Elana Pocress

Isotope Labeling Strategies to Assess Metabolic Pathways of Riboside Derivatives

Chemistry

Research Plan

A. RATIONALE

Dihydronicotinamide riboside, NRH, is an established potent NAD+ precursor. NAD+ is particularly scientifically relevant because it has been investigated for genes and enzymes that might regulate lifespan. To purify NRH, thin layer chromatography plates will be prepared, which is a commonly executed procedure throughout biochemical laboratories.

B. HYPOTHESIS (ES), RESEARCH QUESTION(S), ENGINEERING GOAL(S), and/or EXPECTED OUTCOMES.

- Engineering Goals: Purify NRH and EtONaR through thin layer chromatography
- Expected Outcomes: Once a purified form is produced, NMR readings will be run to observe proton spectra.

C. RESEARCH METHODS

- Procedures
    - In a burette, 250 mL methanol will flow through to prime it. Subsequently, 1 g of NRH will be dissolved in H_2O and added to the burette slowly. 250 mL of H_2O will then be added to the burette after this. 26 fractions of 13 mL will be collected, labelled numerically, and stored at - 20º C. Thin layer chromatography (TLC) plates will be used to separate non volatile mixtures through reverse phase chromatography in which the polarity of a substance will dictate its position after

it is run through a column with a mixture of acetone and methanol in a 3:1 ratio. These plates will then be charred by dipping into an acid bath of 5% H2SO4 in methanol, drying almost completely, then placing on a hot plate at 200ºC until black spots emerge. This charring process will then be repeated with 100 mg ribose in 2 mL methanol ran on a TLC plate as well. Since different compounds have different polarities, a new solution in the chromatography column must be prepared. A 3:1 ratio of CH_2Cl_2 is determined to be most optimal after testing a 1:1 ratio of acetone and CH_2Cl_2 , and a 100% acetone solution. All TLC plates will be viewed under UV light at 235 nm.

- Subsequently, a similar reaction involving ribose will be prepared. This consists of 50 mg EtoNaR dissolved in 1 mL methanol as the starting material. Then 1 pellet of NaOH (.0947g) will be dissolved in .6 µL water. 500 µL of the starting material will be pipetted into a separate scintillation vial. To this, 10 µL of the dissolved sodium hydroxide will be added. Separately, the same volume of the same starting material will be dissolved in 10 µL of 1 pellet of NaOH dissolved in a 1:9 ratio of methanol:water. SCR TLC plates will be ran in which S corresponds to the starting material (50 mg EtoNaR in 1 mL methanol), R corresponds to the product (500 µL S + 10 µL NaOH), and C corresponds to the combined spotting of both S and R.
- Following the previous two reactions, an isotope ribose synthesis will be prepared. In a round bottom 50 mL flask, 100 mg isotope ribose will be dissolved in 2 mL methanol. To this, 375 µL of 5% H_2SO_4 in methanol will be added. After

this solution is dissolved completely, SCR TLC plates will be run as well; the starting material will be 50 mg non isotope ribose in 1 mL.

- NMR will be ran on each final product. To do so, the scintillation vial in which it solution is held will be weighed and the contents will be evaporated in a rotary evaporator. A clear gel will remain which is to be pipetted out and dissolved in deuterium oxide in an NMR tube. This will then be placed in a SampleJet automated machine.

- Risk and Safety: No human participants, vertebrae animals or potentially hazardous biological agents will be used in experiments, therefore personal safety guidelines will be implemented such as the use of gloves and lab coats. For hazardous chemicals, eyewear will be worn and all procedures will be carried out under a fumigation hood as needed.
- Data Analysis: Nuclear magnetic resonance, NMR spectroscopy, will be implemented to view the spectra emitted from inputted samples of NRH, alongside accompanying software.

4. HAZARDOUS CHEMICALS, ACTIVITIES & DEVICES:

(ANSWER THE BELOW ITEMS FOR EACH THING USED)

1. CH_3OH
    a. Methanol is highly flammable and toxic, particularly methanol vapor. At most, 2 mL of methanol is used at a time, using a glass or plastic pipette
    b. Direct exposure is avoided at all times, eyewear, gloves, and lab coats worn at all times, usage is within a fumigation hood. Personal protective equipment is used to minimize health risk.

c. Disposable with a noncombustible absorbent material such as sand. Consult Environmental Health and Safety (EHS) if necessary.

2. C_3H_6O

   a. Acetone is a common household solvent and is particularly dangerous when directly exposed to skin. At most, 3 mL of acetone is used at a time, using a glass or plastic pipette.

   b. Personal protective equipment is worn and direct exposure is avoided at all times.

   c. Dispose by absorbing with an inert material and transfer into a suitable container. Consult Environmental Health and Safety (EHS) if necessary.

3. H_2SO_4

   a. Sulfuric Acid is a strong acid that is strongly corrosive and poses several life threatening risks. At most, 10 microliters of sulfuric acid will be used.

   b. Personal protective equipment will be worn. The bottle will be stored in a cabinet clearly labelled containing corrosive material. When in use, the sulfuric acid will be only handled within the confines of a fumigation hood. Acid is always added to a base or water, rather than a base or water being added to the acid.

   c. Dispose by dilution into water. Consulted Environmental Health and Safety (EHS) if necessary.

4. CH_2Cl_2

a. Dichloromethane (Methylene chloride) is highly volatile and poses potential hazards if exposed to skin, eyes, or inhaled. At most, 1 mL of this will be used.
b. Full personal protective equipment will be utilized. All experiments that required dichloromethane were prepared under a fumigation hood. The bottle is kept in a fumigation hood when in use, and stored safely in a cool, dry area when not in use.
c. Never poured down a sink for disposal. Rather, place in a designated waste basket for hazardous material. Consult Environmental Health and Safety (EHS) if necessary.

5. NaOH
    a. Sodium hydroxide is a crystalling odorless solid which can, at worst, be irritative or corrosive. NaOH will be used in pellet form, weighting at most 100 mg
    b. Personal protective equipment will be used.
    c. Since NaOH in solution will be absorbed by liquid and small concentrations of NaOH will be used in experimentation, unused NaOH will be dissolved in excess water and will be poured down a drain.
6. UV Light
    a. Ultraviolet rays can be extremely dangerous as it is a known cause of skin cancer, eye danger, and damage to the immune system. In order to mitigate the effects of this, the time of exposure will be limited to less than 1 minute, as recommended by the National Institute for Occupational Safety and Health

b. Light is used within the confines of the lightbox and is strictly directed towards TLC plates.

D. BIBLIOGRAPHY

- Yang, Y., & Sauve, A. A. (2016). NAD+ metabolism: Bioenergetics, signaling and manipulation for therapy. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1864(12), 1787-1800.
- Yang, Y., Mohammed, F. S., Zhang, N., & Sauve, A. A. (2019). Dihydronicotinamide riboside is a potent NAD+ concentration enhancer in vitro and in vivo. Journal of Biological Chemistry, 294(23), 9295-9307.
- James, T. L. (1998). Fundamentals of NMR. Online Textbook: Department of Pharmaceutical Chemistry, University of California, San Francisco, 1-31.
- Cantó, C., Houtkooper, R. H., Pirinen, E., Youn, D. Y., Oosterveer, M. H., Cen, Y., ... & Gademann, K. (2012). The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell metabolism, 15(6), 838-847.
- Sauve, A. A. (2008). NAD+ and vitamin B3: from metabolism to therapies. Journal of Pharmacology and Experimental Therapeutics, 324(3), 883-893.

THERE ARE NO ADDENDUMS TO THIS RESEARCH PLAN