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NMR characterization of SRG3 SWIRM Domain Mutant Proteins.

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Abstract : SWIRM domain, a core domain of SRG3 is well conserved in SWI3, RSC8, and MOIRA family proteins. To understand structural basis for cellular functions of the SWIRM domain, we have initiated biochemical and structural studies on SWIRM domain and mutants using gelfiltration chromatography, circular dichroism and NMR spectroscopy. The structural properties of the mutant SWIRM domains (K34A and M75A) have been characterized, showing that the structures of both wild-type and mutant proteins are α-helical conformation. The data conclude that mutations at interaction sites of its binding partner protein do not affect its secondary and tertiary structure.
Keywords: SRG3, SWIRM domain, circular dichroism, nuclear magnetic resonance, chromatin remodeling complex.

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INTRODUCTION

Homosapiens SRG3, a core subunit of SWI/SNF an ATP-dependent chromatin remodeling complex, is composed of SANT domain, SWIRM domain, leucine-zipper motif, and proline and glutamine rich region (Fig. 1A).[1] SWIRM domain conserved in SWI3, RSC8, and MOIRA family proteins comprised of an 85-residue from the open reading frame of *Mus musculus* with a molecular weight of 11.4 kDa. SWIRM domain is considered as a motif which participates in interaction with partner proteins. SWIRM domain from the human transcriptional adaptor ADA2alpha is similar to mouse SRG3 and the structure

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reveals a five-helix bundle consisting of two helix-turn-helix motifs connected by a central long helix, reminiscent of the histone fold. It has been known that the SWIRM domains of human ADA2alpha bind to double-stranded and nucleosomal DNA by colocalizing with lysine-acetylated histone H3 in the cell nucleus and enhancing accessibility of nucleosomal linker DNA bound to histone H1.[2,3] We have identified the binding residues of the SRG3 SWIRM domain with INI1 protein in the SRG3 SWIRM by NMR spectroscopy. Since the protein folding is important in biological function of mutant proteins, we performed structural characterization of mutant proteins compared with that of wilt type.[4] Here, by CD experiment and NMR experiment we present how mutating the 34th residue, lysine to alanine, and 75th residue, methionine to alanine, affects the overall protein folding (Fig 1A).

EXPERIMENTAL PROCEDURES

Overexpression, isotope labeling and purification.

Wild type SWIRM domain and mutants, K34A and M75A were over-expressed in *Escherichia coli* BL21 (DE3) strain (Novagen Inc). In order to obtain an unlabeled protein, cells were grown on LB broth media at 37℃ until optical density reached 0.6 at 600nm for CD experiment. Then protein expression was induced with 1mM isopropyl β -D-thiogalactoside (IPTG) for 20 hours at 25℃. Cells were harvested by centrifugation at 6000 x g for 25 minutes. The cell pellets were suspended in 15ml binding buffer (25 mM sodium phosphate, 100 mM sodium chloride, 5 mM β-mercaptoethanol, in pH 6.8) and lysed by the sonicator. The lysate was then centrifuged for 30 minutes at 14000 x g to gain soluble supernatant. Wild type and mutant SWIRM domains fused to MBP was purified with Ni^{2+} affinity chromathgraphy (Amersham Pharmacia Biothech, Uppsala, Sweden). The proteins bound to the column were washed with 40 mM imidazole washing buffer and eluted with 500 mM imidazole elution buffer. The MBP was cleaved with tobacco edge virus (TEV) for 15 hours at 25℃. The TEV enzyme and MBP were separated with Ni^{2+} affinity chromatography. The purified protein was filtrated through gel filtration chromatography.[5] For obtaining a ^{15}N-labeled protein, cells were grown on M9 minimal media with $^{15}NH_4Cl$ at 37℃, and thiamine 0.1%, glucose and ampicillin 5% were added in the media to support

optimal growth. After the optical density reached 0.6 at 600 nm, the cells were harvested by centrifugation about 20h after induction. The ^{15}N-labeled proteins were purified by the same method as unlabeled protein purification. The final CD sample was approximately 0.3 mM concentration for near-UV experiment and 0.1 mM concentration for far-UV experiment with 30 mM sodium phosphate and 5 mM β-mercaptoethanol at pH 6.8. The final NMR sample was approximately 0.3 mM concentration with 100 mM sodium chloride, 2 mM dithiothreitol (DTT) and 10 mM HEPES buffer containing 0.01% NaN_3 at pH 6.8.

Circular dichroism

All CD spectra of the wild type and mutant SWIRM domains were collected at 20℃ on a Jasco 810 spectropolarimeter.[6] Far-UV CD spectra were collected from 190 to 250 nm and near -UV spectra were monitored from 240 to 340 nm using quartz cell having path-length of 0.1 cm. Data were collected at response time of 1 s, and a scan speed of 50nm/min. Spectra were recorded as an average of eight scans.[7]

NMR spectroscopy

One-dimensional proton and 2D [^1H-^{15}N] HSQC experiments[8] were carried out on a Bruker DRX 500 MHz spectrometer equipped with a 5 mm cryoprobe™ at 25℃, pH 6.8. Spectra were processed with the NMRPipe/nmrDraw software package and analyzed using the Sparky program.[9,10]

RESULTS and DISCUSSION

In SDS-PAGE, purified wild type and mutant SWIRM domains (K34A and M75A) are not ascertained as 11.4 kDa but about 55.4 kDa, because all proteins were not treated with TEV enzyme (Fig. 1B). Concluding from the highest peak of gel filtration and SDS-PAGE profile of TEV cleavage, it is clear that the molecular weight of SWIRM domains is about 11.4 kDa (Fig. 2.). To confirm the molecular weight of the purified SWIRM domains by gel filtration chromatography, a calibration curve relating elution volume and molecular weight was used with known molecular weights (Fig. 2.).

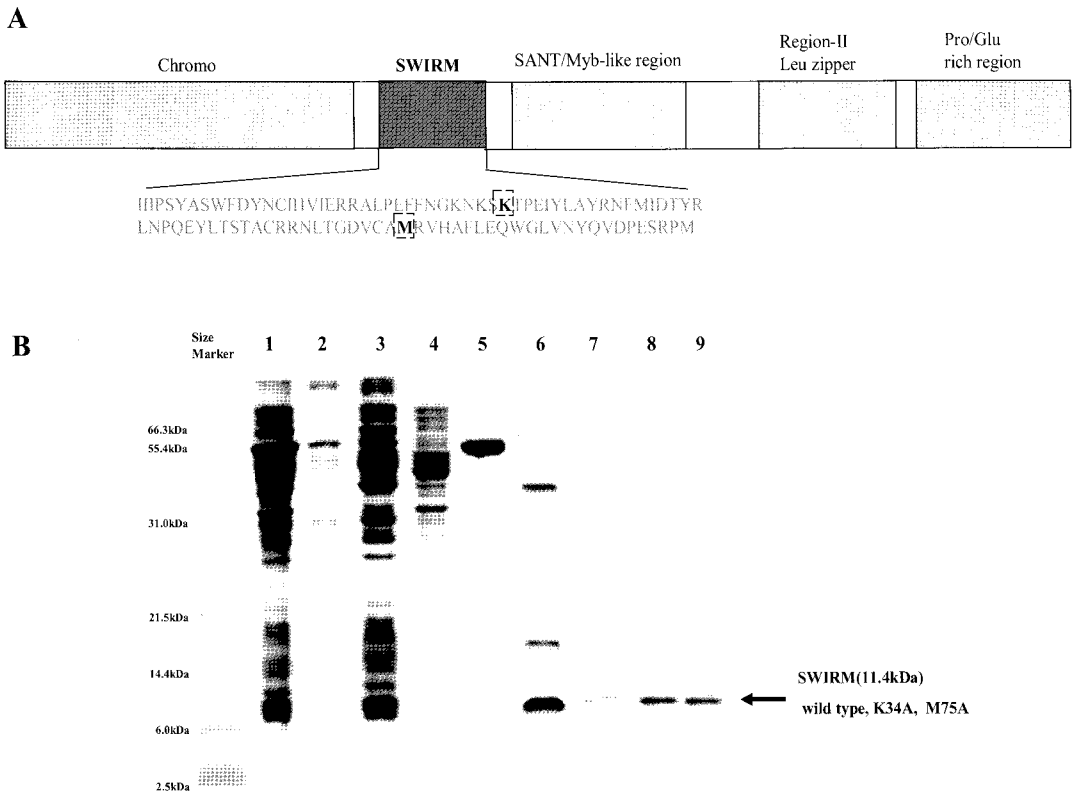

Fig. 1. (A) Domain Organization of SRG3. (B) SDS-PAGE analysis at each step of purification for wild type and mutant SWIRM domains is displayed. (1), supernatant after centrifugation; (2), precipitant after centrifugation; (3), flow through in Ni^{2+} affinity chromatography step; (4), after washing; (5), elution sample; (6), after TEV cleavage experiment; (7), after gel filtration (wild type); (8), after gel filtration (K34A); (9), after gel filtration (M75A).

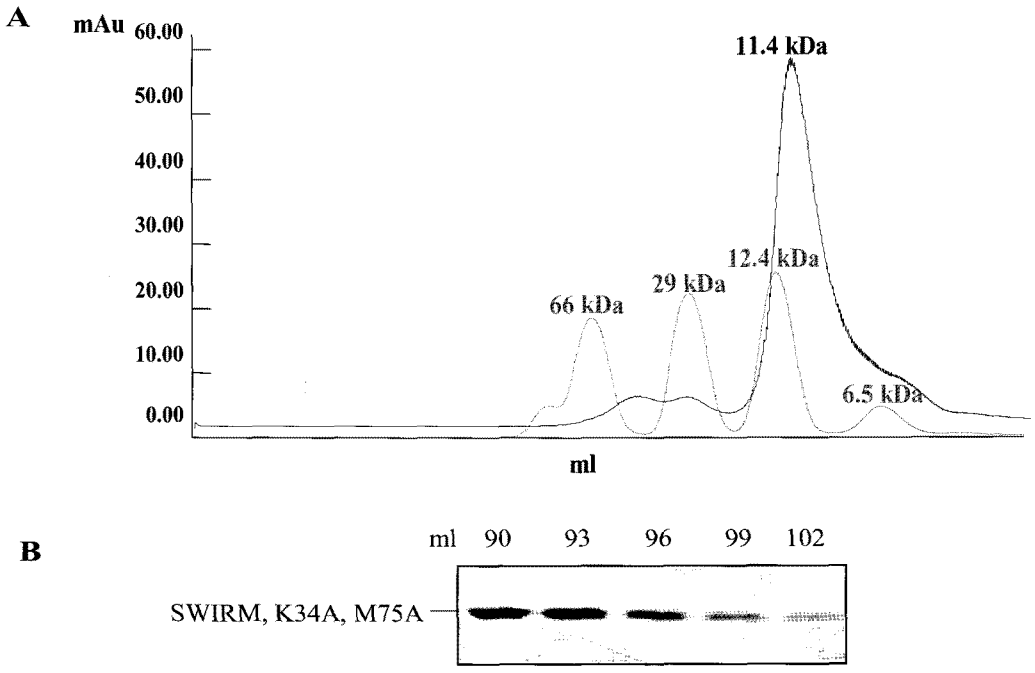

Fig. 2. (A) Gel filtration profile on Superdex 75; Wild type and mutant SWIRM domains (K34A and M75A) are displayed. Standard molecular weights (gray curve) were used to estimate the molecular weight of the SWIRM domains. (B) SDS-PAGE analysis after gel filtration of the SWIRM domains appears as 11.4kDa molecular weight.

From one-dimensional ^{1}H NMR spectra of the wild type and mutant SWIRM domains (K34A and M75A) at 298K, we observed that the spectra have almost same, implying that the fold of all SWIRM domains was not changed by mutation (Fig. 3A, C, E.).

2D [^{1}H-^{15}N] HSQC spectra of the mutant SWIRM domains (K34A and M75A) demonstrated that there are few chemical shift changes compared with those of the wild-type SWIRM domain (Fig. 3B, D, F). In addition, the far-UV CD spectra of the wild type and mutant SWIRM domains (K34A and M75A) indicate that all SWIRM domains are constituted of mostly α-helix form (Fig. 4A). Far-UV CD spectra of mutants, K34A and M75A show that there are not significant changes observed in secondary structures compared with that of wild type SWIRM domain. As we observe in the far-UV CD spectra,

tertiary structures of the wild type and two mutant proteins have almost similar pattern at the near-UV CD spectra (Fig. 4B)

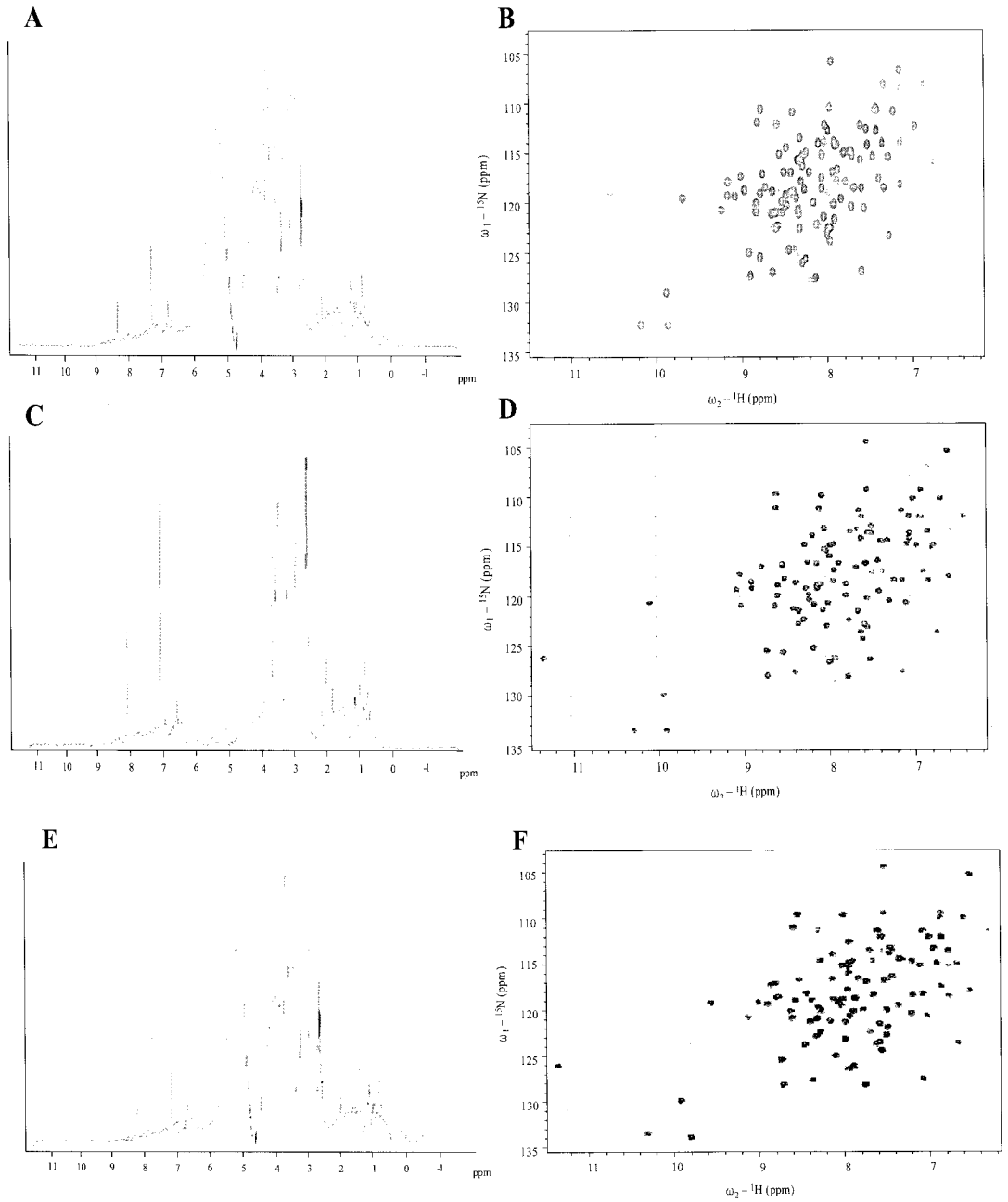

Fig. 3. One-dimensional ^{1}H-NMR spectrum of wild-type SWIRM domain for (A) and mutant SWIRM domains K34A for (C) and M75A for (E) recorded on a bruker DRX 500 MHz spectrometer at 25℃. 2D-[^{1}H-^{15}N] HSQC spectrum of wild type SWIRM domain for (B) and mutant SWIRM domains K34A for (D) and M75A for (F) recorded on a bruker DRX 500 MHz spectrometer at 25℃. Some residues were not appeared in [^{1}H-^{15}N] HSQC and several residues' chemical shift values are changed.

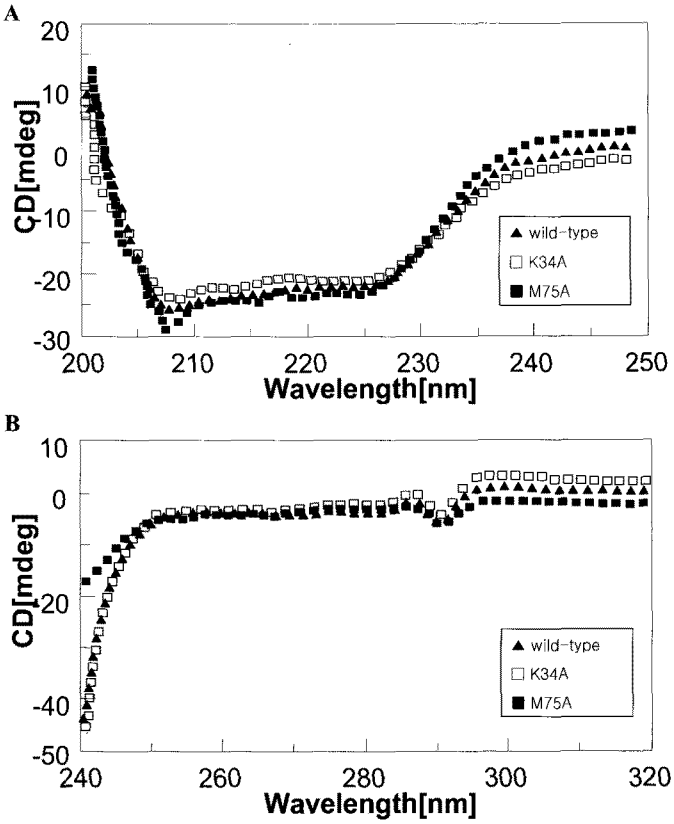

Fig. 4. (A) Far-UV CD spectra of wild type and mutant SWIRM domains (K34A and M75A) are displayed for comparison. (B) Near-UV CD spectra of wild type and mutant SWIRM domains (K34A and M75A) are shown.

Taken together, overall secondary and tertiary structure of the SWIRM mutants were not observed in either CD or NMR spectra. In this study, the conformational changes of the

purified mutant SWIRM domains have been examined using CD and NMR spectroscopy. This result will provide useful information for the study of SRG3-INI1 interaction related to their function.[11]

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