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Backbone and side-chain ^{1}H, ^{13}C, and ^{15}N resonance assignments of the human Sin3A PAH2 domain (residues 295-395)

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Abstract

Sin3A is a conserved transcriptional coregulator that coordinates transcriptional repression through interactions with histone deacetylase complexes and diverse transcription factors. Its paired amphipathic helix 2 (PAH2) domain functions as a protein–protein interaction module that recognizes Sin3-interacting domains from multiple regulatory proteins. Here, we report the backbone and side-chain ^{1}H, ^{13}C, and ^{15}N resonance assignments of the human Sin3A PAH2 domain spanning residues 295-395. The construct yielded a well-dispersed ^{1}H-^{15}N HSQC spectrum and enabled nearly complete backbone assignment. Overall backbone resonance completeness was 98.6%, with 99.0% assignment of backbone amide ^{1}H and ^{15}N resonances, 99.0% of Cα, 99.0% of Cβ, and 97.0% of carbonyl resonances. Extensive side-chain assignments were also obtained. The assigned chemical shifts were used for CS-Rosetta modeling, yielding a predominantly α-helical model characteristic of the PAH fold. Structural superposition with the PAH2 component of the solution NMR structure deposited as PDB 1S5Q gave a Cα RMSD of 2.514 Å over 70 matched Cα atoms, indicating conservation of the overall PAH2 architecture with local deviations in terminal and loop regions. These assignments provide a foundation for future studies of Sin3A PAH2 conformational dynamics and molecular recognition.

Keywords: NMR resonance assignment · Sin3A · PAH2 domain · CS-Rosetta · conformational dynamics · protein–protein interaction

Biological context

Sin3A is an evolutionarily conserved transcriptional coregulator that participates in transcriptional repression by recruiting histone deacetylase-containing complexes to specific genomic loci (Kadamb et al. 2013). Rather than directly recognizing DNA, Sin3A primarily functions as a multidomain protein-protein interaction scaffold that organizes transcription factors and chromatin-modifying enzymes. Human Sin3A contains three paired amphipathic helix domains (PAH1, PAH2, and PAH3) together with additional regions involved in the assembly of histone deacetylase complexes.

The PAH domains are compact interaction modules that recognize short helical Sin3-interacting domains (SIDs) present in diverse transcriptional regulators. PAH2 interacts with SIDs derived from proteins including Mad1/Mxd1, TGIF1, Pf1, and HBP1 (Brubaker et al. 2000; Kumar et al. 2011; He et al. 2021). Structural studies of PAH2–SID complexes have shown that different SID peptides engage a common hydrophobic surface while adopting distinct binding orientations, highlighting the adaptability of the PAH2 recognition interface.

The unliganded PAH2 domain also displays appreciable conformational plasticity. Previous NMR and biophysical studies have shown that free Sin3A PAH2 can populate distinct conformational states and undergo exchange on the millisecond time scale (Zhang et al. 2006), while related behavior has been reported for the PAH2 domain of Sin3B (van Ingen et al. 2006). Such dynamics are potentially relevant to molecular recognition but can complicate residue-specific NMR analysis. Comprehensive chemical-shift assignments are therefore important for characterizing the conformational landscape of PAH2. In the present study, we focus exclusively on human Sin3A PAH2 residues 295-395 and report nearly complete backbone and extensive side-chain resonance assignments together with a chemical-shift-guided CS-Rosetta structural model.

Methods and experiments

Protein expression and purification

The DNA fragment encoding human Sin3A PAH2 residues 295-395 was cloned into a pET-derived expression vector containing an N-terminal His_6 tag followed by a TEV protease cleavage site and transformed into Escherichia coli

BL21(DE3) cells. Uniformly $^{13}C/^{15}N$-labeled protein was expressed in M9 minimal medium containing $^{15}NH_4Cl$ and $^{13}C_6$-glucose as the sole nitrogen and carbon sources. Cells were grown at 37°C to an OD_{600} of approximately 0.8, and protein expression was induced with 1 mM IPTG at 16 °C overnight.

The His_6-tagged protein was purified by Ni^{2+}-NTA affinity chromatography, followed by TEV protease cleavage to remove the affinity tag. The protein was further purified by size-exclusion chromatography on a Superdex 75 column equilibrated in NMR buffer containing 20 mM Tris-HCl (pH 6.8), 150 mM NaCl, 1 mM DTT, and 0.02% NaN_3. Purified Sin3A PAH2(295-395) was concentrated to approximately 0.8 mM for NMR measurements. Protein purity was assessed by SDS-PAGE and identity was verified by mass spectrometry.

NMR spectroscopy

All NMR spectra were recorded at 298 K on Bruker Avance spectrometers operating at a ^1H frequency of 800 MHz and equipped with cryogenic probes. The NMR sample contained approximately 0.8 mM uniformly $^{13}C/^{15}N$-labeled Sin3A PAH2(295-395) in 20 mM Tris-HCl (pH 6.8), 150 mM NaCl, 1 mM DTT, 0.02% NaN_3, and 10% D_2O.

Sequence-specific resonance assignments were obtained using 2D ^1H-^{15}N HSQC and ^1H-^{13}C HSQC spectra together with 3D HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, HBHA(CO)NH, HCCH-TOCSY, ^{15}N-edited NOESY-HSQC, and ^{13}C-edited NOESY-HSQC experiments. The NOESY mixing time was 120 ms. Spectra were processed with NMRPipe and analyzed using Sparky. Sequential assignments were established from intraresidue and interresidue backbone correlations and were extended to side-chain resonances using HCCH-TOCSY and heteronuclear-edited NOESY data.

CS-Rosetta structural modeling

The experimentally assigned chemical shifts of Sin3A PAH2(295-395) were used as input for CS-Rosetta calculations following the chemical-shift-guided structure generation approach described by Shen et al. (2008). The resulting structure is treated here as a chemical-shift-guided structural model rather than as a conventional high-resolution solution NMR structure determined using an extensive set of experimental long-range distance restraints.

Extent of assignment and data deposition

The assigned ^1H-^{15}N HSQC spectrum of uniformly $^{13}C/^{15}N$-labeled Sin3A PAH2(295-395) showed good chemical-shift dispersion and relatively uniform peak intensities, consistent with a folded sample suitable for sequence-specific resonance assignment (Fig. 1). The HSQC labels in Fig. 1 follow the construct numbering (1-101), corresponding to residues 295-395 of full-length human Sin3A; construct residue n therefore corresponds to Sin3A residue n + 294.

The construct contains 101 residues, including four proline residues. Excluding prolines, 97 backbone amide ^1H-^{15}N correlations are expected. Backbone amide ^1H and ^{15}N resonances were assigned for 96 of these 97 sites, corresponding to 99.0% completeness for both HN and ^{15}N assignments. The backbone amide resonance pair of A383 remained unassigned. Assignments were obtained for 100 of 101 Cα resonances (99.0%), with S295 unassigned. Because glycine residues do not contain Cβ atoms, 97 Cβ resonances are expected; 96 were assigned (99.0%), again with S295 unassigned. Carbonyl resonances were assigned for 98 of 101 residues (97.0%); the carbonyl resonances of T350, S386, and T393 remained unassigned.

Taken together, 486 of 493 expected backbone HN, N, Cα, Cβ, and CO resonances were assigned, corresponding to an overall backbone assignment completeness of 98.6%. Extensive side-chain assignments were also obtained. When non-exchangeable proton-bearing side-chain sites represented in the assignment dataset were considered, 344 of 394 expected side-chain ^1H resonances were assigned (87.3%), together with 224 of 262 corresponding proton-bearing ^{13}C resonances (85.5%). This corresponds to a combined completeness of approximately 86.6% for this defined set of proton-bearing side-chain $^1H/^{13}C$ resonances. The remaining unassigned side-chain resonances were concentrated mainly in distal and aromatic side-chain positions.

The complete chemical-shift assignments of human Sin3A PAH2(295-395) will be deposited in the Biological Magnetic Resonance Data Bank (BMRB). BMRB accession number: [to be inserted after deposition].

Chemical-shift-guided structural analysis

The high completeness of the backbone assignments enabled chemical-shift-based structural analysis of the PAH2 domain. The assigned ^1H, ^{13}C, and ^{15}N chemical shifts were used as input for CS-Rosetta calculations, yielding a compact and predominantly α-helical model characteristic of the Sin3 PAH fold (Fig. 2A). For structural comparison, the PAH2 component was extracted from the solution NMR structure of the Mad1 SID–mSin3A PAH2 complex deposited as PDB 1S5Q after removal of the SID peptide (Fig. 2B; Swanson et al. 2004). The overall arrangement of the major helices was comparable between the two structures.

To assess the structural agreement between the two models, the CS-Rosetta structure and the PAH2 reference structure from PDB 1S5Q were superimposed using Cα atoms (Fig. 2C). The structural alignment yielded a Cα RMSD of 2.514 Å over 70 matched Cα atoms. The central α-helical framework shows overall agreement, whereas more pronounced deviations are visible in several terminal and interhelical loop regions. These local differences may reflect greater conformational flexibility in solvent-exposed segments and the distinct structural information underlying the two models, because the CS-Rosetta structure is primarily guided by experimental chemical shifts rather than by an extensive set of long-range distance restraints.

Overall, the comparison indicates that the chemical-shift-guided CS-Rosetta model reproduces the general PAH2 fold while retaining local structural differences relative to the reference solution structure. The resonance assignments and associated structural analysis provide a residue-specific framework for future investigations of PAH2 conformational exchange, ligand-induced structural changes, and recognition of Sin3-interacting domains.

Data availability

The ^{1}H, ^{13}C, and ^{15}N chemical-shift assignments of human Sin3A PAH2(295-395) will be available from the Biological Magnetic Resonance Data Bank under accession number [BMRB ID to be inserted].

Declarations

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Author contributions: [Chang Shu performed the NMR experiments, Shuangli Li analyzed the resonance assignment data, carried out the CS-Rosetta structural calculations, and prepared the manuscript. Xu Zhang supervised the research, contributed to the study design, and revised the manuscript. Danyun Zeng contributed to the experimental work and data analysis. All authors read and approved the final manuscript.].

Competing interests: [The authors declare that they have no competing interests.].

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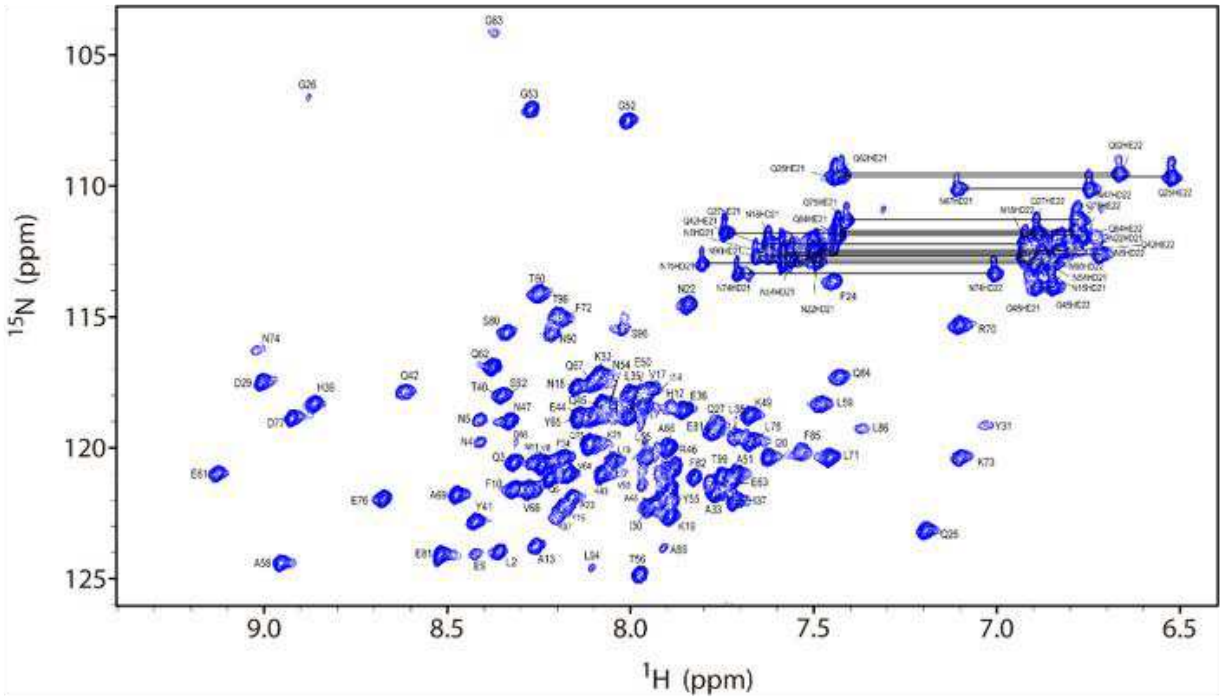


Fig. 1 Assigned ^{1}H-^{15}N HSQC spectrum of uniformly ^{13}C/^{15}N-labeled human Sin3A PAH2 (residues 295–395), recorded at 298 K on an 800 MHz NMR spectrometer. Sequence-specific amide assignments are indicated. Residue labels in the spectrum follow the construct numbering (1-101), corresponding to residues 295–395 of full-length human Sin3A (construct residue n = Sin3A residue n + 294).

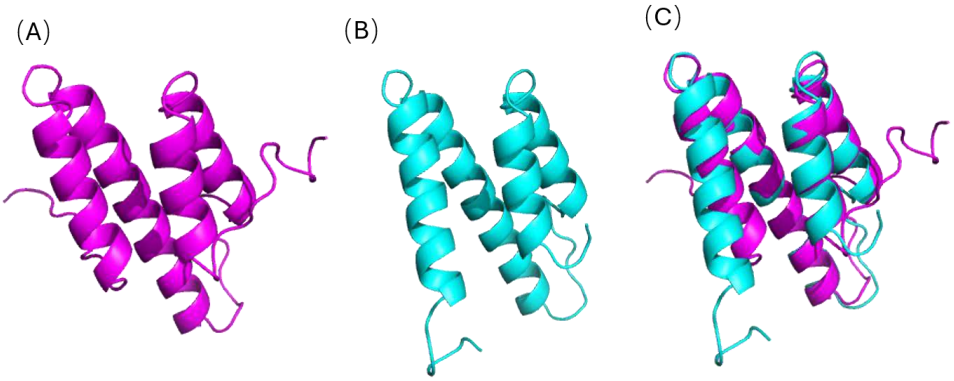


Fig. 2 Structural comparison of the chemical-shift-guided CS-Rosetta model of human Sin3A PAH2(295–395) with a reference solution NMR structure. (A) CS-Rosetta model generated from the experimentally assigned NMR chemical shifts, shown in magenta. (B) PAH2 component of the solution NMR structure of the Mad1 SID–mSin3A PAH2 complex (PDB ID: 1S5Q) after removal of the SID peptide, shown in cyan. (C) Structural superposition of the CS-Rosetta model (magenta) and the reference PAH2 structure (cyan). The major α-helical elements retain a similar overall arrangement, whereas larger deviations are observed mainly in terminal and loop regions. Superposition based on Cα atoms yielded an RMSD of 2.514 Å over 70 matched Cα atoms.