Ku70 exhibit high structural similarity to that of PIAS1. T4 endonuclease VII binds to and cleaves unusual DNA structures such as Holiday junction, base mismatches, and heteroduplex loops (35). Ku70 is an essential component of the non-homologous end-joining DNA double-stranded break repair pathway (23, 24). Although the structures of the SAP-related domains of endonuclease VII and Ku70 are similar to the region of the α2- and α3-helices of PIAS1, they both in turn lack the fourth helix of PIAS1. Moreover, the first helix differs in its alignment between T4 endonuclease and Ku70, the former being more similar to PIAS1 than that of the latter (Fig. 7, b versus c). Although the N-terminal domain of transcription terminator Rho (37, 38) reveals a structural similarity to the SAP domain of Ku70, these domains have virtually no sequence similarity to each other (data not shown) and therefore may play different roles.

It is worth noting that the SAP domain of spCCE1 (also called Ydc2, mitochondrial resolvase of Schizosaccharomyces
Several points can be noted from the sequence alignments in Fig. 8. First, the most conserved amino acids are Val18, Leu19, Gly22, Lys34, and Leu72 (colored in red). Second, the conservatively substituted residues are Leu/Phe44, Arg/Lys45, Arg/Lys49, and Leu/Val/Phe45 (colored in sky blue). Finally, the SAP motifs of Ku70 and spCCE1 are truncated at the C and N termini, respectively, in comparison to those of the PIAS family. This finding suggests that the minimal DNA-binding domain may include only the α2- and α3-helices.

The SAP motif of PIAS1 was reported originally as a DNA-binding domain with ~35 amino acids (residues 13–45). The SAF-box (after SAF-A/B) also was proposed by Kipp et al. (21), encompassing residues 11–48. Apparently, the SAF-box is a little longer than the SAP motif. Sequence alignments shown in Fig. 8 include the regions containing the SAP domains of the well-characterized proteins that are involved in chromosomal organization or associated with nuclear bodies. SAF-A and SAF-B are scaffold attachment factors that bind preferentially to the A/T-rich chromosomal region known as scaffold/matrix-attachment regions (21). Acinus is implicated in the activation of caspase-3 that is required for apoptotic chromatin condensation (40). Myocardin is the founding member of a class of muscle transcription factors and provides a mechanism whereby serum response factor can convey myogenic activity to cardiac genes (41, 42). The protein also can bind matrix attachment regions through its SAP domain (41). Ku70 is an essential component of the non-homologous end-joining DNA double-stranded break repair pathway (24), and spCCE1 is a fission yeast mitochondrial resolvase that catalyzes junction branch migration and endonuclease activity (39, 43). In all of the cases, the SAP-related domains are probably involved directly in DNA binding.

**N-terminal Four-helix SAP Domain Interacts with DNA**

Direct evidence that SAP and SAF-box motifs bind to DNA has
come from the studies of SAF-A (21) and Ku70 (23, 24). It was shown that the SAP motifs from SAF-A and Ku70 bind to the scaffold/matrix attachment regions. The SAP motif of the SAF-A is necessary and sufficient for specific binding to DNA, whereas that of the Ku70 is a subdomain for DNA binding. The SAF-A was found to bind to the minor groove of the A-tracts in DNA with a mass binding mode, suggesting that a self-assembly of SAF would be required for DNA binding (21). It was demonstrated by an NMR study of the DNA binding experiment (23) that wide regions of the SAP motif of Ku70 are involved in DNA binding together with its basic N-terminal flexible loop. It is worth noting that such a basic flexible loop in Ku70 is missing in the four-helix SAP domain of Pias1, suggesting that the DNA binding mode would be different between these two domains. The SAP motif of spCCE1 also was investigated (43) and shown to play important roles in the stable complex formation of spCCE1 with Holiday junction and also in the junction resolution, although its SAP motif is not the main DNA binding determinant.

Consistent with the reports that Piasy and Pias1 can bind to DNA with Ab-T rich sequences in nuclear matrix attachment regions (2–4, 14, 22, 24), we have shown direct evidence that Pias1 binds DNA with strong affinity via its four-helix SAP domain (Figs. 2 and 4). The structural analysis by DALI and the sequence alignment of the SAP-related domain indicate that the four-helix bundle is a novel DNA binding motif distinct from many of DNA-binding domains reported so far. Interestingly, unlike the known DNA recognition sites, one end of the helix bundle makes contacts with DNA as if it were wedged into the DNA grooves (Figs. 6 and 9).

Fig. 9 shows the top view of the ribbon model of the four-helix SAP domain (a), strongly perturbed residues in red, and the moderately perturbed residues highlighted in pink (b) as well as the electrostatic surface potential (c). The perturbed residues are located at the N termini of both α2- and α3-helices (Fig. 9b) whose locations are nearly identical to the positive surface potential shown in Fig. 9c, suggesting that the top face of the four-helix bundle is the binding site for DNA.

N-terminal Four-helix SAP Domain Interacts with Transcription Factors—The GST pull-down assays revealed that the RING finger domain is not involved in p53 binding but that the four-helix SAP domain of Pias1 is involved in p53 binding (Fig. 1). Such involvement of the SAP domain in binding to transcription factors also has been reported in which the interaction of Piasy with Wnt-responsive transcription factor Lef-1 was shown (14). It also was demonstrated that the DNA-binding domain, the high mobility group box, of Lef-1 was attributed to the binding site for the SAP motif. Similar results were obtained for a T-cell factor 4 belonging to the T-cell factor family of proteins with the high mobility group DNA binding motif (26).

The DNA binding ability of Pias1 is rational in light of the ideas that the Pias family associates with the scaffold/matrix attachment regions or nuclear bodies. It has been shown that Piasy sumoylates transcription factors and targets them into specific nuclear bodies such as PML bodies. The localization and function of PML proteins appear to be strongly influenced by the conjugation with SUMO-1, i.e. sumoylated PML protein is localized selectively to PML bodies (1–4, 10). Although specific SUMO ligase for the PML protein has not been identified, the Pias family members might be potential candidates for PML sumoylation ligase.

In conclusion, our experiments provide insight into the structure and function of the N-terminal domain of Pias1 that exhibits the binding ability for both DNA and specific transcription factors. Such binding duality may facilitate the localization of the Pias protein family at the vicinity of nuclear bodies and make the target transcription factors accessible to sumoylation by E3 ligase Pias. Therefore, it is tempting to speculate that sumoylation may be enhanced at nuclear bodies and that the sumoylated target proteins would be recruited into specific subnuclear bodies.

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NMR Structure of the N-terminal Domain of SUMO Ligase PIAS1 and Its Interaction with Tumor Suppressor p53 and A/T-rich DNA Oligomers
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