

THE CONTRIBUTIONS OF VARIOUS PATHWAYS TO DIKETOPIPERAZINE FORMATION FROM PEPTIDES CONTAINING ASPARTIC ACID RESIDUES IN THE PENULTIMATE POSITION

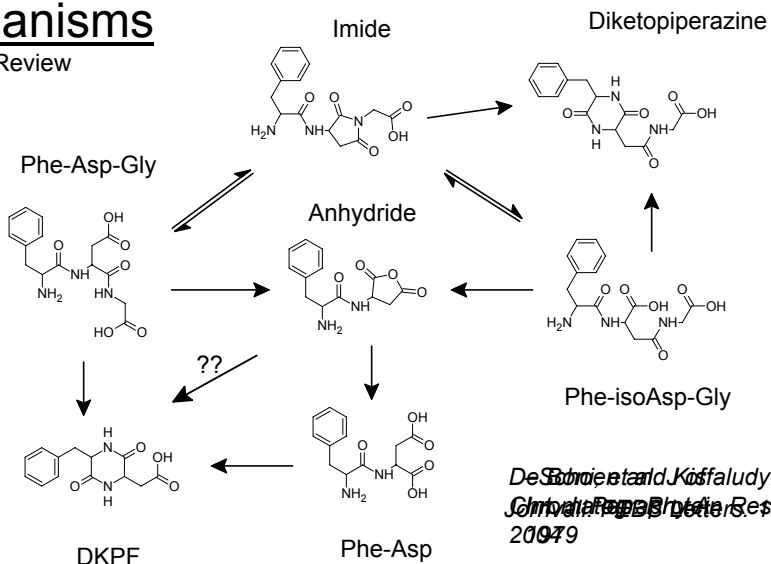
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Protein Stability

- Aspartic Acid is a Hot spot
- Peptide Synthesis
- Peptide Formulation
 - Human Growth Hormone
 - Interleukin-11
- Disease States
 - Alzheimer's Disease
 - Cataract
 - Parkinson's Disease

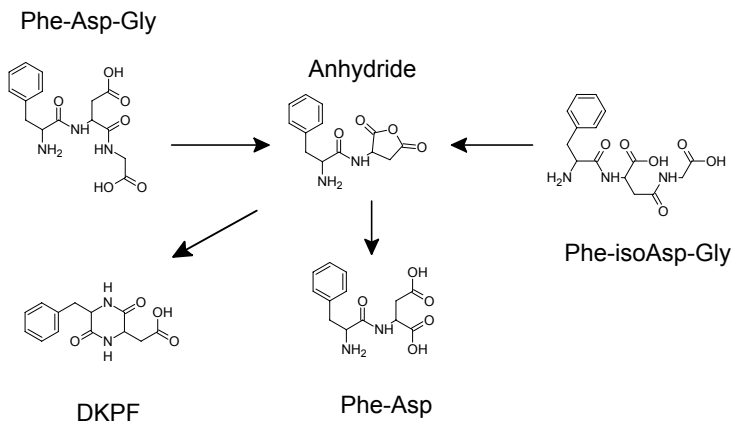
Mechanisms

Literature Review



Mechanisms

New Mechanism



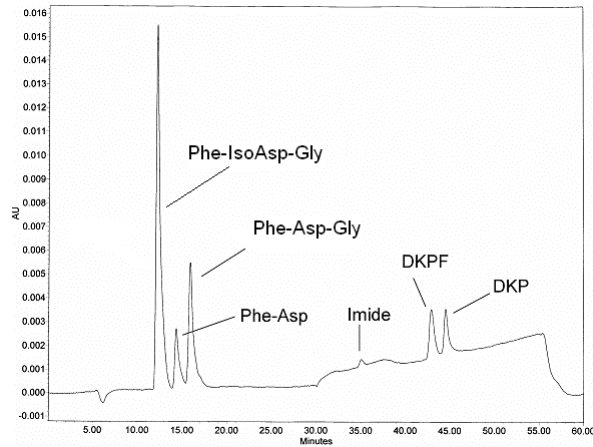
Hypothesis

- The anhydride generated during peptide fragmentation at aspartyl residues is susceptible to nucleophilic attack by the N-terminus.
- DKPF forms via the anhydride.
- pH will greatly affect the formation of DKPF and DKP

Materials and Methods

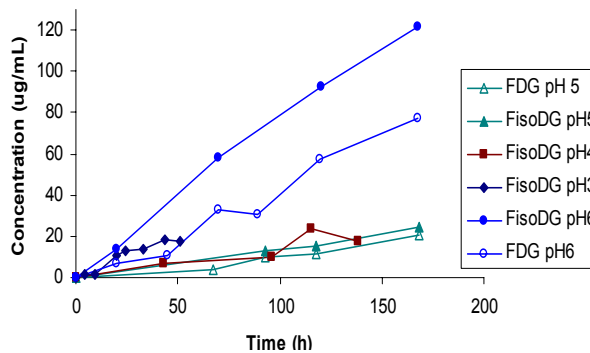
- 50mM Buffers from pH 3-6
- HPLC Analysis
 - isocratic; UV detection (257nm)
- Phe-Asp-Gly and Phe-isoAsp-Gly as Starting Compounds (200 μ g/mL)
- Differential Equations
 - Based on the Previous Scheme

HPLC Chromatogram



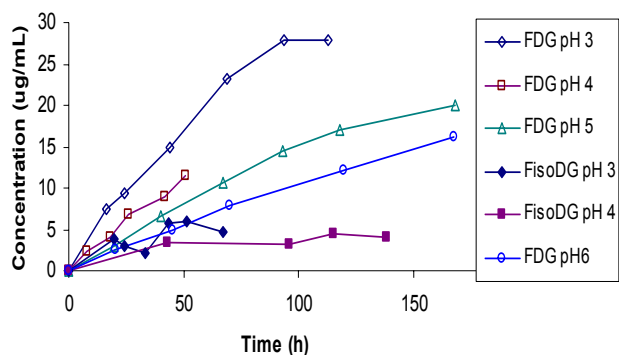
DKP Formation

- DKP formation is greatest when FisoDG is the starting compound
- U-shaped pH curve
- No DKP from FDG at acidic pH



DKPF Formation

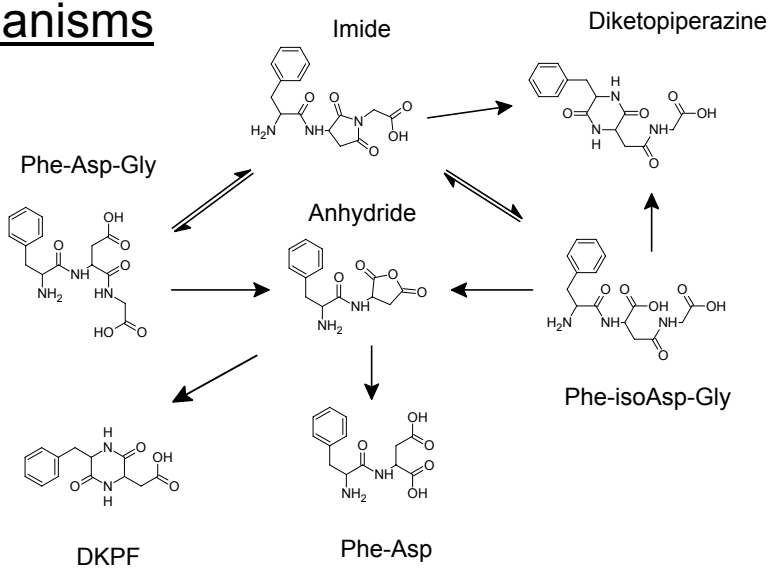
- DKPF formation is greatest when FDG is the starting compound
- Increase in pH decreases DKPF
- No DKPF from FisoDG above pH 4



Aspartate Degradation at pH 3.00

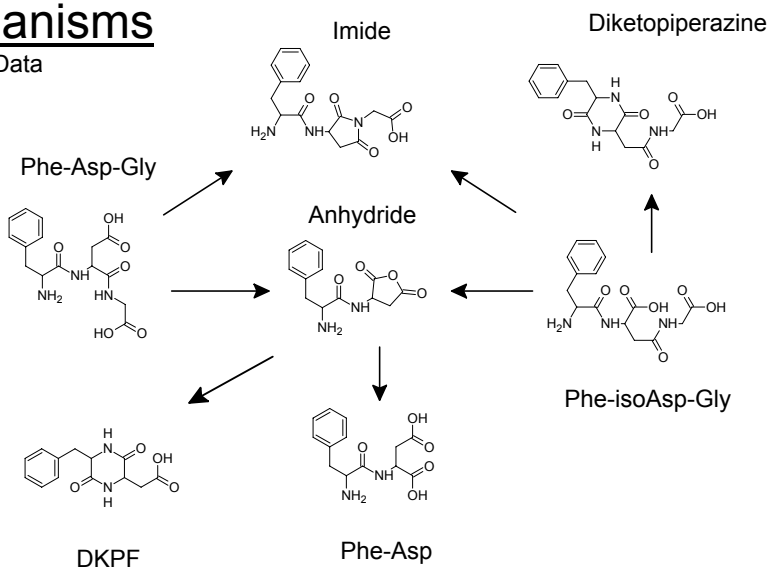
- Phe-isoAsp-Gly (FisoDG)
 - No isomerization to Aspartate
 - Formation of DKPF and DKP
 - Similar Rate of loss as FDG
- Phe-Asp-Gly (FDG)
 - Minimal isomerization to isoaspartate (<0.7%)
 - Formation of DKPF but no DKP
 - Similar Rate of loss as FisoDG

Mechanisms

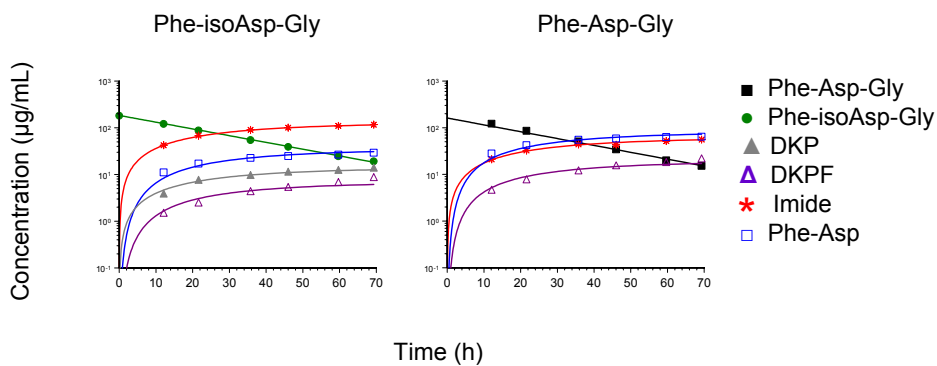


Mechanisms

Based on Data

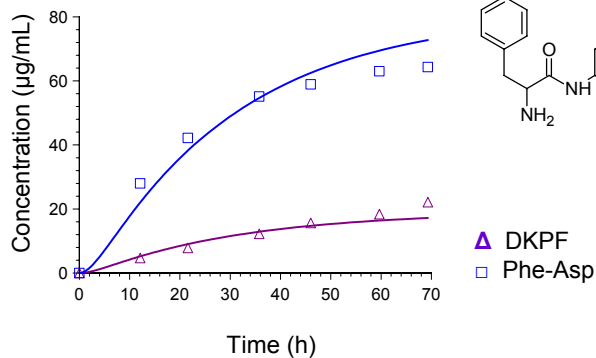
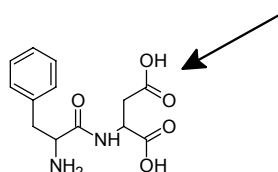
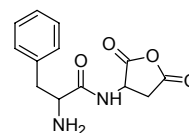
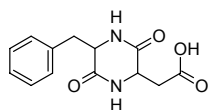


Aspartyl Peptide Degradation



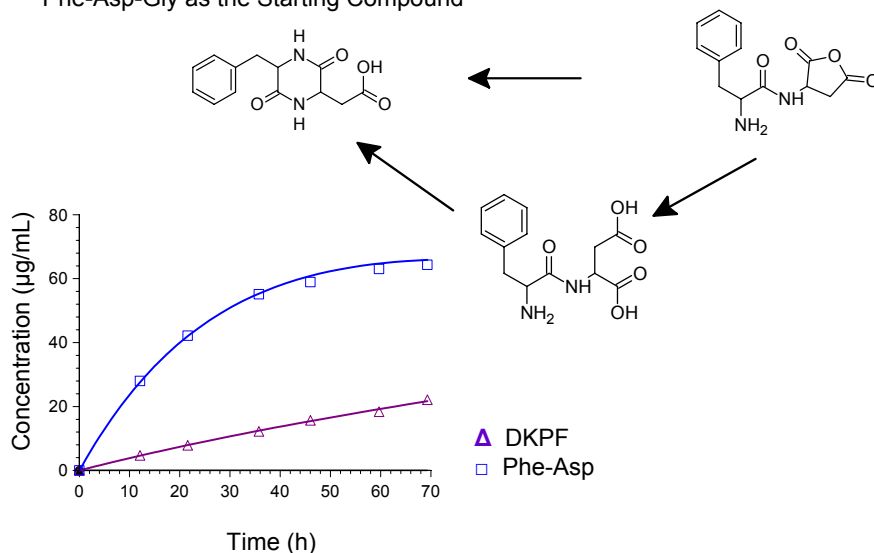
Anhydride to DKPF and Phe-Asp

Phe-Asp-Gly as the Starting Compound



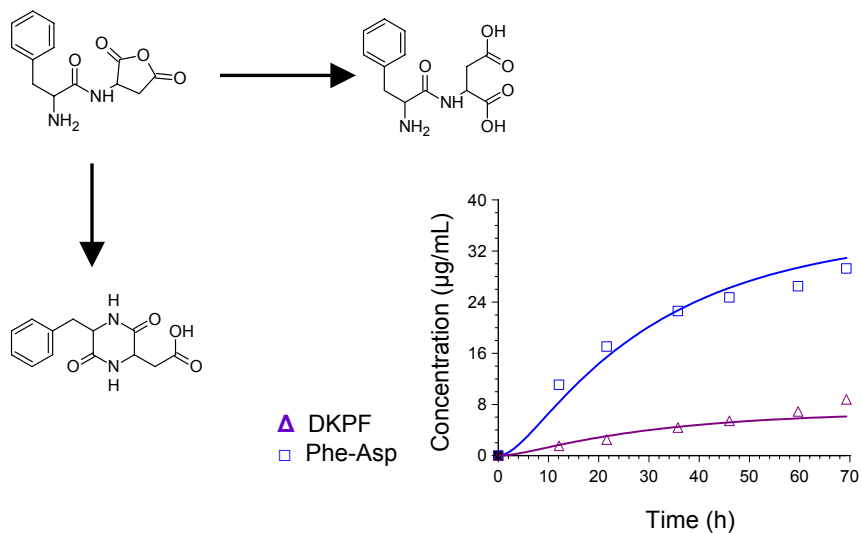
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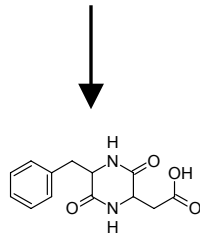
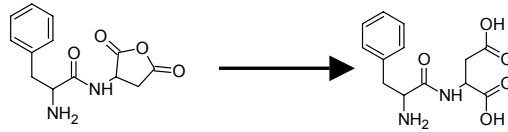
Anhydride to DKPF and Phe-Asp

Phe-isoAsp-Gly as the Starting Compound

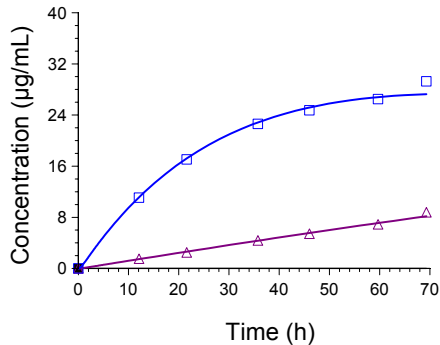


Anhydride to DKPF and Phe-Asp

Phe-isoAsp-Gly as the Starting Compound



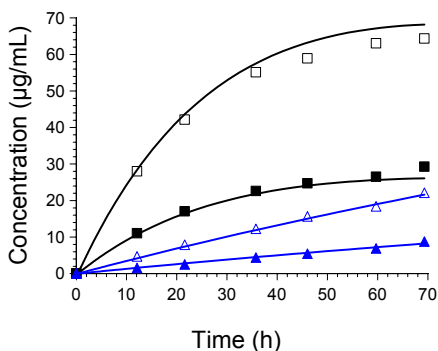
▲ DKPF
□ Phe-Asp



Addition of Parameters

- Fit Any Line
- Mechanism of Formation Based on Structures of the Compounds
- Simultaneous Fit
 - Combine the Phe-Asp-Gly and Phe-isoAsp-Gly Data
 - 9 Equations and 9 Parameters

Simultaneous Fitting



Plot of DKPF Formation from Anhydride and Phe-Asp

- □ - Phe-Asp from Phe-Asp-Gly
- ■ - Phe-Asp from Phe-isoAsp-Gly
- △ - DKPF from Phe-Asp-Gly
- ▲ - DKPF from Phe-isoAsp-Gly
- Anhydride to DKPF Only
– 0.618 SSD
- w/ Phe-Asp to DKPF
– 0.183 SSD
- Above w/ Phe-Asp-Gly to DKPF
– 0.137 SSD

Data Supporting Anhydride

- Anilide trapping of anhydride from dipeptide Asp-Phe
- Loss of Asp-Phe did not increase with increasing aniline concentration
- Found two anilide compounds
 - Alpha and Beta
- Formation of Phe did not decrease
 - Multiple pathways of degradation

Anjali Joshi, Ramil Menzeleev, and Biren Joshi. AAPS Poster, 2005

Conclusions

- Models Were Derived that Implicate the Anhydride Intermediate
- There are Multiple Pathways for DKPF and DKP Formation
 - DKPF – Anhydride and Phe-Asp cyclization
 - DKP – Imide and N-terminus/isoaspartate cyclization
- pH can turn off certain pathways
- Applications to proteins

Future Experiments

- Aniline Experiment
 - FDG and FisoDG as the starting compounds
 - Reduction of Phe-Asp and DKPF
- Phe-Asp to DKPF
 - Adds More Information to the Model
- Solid-State Kinetics
 - Intramolecular reaction dependent on Tg

Acknowledgements

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