Delivery of an MK2 Inhibitor Utilizing Peptide Stapling

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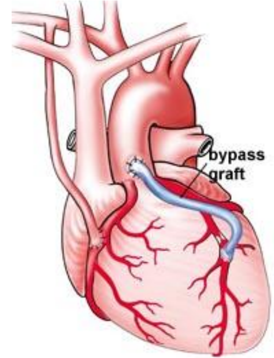
Abstract

Coronary artery bypass grafting is an effective treatment for ischemic heart diseases, but long-term patency remains a significant problem. Graft failure is primarily attributed to intimal hyperplasia, a pathological process in which vascular smooth muscle cells (VSMCs) migrate, proliferate, and deposit extracellular matrix into a neointima. MAPKAP kinase II (MK2) is an upstream regulator of heat shock protein 27 which has been shown to play a major role in the transition of VSMCs to the pathological, proliferative phenotype characteristic of intimal hyperplasia. Therefore, we hypothesize that successful inhibition of MK2 will prevent intimal hyperplasia and ultimately improve graft patency. A previous study has identified a peptide sequence, MK2i, which effectively inhibits MK2 at a concentration range of 8.1-134 µM. However, efficient intracellular delivery of the peptide remains a significant barrier. The overall goal of this project is to determine if peptide stapling can be utilized to enable intracellular delivery of the MK2i peptide. Peptide stapling uses a ring closing reaction to add a hydrocarbon "staple" to successive turns of an α-helix. Stapled peptides have increased helicity, potency, protease resistance and most importantly, cell permeability. The specific aim of this project was to determine if MK2i was a suitable candidate for peptide stapling and develop a protocol for the synthesis of the stapled MK2i peptide. Mk2i was synthesized using solid-phase peptide synthesis (SSPS) with Fmoc chemistry. Circular dichroism was used to assess secondary structure of MK2i in both water and trifluoroethanol which is known to induce α- helical secondary structure in peptides. The circular dichroism results showed that the peptide was 6% α-helical in water and 13% α-helical in trifluoroethanol. These findings suggest that MK2i may be a suitable candidate for peptide stapling. This hypothesis will be further tested by implementing the developed peptide stapling protocol to determine if stapled MK2i peptides showed increased potency, specificity and cell permeability for potential use as a therapeutic in coronary artery bypass grafting.



Significance

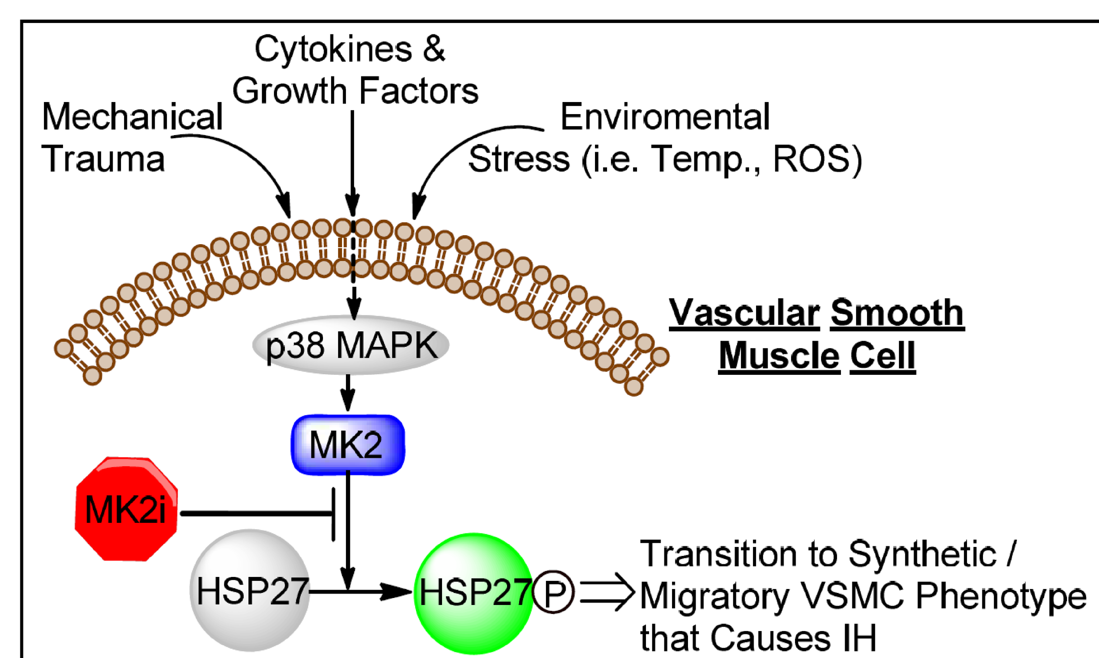


Figure 1: p38 Protein Pathway and VSMC Pathogenesis

- Greater than 45% of the over 500,000 coronary artery bypass graft surgeries fail within the first 18 months
- Intimal hyperplasia is the primary cause of graft failure
- MAPKAP Kinase II, MK2, is involved in the pathogenesis of intimal hyperplasia (Figure 1)

Purpose

- To determine if peptide stapling can be used to enable intracellular delivery of a MK2 inhibitor
- Peptide stapling introduces a hydro-carbon link between successive turns of a α-helix (Figure 2)
- Benefits of peptide stapling
  - Stabilized Structure
  - Improved Potency
  - Improved Protease Resistance
  - Improved Cell Permeability
- The consensus sequence phosphorylated by MK2 is HyXRXXSXX where "Hy" is a hydrophobic amino acid
- **The specific goal of this study was to analyze the secondary structure of a peptide inhibitor of MK2i verify that it was a suitable candidate for peptide stapling**

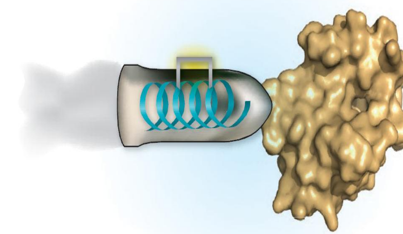
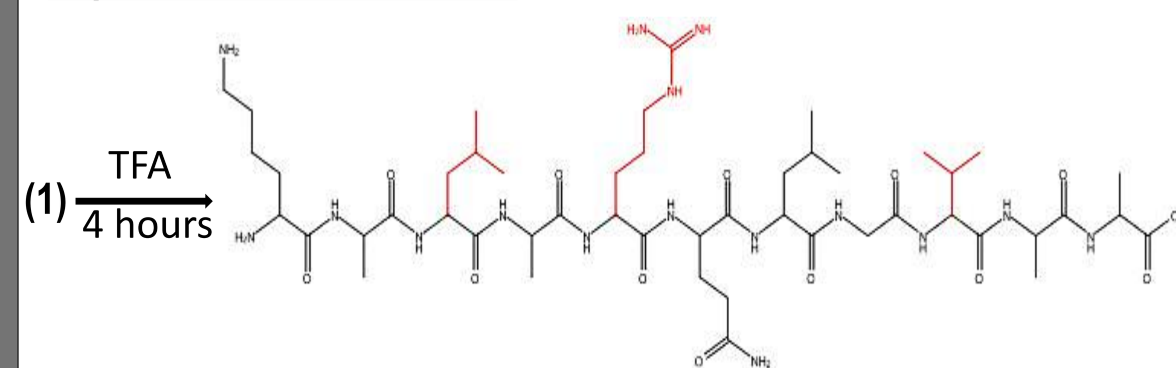


Figure 2: Illustration of Peptide Stapling

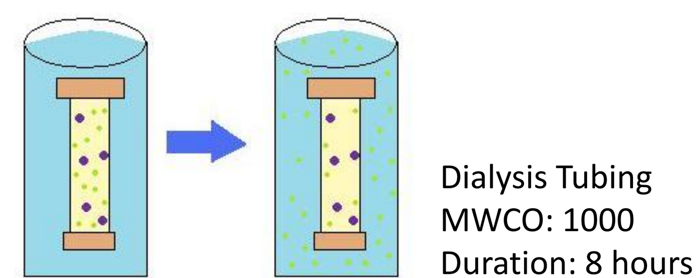
Materials and Methods

Solid Phase Peptide Synthesis with Fmoc Chemistry

Peptide Cleaved from Resin



Peptide Purification



Dialysis Tubing
MWCO: 1000
Duration: 8 hours

Circular Dichroism(CD)

- Differential absorption of right and left circularly polarized light
- Performed in the "far-UV" spectral region (190-250 nm)
- CD spectrum correlates to secondary structure (Figure 4)

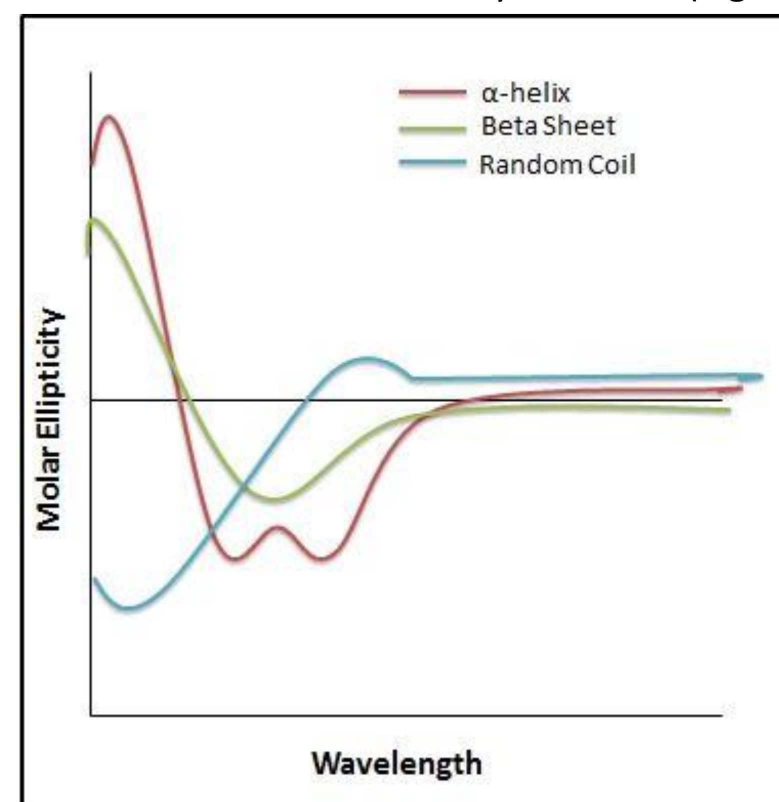


Figure 4: Representative CD Spectra of Different Secondary Structures

Results

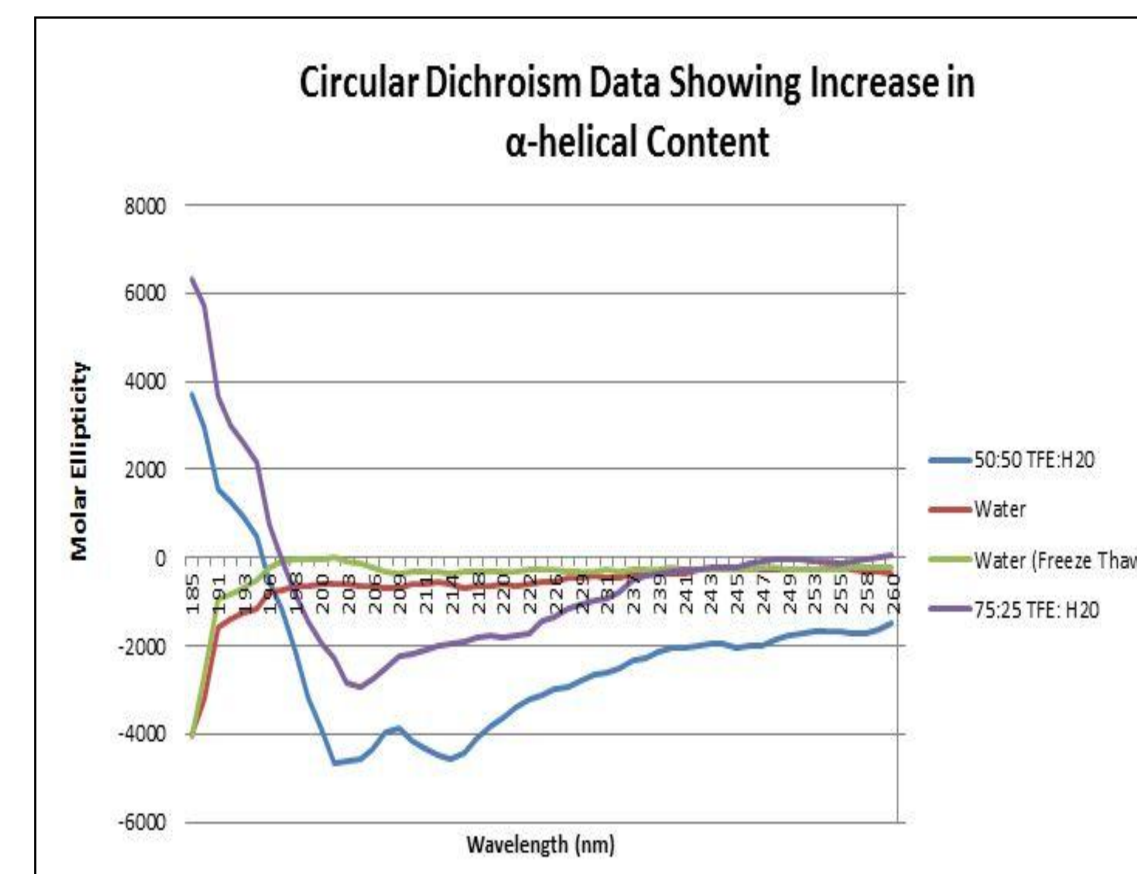


Figure 5: Circular Dichroism Spectra

	Water	Water (Freeze Thaw)	50:50 TFE:H2O	75:25 TFE:H2O
α-helix	5.98%	5.90%	13.60%	7.80%
Beta Sheet	17%	16.30%	18.90%	17.00%
Random Coil	30.30%	29%	31.30%	25.30%

Table 1:CD Data for the 190-260 nm Region

Conclusions

- The percent α-helical secondary structure in the water and 50:50 TFE:water samples were 5.98% and 13.6%, respectively. (Table 1)
- TFE concentration affected the secondary structure of the peptide whereas freeze thawing had no effect (Table 1)
- The presence of some α -helical structure and the enhanced alpha helical structure in TFE indicate that the peptide has sufficient alpha helical nature to benefit from peptide stapling

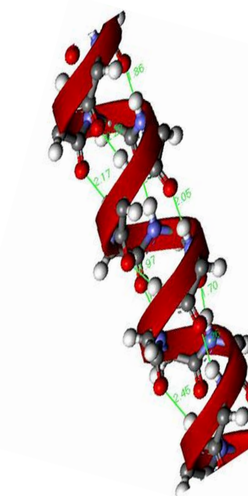
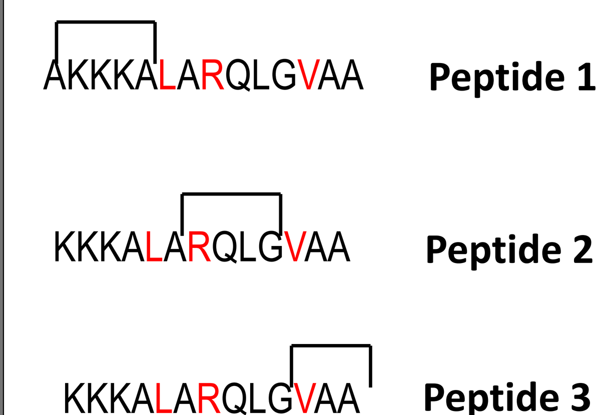


Figure 6: Illustration of Peptide Helix

Future Work

Preparation of stapled MK2i peptides



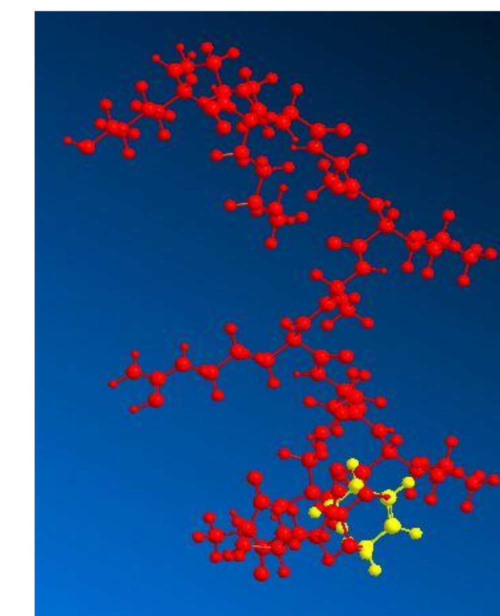
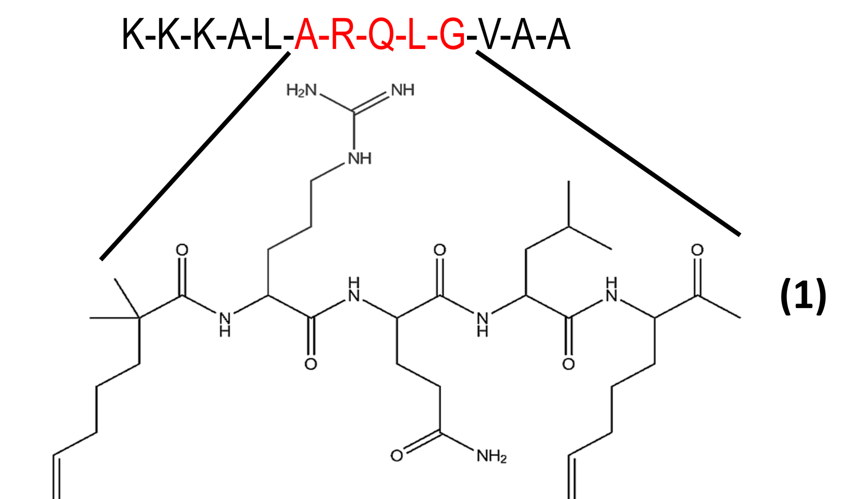


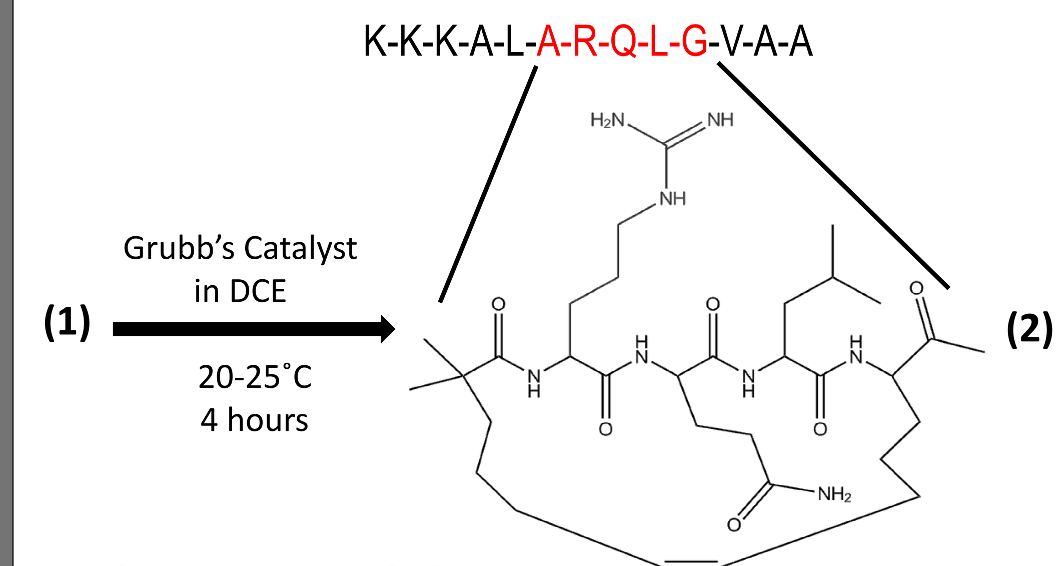
Figure 7: 3D Representation of Peptide 3 (Staple in Yellow)

- Peptide 1 places staple away from binding pocket in order to avoid interference with the MK2 binding domain
- Peptides 2 and 3 place the staple across the binding pocket
- Peptide 3 (Figure 7) uses an i and i+3 staple placement as opposed to the i and i+4 placement in peptides 1 and 2

Synthesize Peptides Using Olefin Modified Amino Acids



Perform Ring-closing Olefin Metathesis (RCM)



Stapled Peptide Analysis

- Verify the RCM by analytical high performance liquid chromatography
- Determine the secondary structure of the stapled peptides using CD
- Test cell permeability with fluorophore conjugated stapled peptide and determine potency with Kinase activity assay

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