Synthesis and Characterization of a Pro-Thr Dipeptide

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Introduction

IgA1 Proteases are proteins that exist in bacteria and are known to cleave human IgA1 (Immunoglobulin A, subclass 1) proteins, which are key antibodies in all major human secretions such as tears, saliva, bronchial mucus, and intestinal mucus [8]. They serve as a first line of defense in the human body by attaching to invading cells. IgA1 proteases target this protein by cleaving this protein in the hinge-region, particularly in the following sequence region: TPPTPSPSTPPTPSPS (T, P, S being threonine, proline, and serine) which are across the top of the IgA1 protein. In an attempt to learn about protein synthesis, and to learn about protein characterization, the primary cutting region in the previous sequence for the IgA1 protease is between the Threonine and the Proline in the center of that region. This proposal presents the procedure to synthesize the dipeptide region of interest in IgA1 proteins, namely the Thr-Pro sequence, and then learn how to characterize it using NMR NOE spectroscopy techniques.

Methods

Synthesize

Dipeptide synthesis is a well known reaction mechanism. This project concerns making the dipeptide Thr-Pro sequence by protecting the N-terminal of the Thr amino acid with a t-Boc protecting group. The carboxyl end of the resulting t-Boc-Thr amino acid is activated by washing with DCC (dicyclohexylcarbodiimide. The second amino acid, Proline, is introduced to the solution and its N-terminal attacks the activated c-terminal of the Thr, and dicyclohexylurea and the t-Boc-Thr-Pro amino acid sequence are the resultant products. Adding a weak, dilute acid solution to the t-Boc-Thr-Pro will cause the t-Boc group to fall off of the Thr, leaving the Thr-Pro sequence. This process is described in a more detailed manner in *Burton et. al.* [2].

Characterize

NOE analysis comes in many forms, but at a basic level attempts to identify a molecule by judging the distance between proton signals in a 1D proton spectrum. First a 1D proton spectrum is taken, and then the standard NOESY experiment is run with the spinner off, and the 1D results are ported into the NOESY results for spectrum comparison. Not much more is known at this point on how to do a NOE experiment on the NMR, but I propose to learn how to do it.

Potential Results

If the synthesis is successful, the challenge will be to learn how to characterize the dipeptide using NOE analysis in Hampden-Sydney's 400Mhz NMR Spectrometer. If the project is fully successful, a new NOE procedure will have been developed for the H-SC Chemistry department, and more longer peptides can be studied at a later date.

References

[1] Wood, S.; Lynch, M. Plaut, A. G., Burton, J. (1989). Tetrapeptide Inhibitors of the IgA1 Proteinases from Type I Neisseria gonorrhoeae. *J. Med. Chem.* **32,** 2407-2411.

[2] Burton, J.; Wood, S.G.; Lynch, M.; Plaut, A.G. (1988). Substrate Analogue Inhibitors of the IgA1 Proteinases from Neisseria gonorrhoeae. *J. Med. Chem.* **31,** 1647-1651.

[3] Mistry, D.; Stockley, R.A. (2006). IgA1 Protease (Molecules in Focus). *International Journal of Biochemistry and Cell Biology.* **38,** 1244-1248.

[4] Parsons, H.K.; Vitovski, S.; Sayers, J.R. (2004). Immunoglobulin A1 Proteases: A structure-function update. B*iochemical Society Transactions.* **32,** part 6.

[5] Frangione, B.; Wonfenstein-Todel, C. (1973). Partial Duplication in the "Hinge" Region of IgA1 Myelomea Proteins. *Proc. Nat. Acad. Sci.* **69,** *No. 12.* 3673-3676.

[6] Vartak, A.P.; Johnson, R.L. (2006). Concerted Synthesis of a Spirobicyclic Type-VI β-Turn Mimic of Pro-Pro-Pro-NH_2. *Organic Letters.* **8,** *No. 5.* 983-986.

[7] Sahai, M.A.; Viskolcz, B.; Pai, E. F.; Csizmadia, I.G. (2007). Quantifying the Intrinsic Effects of Two Point Mutation Models of Pro-Pro-Pro Triamino Acid Diamide. A First-Principle Computational Study. *J. Phys. Chem. B.* **111,** 13135-13142.

[8] Bax, A. (2003). Weak Alignment Offers New NMR Opportunities to Study Protein Structure and Dynamics. *Prot. Sci.* **12,** 1-16.

[8] Berg, J.M.; Tymoczko, J.L.; Stryer, L. (2005) Biochemistry. Sixth Edition. (pp. 951)

[9] Senior, B.W.; Woof, J.M. (2005). The influences of Hinge Length and Composition on the Susceptibility of Human IgA to Cleavage by Diverse Bacterial IgA1 Proteases. *The Journal of Immunology.* **174,** 7792-7799.

[10] Bax, A. (2003). Weak Alignment Offers New NMR Opportunities to Study Protein Structure and Dynamics. *Protein Science.* **12,** 1-16.