Synthesis of a fluorescent antimicrobial peptide derivative UBI 31-38 via click reaction

Soraya Maria Zandim Maciel Dias Ferreira[a], Hugo Vinícius de Andrade Lara[a], Rosemeire Brondi Alves[a], Jarbas Magalhães Resende[a], Rossimiriam Pereira de Freitas[a]*

[a]*Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*

**rossipdf@yahoo.com.br*

Keywords: ubiquicidin, coumarin, click reaction

INTRODUCTION

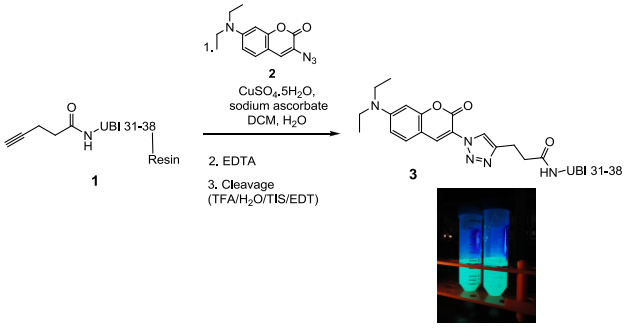
Ubiquicidin (UBI 1-59) is a cationic peptide of human origin which has shown to be antibacterial against a broad spectrum of pathogens, including methicillin resistant *S. aureus* (MRSA). The smaller synthetic fragment UBI 31–38 (RAKRRMQY) also exhibits strong activity against multi-drug resistant micro-organisms[1] and offers a promising alternative for antimicrobial therapy and detection of infections in humans, as it is easy to be synthesized and produced under Good Manufacturing Practices. Although the basis of its antimicrobial activity is the interaction of the cationic domains of the peptide with the negatively charged surface of micro-organisms, the number of cationic residues itself cannot fully explain the antimicrobial activity of antimicrobial peptides.[1] The physiological relevance, mechanism of action and antimicrobial spectrum of ubiquicidin are not well understood.[2]

The bioconjugation of peptides with pro-fluoropheres can be a good strategy to produce biologic probes.[3] Coumarin derivatives have been investigated as pro-fluorophores since they are small in size, biocompatible, and easy to manipulate synthetically. Copper-catalyzed azide-alcine cycloaddition (CuAAC) reaction has been largely employed for bioconjugating fluorescent molecules to peptides under mild conditions and has applications in the field of cell biology and functional proteomics.[4]

We aimed to develop a synthetic route for the preparation of a fluorescent antimicrobial peptide UBI 31-38 to better identify cell interaction mechanisms and evaluate its intrinsic biological activity.

RESULTS AND DISCUSSION

The peptide UBI 31-389 (RAKRRMQY) was prepared by classical Fmoc solid phase peptide synthesis using Rink amide resin. The alkine-decorated peptide **1** (scheme 1) was obtained by coupling 4-pentynoic acid to the peptide during the solid-phase synthesis. The pro-fluorophore 3-azido-7-diethyl-aminocoumarin **2** was synthesized in 3 steps from ethyl nitroacetate using a Knoevenagel condensation as key step. The click reaction between **1** and **2** in the presence of Cu (I) yielded the 1,2,3-triazole peptide derivative **3.** Cleavage of peptide-resin and removal of side chain protecting groups were accomplished with a solution of trifluoroacetic acid: water: tri-isopropylsilane: 1,2-ethanedithiol 94.0:2.5:2.5:1.0. The new derivative obtained displayed high fluorescence, as shown in Scheme1.



Scheme 1. Synthesis of fluorescent peptide UBI 31-38 by CuAAC reaction

CONCLUSION

CuAAC reaction was successfully employed for the conjugation of the synthetic antimicrobial peptide UBI 31-38 to a pro-fluorophore coumarin derivative. The new peptide obtained was fluorescent and might prove useful as an imaging probe for elucidation of biological mechanism of ubiquicidin in infections.

ACKNOWLEDGEMENTS

We thank FAPEMIG for financial support and CNPq for fellowships to H.V.A. L. and R.P.F.

REFERENCES

[1] Brouwer, C.; Bogaards, S.; Wulferink, M.; Velders, M. e Welling, M. *Peptides* **2006**, *27*, 2585.
[2] Wiesner, J. e Vilcinskas, A. *Virulence* **2010**, *1(5)*, 440.
[3] Kalia, J. e Raines, R. *Curr. Org.Chem.***2010**, *14(2)*, 138.
[4] Rostovtsev, V.; Green, L.; Fokin, V. e Sharpless, K. *Angew. Chem.***2002**, *41*, 2596.