The Biotin Carboxylase-Biotin Carboxyl Carrier Protein Complex of *E. coli* Acetyl-CoA Carboxylase

Choi-Rhee et al. 2003

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REACTION MECHANISM OF ACETYL-COA CARBOXYLASE (ACCase)

Gene  Protein  Enzyme activity

accA  AccA  Acetyl-CoA carboxylase, carboxyltransferase α-subunit

accB  AccB  Acetyl-CoA carboxylase, carboxy-biotin carrier protein

accC  AccC  Acetyl-CoA carboxylase, biotin carboxylase

accD  AccD  Acetyl-CoA carboxylase, carboxyltransferase β-subunit

*Cronan et al. 2000
PURIFICATION OF THE BC:BCCP COMPLEX USING A MONOMERIC AVIDIN COLUMN

- BCCP-has a natural affinity tag, biotin moiety
  - Monomeric avidin column
  - Eluted with 2mM biotin

- Overproduced *E. coli* biotin protein ligase (BirA) protein
  - Attaches biotin to lysine 122 of BCCP
Figure 1

Lane 1: Crude extract
Lane 2: Purified protein
BC:BCCP COMPLEX LABELED WITH L-[\(^{35}\text{S}\)]METHIONINE

- **Question:**
  - What is the protein stoichiometry of the BC:BCCP complex, examining BCCP

- Rifampicin added to block protein synthesis of chromosomal genes.
  - L-[\(^{35}\text{S}\)]methionine

- Purified BC:BCCP complex through monomeric avidin column
  - Native PAGE
  - SDS-PAGE

- Phosphorimaging device
FIGURE 2

BC:BCCP
Lane 1: Crude extract-molar ratio of 1:2
Lane 2: Band 1-molar ratio of 1:1.95
Lane 3: Band 2-molar ratio of 1:1.3
Lane 4: Band 3-only BCCP
ANALYSIS OF BC:BCCP COMPLEX BY CHROMATOGRAPHY THROUGH IMMOBILIZED AVIDIN AND/OR IMMOBILIZED NICKEL CHELATE COLUMN

- Question: What is the protein stoichiometry of the BC:BCCP complex, examining BC

- N-terminal His-tag BC protein

- Purified BC:BCCP complex through nickel chelate column
  - Native conditions

- Purified BC:BCCP complex through tetrameric avidin column
BC:BCCP

- **Lane 1**: Crude extract-molar ratio 1:2
- **Lane 2**: Avidin column-molar ratio 1:3
- **Lane 3**: Nickel chelate column-molar ratio 1:1.4
- **Lane 4**: (5μL) Eluted from both columns-1:2
- **Lane 5**: (10μL) Eluted from both columns-1:2
**Analysis of the purified complex by native PAGE**

- Question
  - How many copies of each protein is present in the BC:BCCP complex

- Purified the complex on non-denaturing gels with purified BC as a standard
Figure 4

Lane 1: Bovine Serum Albumin (67kDa)
Lane 2: Ovalbumin (43kDa)
Lane 3: His-tagged BC
Lane 4: BC:BCCP complex
Effect of deletions within the BCCP N-terminus on formation of the BC:BCCP complex

Question:
- What is the role of the N-terminal region in BC:BCCP interaction

- Constructed a series of four aa residue deletions within the N-terminal region

- Assayed the ability of the mutated protein to interact with BC
Figure 5

A. N-Terminal sequence

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B. Ladder assay

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BIOTINYLYATION OF THE BC:BCCP COMPLEX IN VITRO

- Question: Does biotinylation of BCCP occur before or after assembly of the protein into the BC:BCCP complex?

- Purified an under-biotinylated BC:BCCP complex on a nickel chelate column

- Biotinylated *in vitro* with purified BirA, ATP, and biotin

- Purified the complex using an avidin column
Lane 1: Crude extract
Lane 2: Nickel chelate-purified complex
Lanes 3 and 5: Samples of the complex that were not biotinylated \textit{in vitro}
Lanes 4 and 6: Samples of the complex that had been biotinylated \textit{in vitro}. 