Methodizing HPLC for Stereoisomeric Peptide Analysis

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Background and Purpose

Our research focus is on unregulated concentrations of iron in the body. A derivation of apoptosis, known as ferroptosis is a triggered cell death based on overabundance of iron.[1] The Fenton reaction is a process in which iron reacts with peroxide to create reactive oxygenated species (ROS) which is really threatening to the body.[2]

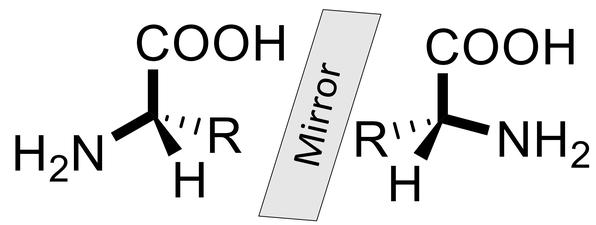
$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + HO^{\bullet} + OH^{\bullet}$$

$$O_2^{-\bullet} + H_2O_2 \xrightarrow{Fe^{II}/Fe^{III}} O_2 + OH^- + OH^{\bullet}$$

To sequester iron found within organelles, we are using a modified Szeto Schiller (SS-20) peptide for iron detection and capturing.[3,4]
Solid phase peptide synthesis (SPPS) was used to create the peptide for our SS-20 peptide chelator. This procedure was derived from Dr. Bruce Merrifield.[5]

By utilizing SPPS, the N-terminus of an amino acid is covalently bounded to a rink amide resin. Each residue is deprotected at their N-terminus and attached to the C-terminus of the bounded amino acid. The protecting group is important because it prevents the excess materials in the reaction chamber from reacting with the amino group of the residue preemptively.[5]

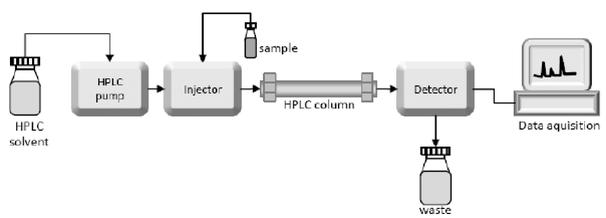
Asymmetric carbons with unique attachments are known as chiral carbons and they can exist as non-superimposable mirror images. These mirror images are known as stereoisomers.[6] Incorrect stereochemistry can result in vastly different chemical properties despite having the same composition.



(L)- Amino Acid (D)-Amino Acid

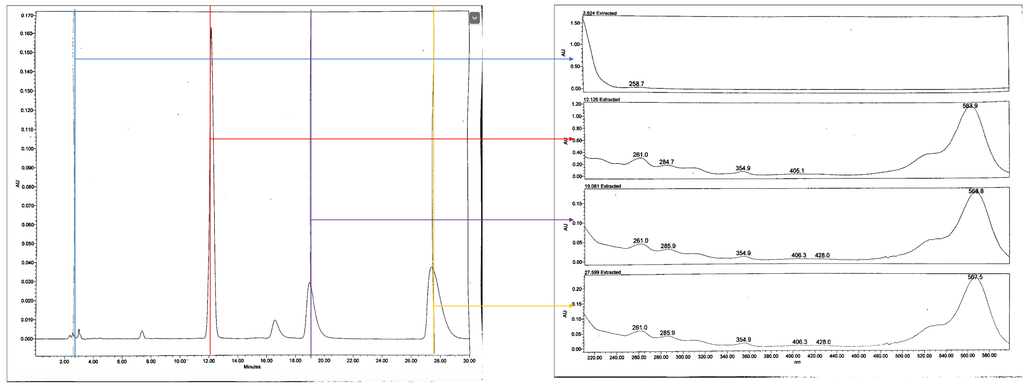
Goals with this project

- Refurbish and optimize HP-1050 HPLC
- Develop built in method for peptide analysis
- Convert the instrument to a normal phase composition
- Condition instrument for stereoisomer separations
- Successfully separate and resolve stereoisomeric peptide mixtures so that we can apply it to our peptide.



Racemization and Chiral Chromatography

Using Reverse phase HPLC analysis, the presence of stereoisomers was revealed. Multiple elution peaks contained the same Uv-vis signal, indicating potential isomers. We believe that the use of base diisopropylethylamine during the coupling process may have caused this phenomenon. LC-MS was used to validate this hypothesis.



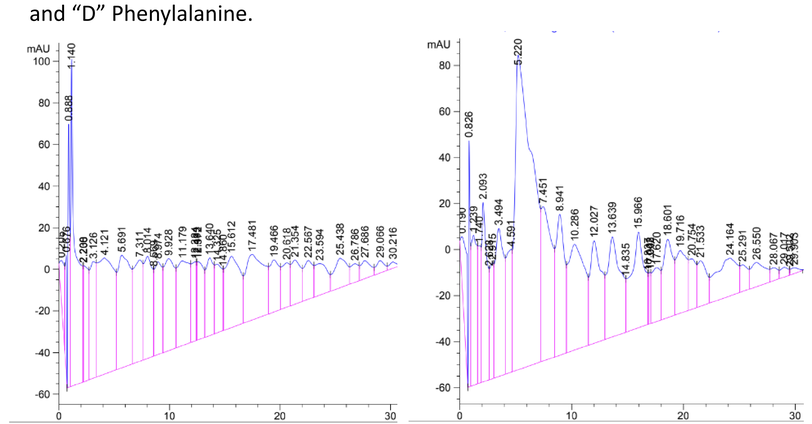
Chiral HPLC uses a chiral stationary phase and nonpolar mobile phase to purify stereoisomers. Depending on the stereochemistry of our peptide, it will elute out of the column at different retention times. This is a valuable technique because chiral HPLC can be used to isolate the two stereoisomers since only one of the stereoisomers is valuable to our work.[7]

HP-1050 HPLC Troubleshooting

- Mobile phase conditions
- Primed quaternary pump
- Pump and column leaks
- Instrument and Computer communication
- Method reading configurations
- Manual injection and run start
- Gradient analysis
- Waste Storage

Current work

I have successfully separated various peptide samples with the refurbished instrument. Work with ion-pairing agent trifluoroacetic acid is being tested to increase chromatogram resolution of various peptide samples. The mobile phase conditions of the instrument have been swapped to a nonpolar “normal phase” for chiral separations. Currently I am working on resolving clear separations of “L” and “D” Phenylalanine.



References

[1]Fairbanks, V.F. and Beutler, E.R.N.E.S.T., 1995 *William Hematology*.[2] Eid R, Arab NT, Greenwood MT. Biochim Biophys Acta Mol Cell Res. 2017. [3] Wayne Mitchell, Emily A. Ng, Jeffrey D. Tamucci, Kevin J. Boyd, Murugappan Sathappa, Adrian Coscia, Meixia Pan, Xianlin Han, Nicholas A. Eddy, Eric R. May, Hazel H. Szeto, Nathan N. Alder Journal of Biological Chemistry, Volume 295, Issue 21, 2020, Pages 7452-7469.[4] Abbate V, Reelfs O, Hider RC, Pourzand C. Biochem J.[5] Merrifield, R.B., 1963. *Journal of the American Chemical Society.* [6] Hahn, D., Wang, W., Choi, H. *et al. Sci Rep* **12**, 10285 (2022).[7] Izuru Kawamura, Batsaikhan Mijiddorj, Yohei Kayano, Yuta Matsuo, Yumi Ozawa, Kazuyoshi Ueda, Hisako Sato, Volume 1868, Issue 8, 2020