Nitric oxide (NO) is a product of nitric oxide synthase (NOS). NOS catalyzes the oxidation of L-arginine to form NO. NO plays important roles in the cardiovascular, nervous, and immune systems. To form NO, there must be an electron transfer between the oxygenase and reductase domains. The electron flows from NADPH to FAD, FMN, and the oxygenase domain heme during catalysis. The NO that is released can be further oxidized to form nitrite, while heme-NO complex can also be converted to nitrate.

Design & Procedure

1. Initially, the reactions were performed in conical tube and the reaction mixtures were stored at 80°C.

2. The experiment was conducted to measure the ratio of nitrate and nitrite produced.

A 96 well plate was filled following the sequence depicted in Table 2, and incubated at 37°C for 2 hours.

A 1:1 ratio LDH/Napryurate mixture and 100 µl of Griess reagent (salicylaldehyde and N-1-naphthylethylenediamine dihydrochloride (NED), which is a pink indicator detecting the presence of nitrite ions in a solution) were added to all wells (Figure 5).

The 96 well plate was read at 550 nm and 650 nm in a spectrophotometer (Figure 6).

Results

The data obtained from the spectrophotometer was used to:

- Create a standard curve (Graph 1). The higher the R² value (closer to 1.0), the better the results of the experiment.
- The numbers obtained from the spectrophotometer were used to calculate the different percentages/concentrations of nitrate and nitrite (Graph 2).

Conclusions

- Only NO₂⁻ is produced in the NOS-12 solution.
- In the absence of NADPH, the presence of enzyme doesn’t change the nitrite production from NOC-12.
- NADPH converts half of the NO₂⁻ to NO₃⁻.
- In NO synthesis from nNOS or iNOS, more NO₂⁻ vs. NO₃⁻ was generated in the presence of NOS-12.
- Cofactors and other chemicals in the assay buffer do not affect the reaction ratio of nitrite to nitrate.

References


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