Papain-Catalyzed Synthesis of Alternating Arginine-Tryptophan Peptides for Antimicrobial Applications

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Abstract

- In this study, we investigate an environmentally friendly and efficient approach to synthesizing peptides using protease-catalyzed peptide synthesis (PCPS) to generate short arginine-tryptophan (Arg-Trp) peptides, molecules known to possess antimicrobial properties. In addition to the synthesis, we'll be examining the Arg-Trp-Oet dipeptide's ability to inhibit the bacterial growth of E.Coli and S. Epidermidis.
- We examine the enzymatic coupling of Arg-Trp dipeptides using papain, promoting peptide bond formation through aminolysis rather than hydrolysis.

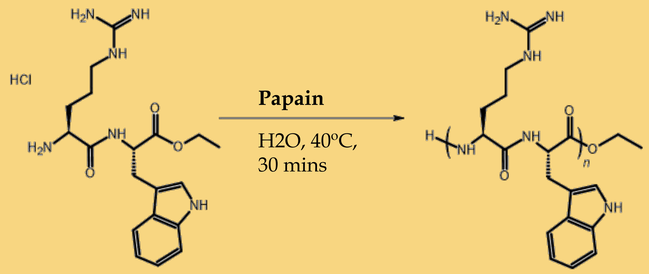


Figure 1. Papain catalyzed coupling of Arg-Trp under mild conditions.

Background

- Antimicrobial peptides (AMPs) have gained considerable attention due to their reduced susceptibility to the resistance mechanisms of bacteria. AMPs typically exert their effects through disruption of microbial membranes, a mechanism that is less dependent on specific molecular targets.
- Short cationic peptides rich in arginine and tryptophan residues have been identified as particularly effective AMPs.[1,2]
- Traditional peptide synthesis methods, such as solid-phase peptide synthesis (SPPS), are widely used but often require large quantities of organic solvents and specialized reagents, which are costly and environmentally wasteful.[3]
- Protease-catalyzed peptide synthesis (PCPS) has emerged as a promising alternative, leveraging the catalytic capabilities of enzymes to form peptide bonds under mild, aqueous conditions.[3] Enzymes such as papain, have been shown to facilitate peptide coupling with reduced need for harsh reagents.[4]

Experimental Conditions

PCPS

- 85.1mg of Arg-Trp-OEt was added to a dram vial, followed by 2mL H2O.
- 50mg of papain was then added to the mixture, along with a stir bar.
- The solution proceeded to stir at 40ºC for 30 minutes.
- 0.5mL aliquots of the solution were taken at 5,10,15, and 30 minutes and placed in microcentrifuge tubes.
- 1mL 5% TCA solution was added to each aliquot sample and then centrifuged to separate the supernatant from the solution.
- The isolated product was stored in the freezer, awaiting analysis.

Antimicrobial Assay

- E.Coli and S. Epidermidis were inoculated for 3 days in 5mL Mueller-Hinton Broth (MHB) at 37ºC.
- In 12 separate culture tubes, 1mL of MHB was added to each, 6 for each strain of bacteria (including control).
- Arg-Trp-OEt/H2O solution of varying concentrations (50, 100,150,250, 500µg) was added to the culture tubes.
- 100µL of each bacterial suspension was added to each of the tubes and placed in an incubator at 37ºC overnight.

Data

PCPS

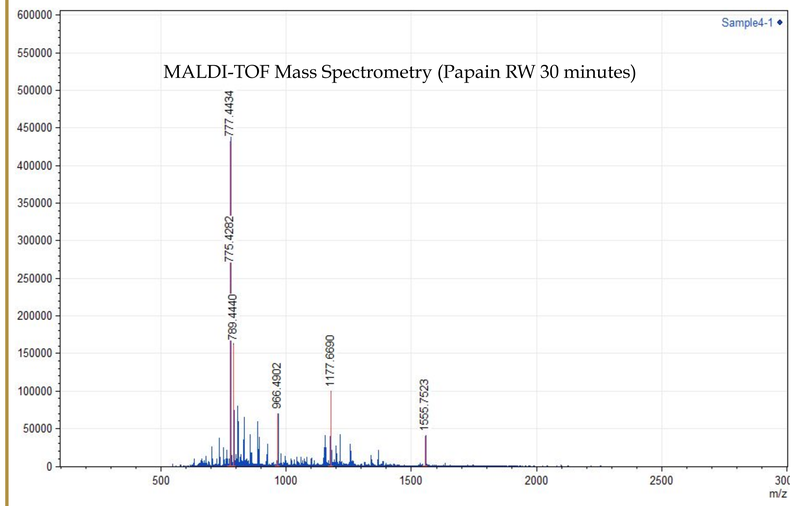


Figure 2. MALDI-TOF mass spectrometry graph of papain catalyzed RW sample at 30 minutes reaction time.

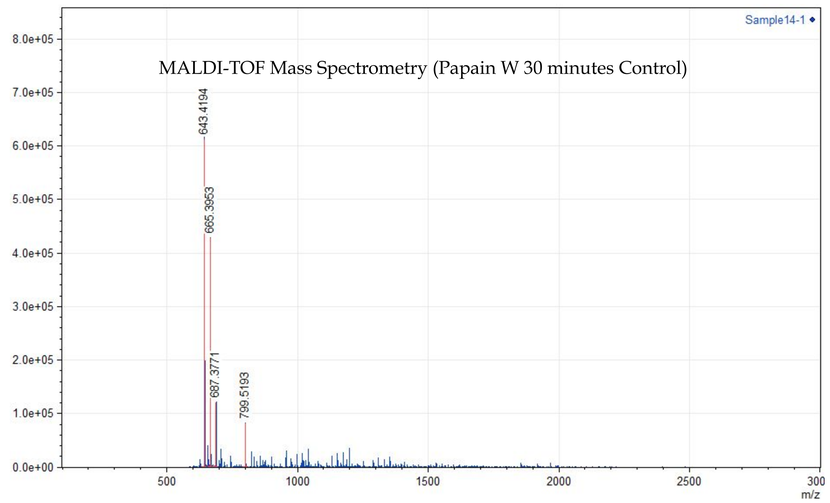


Figure 3. MALDI-TOF mass spectrometry graph of papain catalyzed W sample (control) at 30 minutes reaction time.

Antimicrobial Assay

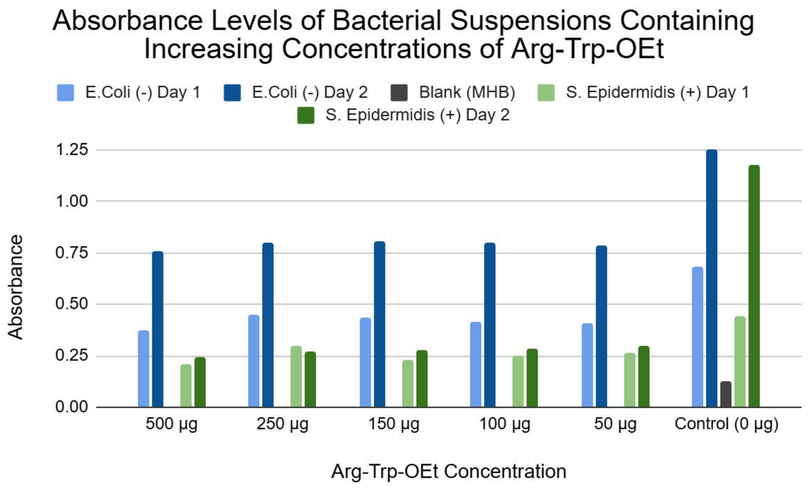


Figure 4. Agilent BioTek microplate reader absorbance readings from RW (varying concentrations) treated E.Coli and S.Epidermidis incubated samples.

	500 µg	250 µg	150 µg	100 µg	50 µg	Control (0 µg)
E.Coli (-) Day 1	0.375	0.447	0.438	0.413	0.405	0.682
E.Coli (-) Day 2	0.755	0.796	0.803	0.798	0.788	1.25
Blank (MHB)						0.124
S. Epidermidis (+) Day 1	0.207	0.299	0.232	0.248	0.265	0.444
S. Epidermidis (+) Day 2	0.244	0.268	0.28	0.286	0.3	1.178

Table 1. Agilent BioTek microplate reader absorbance data correlating to *Figure 4.*

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References