Published in final edited form as:

Biomol NMR Assign. 2014 October ; 8(2): 279–281. doi:10.1007/s12104-013-9500-8.

^1H , ^{13}C , and ^{15}N NMR assignments of a *Drosophila* Hedgehog autoprocessing domain

Jian Xie,

Biochemistry and Biophysics Graduate Program, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

Zhenming Du,

Biochemistry and Biophysics Graduate Program, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

Brian Callahan,

Department of Chemistry, State University of New York at Binghamton, Binghamton, NY 13902, USA

Marlene Belfort, and

Department of Biological Sciences, University at Albany, Albany, NY 12222, USA

Chunyu Wang

Biochemistry and Biophysics Graduate Program, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

Chunyu Wang: wangc5@rpi.edu

Abstract

The Hedgehog (Hh) signaling pathway plays important roles in embryonic growth and patterning in different organisms. Abnormal activity of the Hh signaling pathway has been associated to cancers, holoprosencephaly and autism spectrum disorders. The backbone and side chain resonance assignments of a *Drosophila* Hh auto-processing domain have been determined based on triple-resonance experiments with the [^{13}C , ^{15}N]-labeled and [^2H , ^{13}C , ^{15}N]-labeled proteins.

Keywords

Assignments; Autoprocessing; *Drosophila*; Hedgehog; NMR

Biological context

Hedgehog (Hh) is a vital morphogen in development and the Hh signaling pathway is deregulated in many types of cancers (Varjosalo and Taipale 2008). Recently, evidences also show that Hh plays a role in autism spectrum disorders (Al-Ayadhi 2012). Hh is composed of two domains, the N-terminus signaling domain and C-terminus autoprocessing

domain. The Hh signaling domain requires the covalent attachment of cholesterol at its C-terminus (cholesteroylation) for its transport and precise localization, which is catalyzed by the Hh autoprocessing domain. Hh autoprocessing domain has the Hint (hedgehog intein) fold and utilizes the chemistry of N–S acyl shift, as in the first step of intein-mediate protein splicing. Many NMR and structural studies of inteins have been reported (Oeemig et al. 2012; Frutos et al. 2010; Du et al. 2009; Du et al. 2011). In contrast, there are just few mechanistic studies about the Hh autoprocessing domain (Porter et al. 1996a, b; Hall et al. 1997; Chen et al. 2011). The mechanism of Hh cholesteroylation is still poorly understood; in particular, the precise roles of conserved residues (C₁, D₄₆, T₆₉ and H₇₂) in the cholesteroylation (Hall et al. 1997) also remain poorly defined. As a first step towards carrying out structural and mechanistic studies at atomic resolution using solution NMR, we have determined the backbone and side chain resonance assignments of the *Drosophila* Hh (DHH) autoprocessing domain.

Methods and experiments

Drosophila Hh autoprocessing domain gene was cloned into the pET-45b vector between unique PmlI and HindIII restriction sites. The DHH autoprocessing domain expresses in fusion with a N-terminal (His)₆ tag and 3 DHH signaling domain residues VH₆. Uniformly isotopically labeled ([U-¹⁵N] or [U-¹³C; U-¹⁵N] or [~90 % ²H; U-¹³C, U-¹⁵N]) fusion protein was expressed in BL21 (DE3) cells in M9 medium and purified by the Ni–NTA affinity chromatography followed by DTT-catalyzed release of DHH autoprocessing domain from the whole fusion protein. The DHH autoprocessing domain contains 146 amino acids without the sterol recognition region. NMR samples were prepared in 50 mM sodium phosphate, 100 mM sodium chloride, 10 mM DTT in 90 % H₂O/10 % D₂O or 99.9 % D₂O at pH 7.4 or pH 4.5. The final concentrations of the NMR samples are between 0.6 and 0.7 mM.

All NMR experiments were carried out at 25 °C on a Bruker 800 MHz or 600 MHz (¹H) spectrometer equipped with a cryogenic probe. Spectra were processed with NMRPipe software and analyzed using Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco, USA). The ¹H chemical shifts were referenced relative to DSS and the ¹⁵N and ¹³C chemical shifts were referenced indirectly.

The sequence specific backbone ¹H^N, ¹³C^α, ¹⁵N, ¹H^α, ¹³C^γ and side chain ¹³C^β assignments for DHH autoprocessing domain were obtained by standard triple resonance NMR methods, such as HNCO, HNCACO, HNCACB, HNCOCACB, and ¹⁵N TOCSY experiments. Assignments for aliphatic side chains were achieved by standard NMR techniques, including H(CC)(CO)NH, (H)C(CCO)NH, HCCH-TOCSY, and ¹⁵N TOCSY.

Assignments and data deposition

We achieved 93 % backbone assignments at pH 7.4. In order to get more complete assignments, we also performed HNCACB experiment at pH 4.5 and obtained additional assignments for R₄₇, N₄₈, L₄₉, L₇₃, V₈₇, S₁₄₂ and A₁₄₅. Nearly complete assignments of the backbone (98 %) ¹H^N, ¹⁵N, ¹³C^α, ¹H^α, and ¹³C^γ resonances were obtained with the exception of C₁, C₁₄₃ and V₁₄₆, and 6 Proline residues (P₄, P₁₇, P₇₀, P₇₉, P₁₁₀ and P₁₂₇). All

the $^{13}\text{C}^\beta$ resonances were assigned. Aliphatic side chain ^1H and ^{13}C assignments are nearly complete (98 %). Importantly, the assignments of conserved residues C₁, D₄₆, T₆₉ and H₇₂ are complete except for the amide group of C₁. Unassigned resonances include the side chains of L₆₆, L₇₃, L₁₂₈ and some of the ^1H and ^{13}C resonances for the aromatic rings. The chemical shifts have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under ID number 19156 (Fig. 1).

Acknowledgments

The project described was supported by R01GM081408 (to C.W.) and R01GM44844 (to M.B.) from the National Institute of General Medical Sciences.

References

- Al-Ayadhi LY. Relationship between sonic hedgehog protein, brain-derived neurotrophic factor and oxidative stress in autism spectrum disorders. *Neurochem Res.* 2012; 37(2):394–400. [PubMed: 21984201]
- Chen X, Tukachinsky H, Huang CH, Jao C, Chu YR, Tang HY, Mueller B, Schulman S, Rapoport TA, Salic A. Processing and turnover of the Hedgehog protein in the endoplasmic reticulum. *J Cell Biol.* 2011; 192(5):825–838. [PubMed: 21357747]
- Du Z, Shemella PT, Liu Y, McCallum SA, Pereira B, Nayak SK, Belfort G, Belfort M, Wang C. Highly conserved histidine plays a dual catalytic role in protein splicing: a pKa shift mechanism. *J Am Chem Soc.* 2009; 131(32):11581–11589. [PubMed: 19630416]
- Du Z, Zheng Y, Patterson M, Liu Y, Wang C. pKa coupling at the intein active site: Implications for the coordination mechanism of protein splicing with a conserved aspartate. *J Am Chem Soc.* 2011; 133(26):10275–10282. [PubMed: 21604815]
- Frutos S, Goger M, Giovani B, Cowburn D, Muir TW. Branched intermediate formation stimulates peptide bond cleavage in protein splicing. *Nat Chem Biol.* 2010; 6(7):527–533. [PubMed: 20495572]
- Hall TM, Porter JA, Young KE, Koonin EV, Beachy PA, Leahy DJ. Crystal structure of a Hedgehog autoprocessing domain: homology between Hedgehog and self-splicing proteins. *Cell.* 1997; 91:85–97. [PubMed: 9335337]
- Oeemig JS, Zhou D, Kajander T, Wlodawer A, Iwa^ˆ H. NMR and crystal structures of the *Pyrococcus horikoshii* RadA intein guide a strategy for engineering a highly efficient and promiscuous intein. *J Mol Biol.* 2012; 421(1):85–99. [PubMed: 22560994]
- Porter JA, Ekker SC, Park WJ, von Kessler DP, Young KE, Chen CH, Ma Y, Woods AS, Cotter RJ, Koonin EV, Beachy PA. Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell.* 1996a; 86(1):21–34. [PubMed: 8689684]
- Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. *Science.* 1996b; 274(5285):255–259. [PubMed: 8824192]
- Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. *Genes Dev.* 2008; 22:2454–2472. [PubMed: 18794343]

Fig. 1. ^1H - ^{15}N HSQC spectrum of the Hedgehog autoprocessing domain backbone assignments. Spectrum is recorded at 800 MHz and 25 °C