# The Synthesis of RGD Peptides via Solid Phase Peptide Synthesis

Chee Yang, Daniel H. Rose and Dr. Thao Yang ❖ Chemistry ❖ University of Wisconsin-Eau Claire

## Abstract

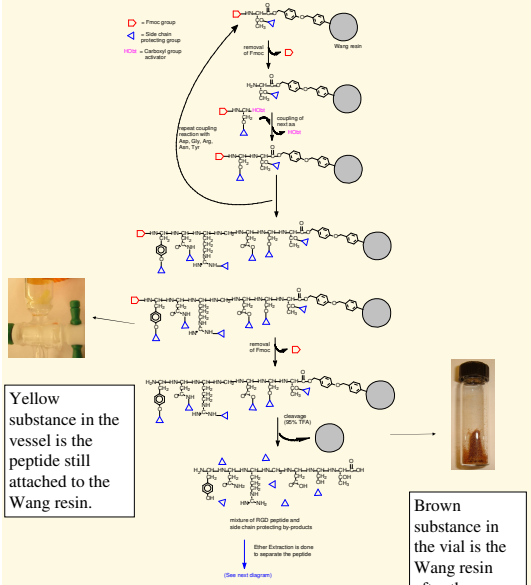
The amino acid sequence Arg-Gly-Asp or RGD is present on several extracellular matrix proteins and known to be a requirement for their binding to integrins, which are a class of cell receptor proteins on cell surface. Some of the well-studied extracellular matrix proteins included fibrinogen, fibronectin, vitronectin, collagen, and laminin, which contain the RGD sequence. Subsequent studies in this project will focus on the conformational structures of the RGD-peptides and their binding properties to integrins. We present here the methodology for the synthesis of two linear RGD peptides using the Solid Phase Peptide Synthesis Method and some preliminary NMR data.

## Introduction

RGD peptides are short peptide fragments derived from the amino acid sequence of several extracellular matrix proteins, such as fibrinogen, fibronectin, vitronectin, collagen, and laminin. The amino acid sequence Arg-Gly-Asp or RGD present on extracellular matrix proteins is known to be a requirement for binding to cell surface receptor proteins, the integrins. The binding of extracellular matrix proteins to the integrin receptors by the RGD sequence involves a number of important cellular processes, such as cell anchorage to the extracellular matrix, cell-to-cell communication, cell growth and migration, blood clotting, and so on (1, 2). Certain unnatural processes such as microbial invasion of cells and tumor metastasis are also involved with some type of ligand-to-receptor binding via the RGD sequence (3). Small RGD peptides such as the ones proposed to be synthesized in this project have been known to have the ability to bind to cell surface receptors just like the native extracellular matrix proteins do. Therefore, these little RGD peptides have been proposed to be used as antagonists to the extracellular matrix proteins (4). In this project we present the synthesis of an RGD peptide derived from the RGD region of fibrinogen, which has the sequence YNRGDST (5). Fibrinogen is a protein involved in the mechanism of blood clotting. The synthesis of this peptide was carried out manually by the Solid Phase Peptide Synthesis Method (SPPS), employing the Wang resin.

## Materials and Methods

**Figure 1. Solid Phase Peptide Synthesis**




This diagram shows the chemical reactions and the steps used in the SPPS method.

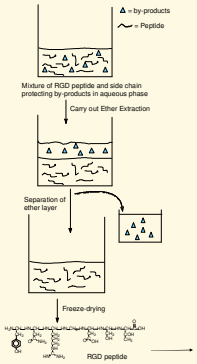
Yellow substance in the vessel is the peptide still attached to the Wang resin.





Brown substance in the vial is the Wang resin after the peptide has been cleaved from it.

This illustration depicts the extraction of the RGD peptide from the by-products.




White solid is the RGD peptide after freeze-drying has been completed.

## Results

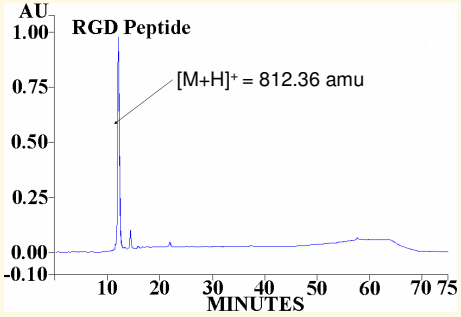



**Figure 2.** This figure is a HPLC chromatogram of the RGD peptide at 220 nm. A concentration of 1 mg/ml was used for analysis. The organic solvent and polar solvent used for the HPLC analysis were acetonitrile containing 0.1% TFA and water containing 0.1%TFA respectively.

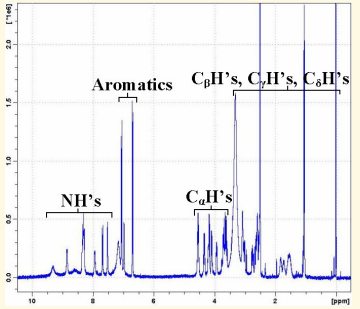



**Figure 3.** 1D $^1$H-NMR spectrum of RGD peptide in DMSO; (no specific $^1$H assignments have been made.)

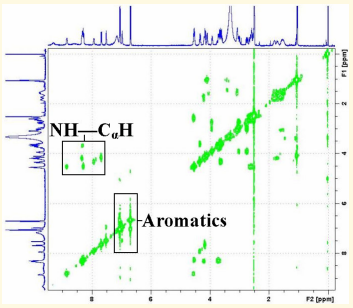



**Figure 4.** This figure shows the COSY 2-D $^1$H-NMR spectrum. This data will be used to make all the proton assignments.

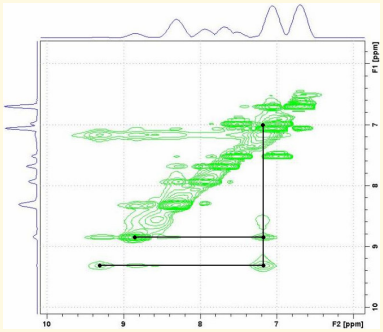



**Figure 5.** This figure shows the 2D NOESY $^1$H-NMR spectrum at the NH—NH region in DMSO, indicating that the peptide backbone is bent.

## Conclusions

Based on the HPLC, NMR, and mass spectral data, we conclude that the RGD peptide has been synthesized.

The 2D NOESY data in the NH-NH region indicated that the peptide backbone is folded.

Immediate future study is to assign all the protons on the RGD peptide molecule and further investigate its structure.

## References

1. Hynes, R. O. (1992) "Integrins: Versatility, Modulation, and Signaling in Cell Adhesion," *Cell.* **69**, 11-25.

2. Lodish, H., Baltimore, D., Arnold, B., Zipursky, L. S., Matsudaira, P., and Darnell, J. (1995) in "Molecular Cell Biology," 3rd ed., W. H. Freeman and Co., New York, 1143-1166.

3. Cheresh, D. A., and Spiro, R. C. (1987) "Biosynthetic and Functional Properties of an Arg-Gly-Asp-directed Receptor Involved in Human Melanoma Cell Attachment to Vitronectin, Fibrinogen, and von Willebrand Factor." *J. Biol. Chem.*, **262**, 17706-17711.

4. Greenspoon, N., Hershkoviz, R., Alon, R., Varon, D., Shenkman, B., Marx, G., Federman, S., Kapustina, G., and Lider, O. (1993) "Structural Analysis of Integrin Recognition and the Inhibition of Integrin-Melated Cell functions by Novel Nonpeptidic Surrogates of the Arg-Gly-Asp Sequence." *Biochemistry*, **32**, 1001-1008.

5. Reed, J., Hull, W. E., von der Lieth, C. W., Kübler, D., Suhai, S., and Kinzel, V. (1988) "Secondary structure of the Arg-Gly-Asp recognition site in proteins involved in cell-surface adhesion. Evidence for the occurrence of nested β-bends in the model hexapeptide GRGDSP." *Eur. J. Biochem.*, **178**, 141-154.

**Acknowledgments**: This research was supported by the UWEC University Research and Creative Activity Grant (2006 - 07) via the ORSP office.