In-vitro Testing of Malic Enzyme 2 Activity in the Presence of Coenzyme-A

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Background

Coenzyme-A (CoA) is a non-protein helper molecule that is needed for the activation of certain enzymes.

It is also involved in the synthesis and oxidation of fatty acids, as well as the oxidation of pyruvate in the citric acid cycle.
Identification of Coenzyme-A (CoA) binding Proteins

Ornella synthesized a Coenzyme-A analog which has a biotin tag that could bind tightly to streptavidin beads.

She did a proteomic study with a human cell line and the molecule.

The result was a list of proteins to investigate. Some proteins are not reported to bind CoA.
NAD-dependent Malic Enzyme (ME2)

Malic enzyme has a dimer of dimers quaternary structure.

There are two isoforms reported on the NCBI database

PBD: 1pj3
Methods—Protein Overexpression and Purification

Prepared 4 flasks, each containing 2L LB media at 20g/L

Added 2mL Kanamycin (KAN) to 2 flasks, then 2mL chloramphenicol (CAM) to remaining 2 flasks

Placed flasks in 37°C

After 3 hours, checked density of bacteria using UV Visible Spectroscopy. Acceptable density is between 0.7-0.8

Added 0.8mL IPTG and left for 4 hours at 37°C

Collected cells using a large centrifuge tube

Stored cell pellet at -80°C overnight

Lyse cells using cell disruptor

Purify protein using nickel purification column

Supplement with FPLC purification column
Methods—Protein Overexpression and Purification

Lyse cells using cell disruptor

Purify protein using nickel purification column

Supplement with FPLC purification column (not shown in image)

gel electrophoresis
Purification of ME2-isoform2 from SoluBl-21 cells

- Fractions 1-3 were combined and stored at -80°C.
- No activity was detected from the purified protein.
Methods—Colony PCR

General Protocol (for 10 colonies)

Master mix contains:

- 480µl H2O
- 60µl EconoTaq® buffer
- 24µl Primer 1 (pCMV T3 forward)
- 24µl Primer 2 (ME2 isoform 1 3’)
- 6µl dNTP
- 6µl Econotaq® enzyme

Aliquot 15µl master mix into each of 19
Methods—Miniprep

Lyse, neutralize, and wash cells. Label samples with “(colony number), plasmid, pcmv, ME2 isoform 1”

Sequence samples
Methods—Transformation

Transformation is the process by which foreign DNA is introduced into the cell.
Methods—Protein Overexpression and Purification

Prepared 4 flasks, each containing 2L LB media at 20g/L

Added 2mL Kanamycin (KAN) to 2 flasks, then 2mL chloramphenicol (CAM) to remaining 2 flasks

Placed flasks in 37°C

After 3 hours, checked density of bacteria using UV Visible Spectroscopy. Acceptable density is between 0.7-0.8

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Stored cell pellet at -80°C overnight

Lyse cells using cell disruptor

Purify protein using nickel purification column

Supplement with FPLC purification column

(See slide 6 for figure)
Purification of ME2-isoform1 from SoluBl1-21 cells

- Fractions 3-6 were combined and stored at -80°C.
Results—Enzyme Activity Assays

Detection of NADH formation

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
</tr>
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<tbody>
<tr>
<td>HEPES pH 7.4</td>
<td>50 mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>150 mM</td>
</tr>
<tr>
<td>MgCl</td>
<td>20 mM</td>
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<tr>
<td>NAD</td>
<td>1 mM</td>
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<tr>
<td>CoA/ATP/ADP</td>
<td>250 uM</td>
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<tr>
<td>ME2</td>
<td>50 nM</td>
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<tr>
<td>Malate</td>
<td>40 mM</td>
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</tbody>
</table>
Results—Enzyme Activity Assays

![Bar Chart showing Relative NADH formation](chart.png)
Conclusions

ME2 iso1 (from SoluBL-21) activity is affected by ATP and ADP but not free CoA.

This is only one set of conditions. Therefore, further investigation is necessary.
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Dr. Stephen Lee
HHMI
CHAMPS
Dr. Hening Lin
Ornella Nelson

http://www.planwallpaper.com/thank-you
Questions?