Preparing a Selenomethionyl Protein

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Purpose

The protocol describes how to prepare selenomethionyl (Se-Met) protein using a regular E.coli strain. Selenium can be used for phase determination in multi-wavelength anomalous diffraction (MAD) method. Se-Met can often replace methionine residues in a protein without affecting the protein's properties, therefore producing a protein advantageous for crystal structure solving. Also, the X-ray absorption edge of selenium is easily accessible by synchrotron radiation, making a Se-Met crystal ideal for collecting anomalous X-ray diffraction data. The Se-Met proteins can also be prepared using insect cells and CHO cells, which will be described in separate protocols.

Materials

- LB media
- antibiotics (1000x conc.)
- 1M IPTG
- M9 media (minimal media)

  1 Liter 5x M9 media: (sterile filtered)
  - 30g Na_2HPO_4 or 64g Na_2HPO_4-$7H_2O$
  - 15g KH_2PO_4
  - 5g NH_4Cl
  - 2.5g NaCl

  Dilute and autoclave before use.
- Amino acid 50x stock

  Use all amino acids EXCEPT Gly, Ala, Pro, Asn, Cys, and Met at a concentration of 2mg/ml

  To help in dissolving the amino acids, autoclave for 10 minutes.
- 20% glucose (sterile filtered or autoclaved)
- 1M $MgSO_4$ (sterile filtered)
- 2M $CaCl_2$ (sterile filtered)
- 0.5% (w/v) Thiamine Solution (sterile filtered)

Procedure

Day 1

1. Prepare a 2mL day culture consisting of 2mL LB media, 2uL antibiotics (1000x conc.), and a single E.coli colony. Grow at 37°C all day.
2. Prepare M9 stock media. Dilute and autoclave before use.
3. Prepare amino acid 50x Stock.
4. Prepare a 150mL overnight culture consisting of 150mL LB, 150uL antibiotics (1000x conc.), and 150uL of day culture. Grow at 37°C overnight.

Day 2

1. To each liter M9 (1x conc.) add:
   10mL 20% Glucose (sterile filtered or autoclaved)
   2mL 1M $MgSO_4$ (sterile filtered)
   0.05mL 2M $CaCl_2$ (sterile filtered)
   0.1mL 0.5% (w/v) thiamine solution (sterile filtered)
   1mL antibiotics (1000x conc.)
   20mL amino acid 50x Stock (If precipitate is seen, heat to 60-70°C and shake.)
2. Inoculate M9 with 50mL overnight culture and grow until an OD_{600}=0.5-0.6. (~2.0 - 2.5 hours)
3. Add 100mg threonine, lysine hydrochloride, phenylalanine to the culture.
   Add 50mg leucine, isoleucine, valine to the culture (all as solid powders).
4. Add 120mg DL-Se-Met or 60mg L-Se-Met to the culture (as a solid powder).
5. Continue to grow the culture for 15 minutes.
6. Induce with 1mL 1M IPTG (final concentration = 1mM).
7. Grow about 6-8 hours (whatever is optimal for the protein of interest).
8. Collect cells as usual and proceed to purification steps.

Expected Results

- Se-Met protein will show slightly larger MW than the native protein in mass spectrum.
- Se-Met protein may behave slightly differently from the native protein in purifications and crystallization.

References

1. Doublie, S. (1997) Preparation of Selenomethionyl Proteins for Phase Determination. *Methods in Enzymology* **276**, 523-530.
2. Deacon, AM., Ealick SE. (1999) Selenium-based MAD phasing: setting the sites on larger structures. *Structure*, **7**, R161-R166
3. Protocol originally obtained from Qing Fan at Don Wiley's Lab.
4. X-ray Anomalous Scattering: Principles, WebTools, and Related Links provided by Ethan A. Merritt at University of Washington.