Methylmalonyl-Coenzyme A Mutase Activity in Vitamin B$_{12}$ Deficiency

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Presentation Overview

- Introduction to Methylmalonyl-CoA Mutase
- Low Activity Implications
- Ways to Measure Mutase Activity
  - Radiometric- Common Method
  - Non-radioactive
- Experimental Overview
  - HPLC
  - Mutase Activity in B_{12} Deficient and Replete Samples
  - Results
- Conclusions
Cobalamin- Dependant Pathways

HoloTC

Adenosyl $\text{B}_{12}$

Methyl $\text{B}_{12}$

MMA

Mitochondrion

L-Methylmalonyl-CoA

$\text{B}_{12}$

MCM

Succinyl-CoA

Purines, DNA, RNA

THF

5'-Methyl THF

dTMP+DNA

SHMT

MTHFR

5'-Methyl THF

MS

B$_{12}$

Methionine

SAM

R

Methylation

RCH

Homocysteine

Homocysteine
Methylmalonyl-Coenzyme A Mutase

- Mitochondrial
- Requires B$_{12}$ Derivative: 5’-adenosylcobalamin (AdoCbl)
Impaired Mutase Activity

- Methylmalonic acidemia: mild-fatal
- Causes:
  - Cobalamin deficiency
  - Defect in genes involved in synthesis of AdoCbl from hydroxocobalamin (cbl mutations)
  - Defect in structural gene for mutase apoenzyme
- Symptoms: ketoacidosis, vomiting, lethargy

\[
\text{Methylmalonyl-CoA} \leftrightarrow \text{Succinyl-CoA}
\]
Uses of Mutase Activity Measures

- In vitro measurements of total and holo enzyme activity (w/ and w/o added AdoCbl)
  - Investigate Cbl pathway
  - Identify *mut* and *cbl* mutations
- Clinical Uses
  - Diagnose Methylmalonic Acidemia
  - When $B_{12}$-Test levels are Low to Normal or hematologic indexes are normal
  - Nutritional status indicator- $B_{12}$ Deficiency
Activity Measurement Methods

- 1 unit of activity = $1 \mu\text{mol Succinyl-CoA/ minute}$

- Radiometric: DL $[\text{CH}_3^{14}\text{C}]$methylmalonyl-CoA produces $[^{14}\text{C}]$succinyl- CoA which is separated and quantified using:
  - Paper chromatography
  - TLC
  - Electrophoresis
  - Potassium permanganate oxidation*
  - Extraction into ethyl acetate
  - Gas chromatography
  - HPLC
Activity Measurement Methods

- Nonradioactive- separate Methylmalonyl-CoA and Succinyl-CoA
  - Reverse-phase HPLC

http://www.galantamine.cn/images/hp-hplc.jpg
High Performance Liquid Chromatography

- Separate, Identify, and Quantify Compounds

- **Column** - Stationary phase, silica binds covalently (BETASIL Phenyl Column)

- **Pump** - Mobile phase (MeOH)

- **UV Detector** - 259nm, Retention times (Varies on Stationary phase, molecules, and solvent)

- **Reverse Phase Chromatography** - Non-polar stationary phase and aqueous, moderately polar mobile phase

http://www.lcresources.com/resources/getstart/2g01.htm
Example Chromatogram
Experimental Assay

- **Hypothesis:**
  - Mutase activity in liver will be reduced in rats with B$_{12}$ deficient diets as compared to normal B$_{12}$ diets.

- **Experimental Design:**
  - 5 B$_{12}$ Deficient Rats
  - 4 B$_{12}$ Sufficient Rats
HPLC Method

- Samples- Homogenized rat liver, centrifuged and collect supernatant
- Added 1mM AdoCbl (Total) or Water (Holo)
- Preincubation (5 min)
- Added 600µM Methylmalonyl CoA
- Long incubation (30 min)
- 10% TCA added to stop reaction
- Centrifuged samples
- Ran HPLC with 75mM Acetic Acid, 100mM KPhos, in 15% MeOH Buffer
Results

Succinyl CoA (nmol/mg) Levels in B12 Deficiency and Sufficiency

Animal No: #70-74  B12 Deficient
#128-131 Normal B12
Results

Mean Succinyl CoA Levels (nmol/mg) in B12 Deficiency and Sufficiency

- Holo Enzyme (w/o added AdoCbl)
- Total Enzyme (w/ added AdoCbl)
Conclusion

- HPLC Method Successful
  - Identify and quantify synthesized Succinyl-CoA
  - Change in holo and total enzyme activity
- No difference in mutase activity between deficient and replete samples
  - Repeat experiment with fresh samples
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Resources


Questions?