Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.272*

Studies Towards the Chemical Synthesis of Sonic Hedgehog

Iván Sánchez-Campillo[1], Judith Palà-Pujadas[2], and Juan B. Blanco-Canosa[1]
[1]Chemical Biology, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain;[2] The Donnelly Centre, University of Toronto, M5S 3E1 Toronto, Canada

Introduction

Sonic Hedgehog (ShhN) is an extracellular protein, responsible for the activation of the Hedgehog (Hh) signaling pathway. Hh is present in all metazoans and it carries out an important role in embryo development, by regulating cell proliferation and differentiation into tissues and organs. In adults, it remains quiescent, while it still regulates cell population and growth (Figure 1) [1].

A quite interesting fact about Hh and ShhN is their role in cancer disease. Aberrant signaling of Hh, especially by overexpression of ShhN, has been related to the development of many types of cancer, i.e. pancreatic, glioblastoma, and basal cell carcinoma, while the inhibition of the Hh activity has been demonstrated to stop cell proliferation. Therefore, Hh proteins are attractive as targets for anti-cancer drug discovery [2].

Out of all the possible targets, ShhN is the most attractive one. It is located in the extracellular matrix, enabling easier drug targeting because it does not need to be delivered inside the cell. Despite that, only 3 inhibitors targeting ShhN have been described [2,3]. ShhN is a challenging target to study due to its hydrophobic nature. Native human ShhN is a 174-mer protein that bears two post-translational modifications (PTM): an N-terminal palmitoylation and a *C*-terminal cholesterol ester. These lipidations impair its recombinant expression and chemical synthesis.

Considering its interest in medicine and the synthetic challenge, this project aims to synthesize native ShhN by chemical means and use the synthetic ShhN for drug discovery.

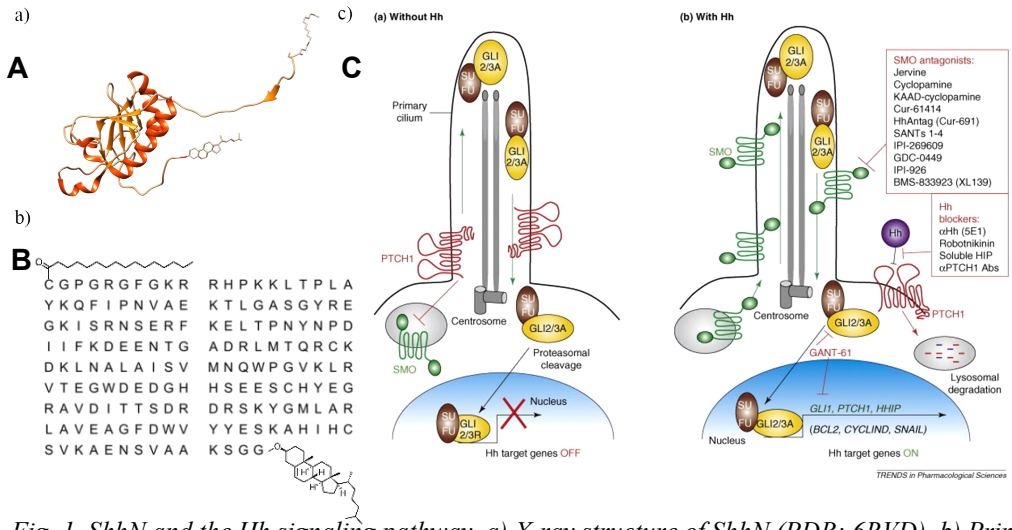

Fig. 1. ShhN and the Hh signaling pathway. a) X-ray structure of ShhN (PDB: 6RVD). b) Primary sequence of ShhN. c) Overview of the Hh pathway and its inhibitors (picture from reference 2).

Methodology

In our group, we rely on the Dbz linkers to synthesize peptide thioesters for native chemical ligation (NCL). First reported by Dawson et. al. [4], these linkers based on *o*-aminoanilides are compatible with Fmoc/*t*Bu SPPS and can be activated by two different pathways, one as *N*-acylbenzimidazolinone (Nbz) and the other as *N*-acylbenzotriazole, both enabling thioesterification and subsequent ligation in the presence of *N*-terminal Cys-containing peptides (Figure 2).

To avoid the branching peptides that can be formed in the *p*-amino position, the methylated MeDbz was developed [5]. This strategy fixed the issue, but it is only reactive through the Nbz (MeNbz) pathway. Most recently, we reported a linker based on 1,2-diaminobenzene which reacts through both pathways, avoids branching, and enables selenoester preparation through the benzotriazole pathway [6].

State of the art

Based on our previous work in which an ShhN analog-bearing biotin at the C-terminus was synthesized [7], the native ShhN is retrosynthetically divided into five fragments (Figure 3). The palmitic acid is introduced in SPPS and the cholesterol is introduced by reaction of Gly-Cholesterol with the fully protected fragment Cys^{160}-Gly^{173}–MeNbz-G. Fragments 2 and 4 bear the MeNbz precursor, the aryloxycarbonyl-*o*-methylaminoanilide (Figure 2, route A), which is cyclized into MeNbz under ligation conditions. These peptides enable kinetically controlled ligations (KCL), as they react slower than those that are prepared as 2-mercaptoethanosulfonic acid thioesters (fragments 1 and 3).

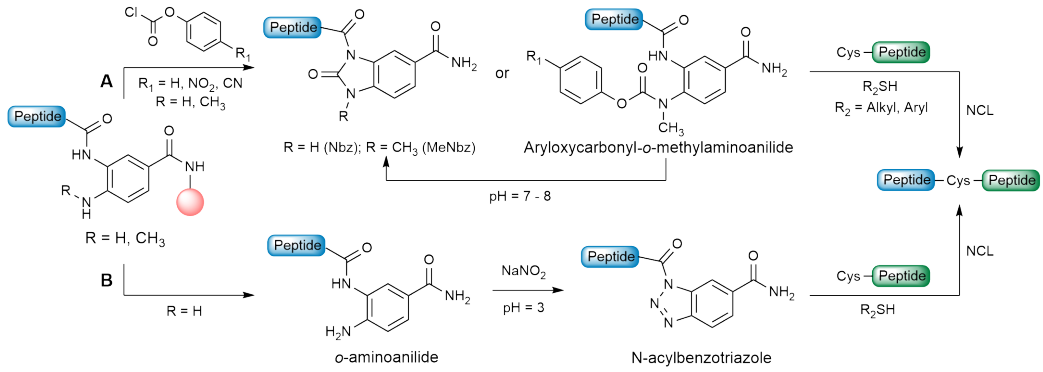

Fig. 2. The Dbz linkers and their application to Native Chemical Ligation (adapted from ref 6). Route A is the Nbz / MeNbz pathway. Route B is the N-acylbenzotriazole pathway.

ShhN only comprises 3 Cys residues, therefore, residues 35 and 122 are mutated into Cys to desulfurize them into native Ala positions after ligation. Moreover, Cys^{1} is protected with Acm to prevent its desulfurization, and Cys^{79} is protected with thiazolidine (Thz) to avoid the intramolecular cyclization. The synthetic scheme pointed out in Figure 3 leaves the ligation of fragment 5 for the last step of the synthesis to minimize the purification steps of this hydrophobic fragment during the synthesis. Remarkably, fragment 1 is still soluble, possibly likely due to the content of Arg and Lys residues in its sequence.

So far, the five fragments have been synthesized and purified, and the first two ligations are being scaled up (Figure 4). Ligations of fragments 1 and 2, as well as fragments 3 and 4, proceed at 10 mM total peptide concentration in guanidinium/-phosphate buffer. 4-Mercaptophenol (4-MPOH) is used as a thiol catalyst. The control of the pH and reaction time enables the two KCL to maintain the MeNbz precursors intact.

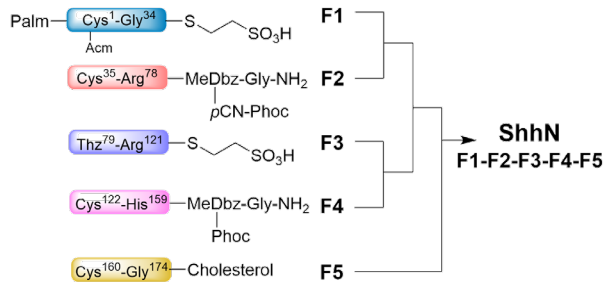

Fig. 3. The proposed synthetic route to ShhN.

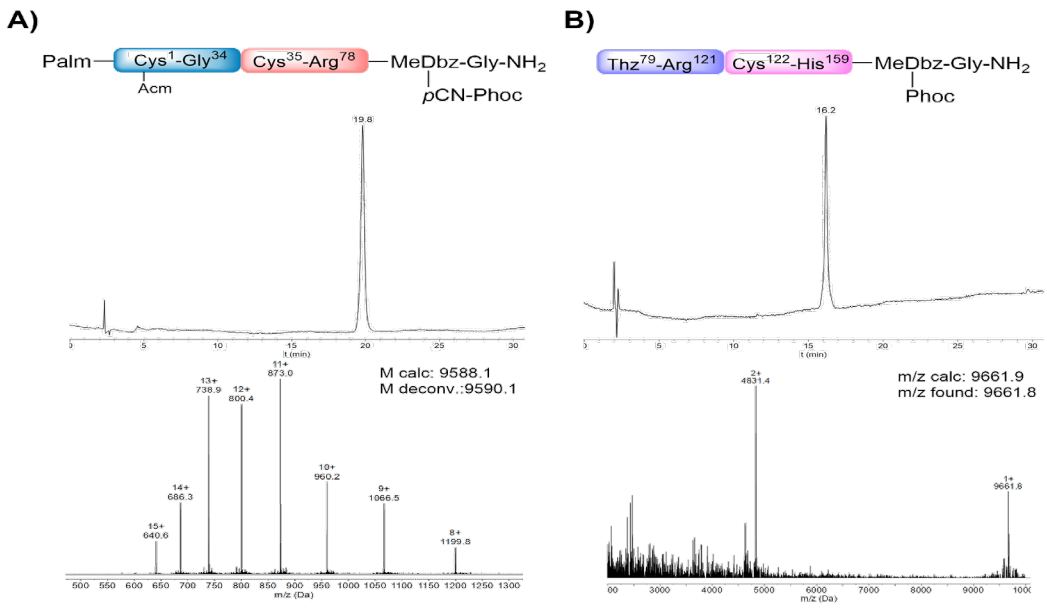

Fig. 4. a) Fragment 1-2. RP-HPLC (C_{18}, 0 to 70% ACN, 30 min) and ESI-MS. b) Fragment 3-4. RP-HPLC (C_3, 0 to 70% ACN, 30 min) and MALDI-TOF.

Acknowledgments

We thank our funding agencies, the Spanish Ministerio de Ciencia and the Agency for Management of University and Research Grants (AGAUR), which supported the research through the National Research Plan (RTI 2018-096323-B-I00) and the FI grant (2021 FI_B 00142).

References

1. Lum, L. and Beachy, P.A. *Science* **304**, 1755-1759 (2004), https://doi.org/10.1126/science.1098020
2. Scales, S.J. and de Sauvage, F.J. *Trends Pharmacol. Sci.* **30**, 303-312 (2009), https://doi.org/10.1016/j.tips.2009.03.007
3. Owens, A.E., de Paola, I., et. al. *J. Am. Chem. Soc.* **139**, 12559-12568 (2017), https://doi.org/10.1021/jacs.7b06087
4. a) Blanco-Canosa, J.B. and Dawson. P.E. *Angew. Chem. Int. Ed.* **47**, 6851-6855 (2008), https://doi.org/10.1002/anie.200705471, b) Mahto, S. K., Howard, C.J. et al. *ChemBioChem* **12**, 2488-2494 (2011), https://doi.org/10.1002/cbic.201100472, c) Zhao, Z., Mousa, R. and Metanis, N. *Chem. Eur. J.* **28**, e202200279 (2022), https://doi.org/10.1002/chem.202200279, d) Maity, S., Jbara, M., Mann, G. et al. *Nat. Protoc.* **12**, 2293-2322 (2017), https://doi.org/10.1038/nprot.2017.049
5. a) Blanco-Canosa, J.B., Nardone, B., et. al. *J. Am. Chem. Soc.* **137**, 7197-7209 (2015), https://doi.org/10.1021/jacs.5b03504, b) Sakamoto, K., Tsuda, S., et al. *Chem. Commun.* **53**, 12236-12239 (2017), https://doi.org/10.1039/C7CC07817J, c) Acosta, G.A., Royo, M., et al. *Tetrahedron Letters* **58**, 2788-2791 (2017), https://doi.org/10.1016/j.tetlet.2017.06.008, d) Gless, H.B. and Olsen, C.A. *J. Org. Chem.* **83**, 10525-10534 (2018), https://doi.org/10.1021/acs.joc.8b01237
6. Sánchez-Campillo, I., Miguel-Gracia, J., et. al. *Chem. Sci.* Advance Article (2022), https://doi.org/10.1039/D2SC04158H
7. Palà-Pujadas, J., Albericio, F., and Blanco-Canosa, J.B. *Angew. Chem. Int. Ed.* **57**, 16120-16125 (2018), https://doi.org/10.1002/anie.201810712