PHYH

PDB:2A1X

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:none
Host:BL21(DE3)

Construct

Prelude: Deletion of N-terminal peroxisome leader sequence (residues 1-30: meqlraaarlqivlghlgrpsagavvahpt)
Sequence:

MSGTISSASFHPQQFQYTLDNNVLTLEQRKFYEENGFLVIKNLVPDADIQRFRNEFEKICRKEVKPLGLTVMRDVTISKSEYAPSEK
MITKVQDFQEDKELFRYCTLPEILKYVECFTGPNIMAMHTMLINKPPDSGKKTSRHPLHQDLHYFPFRPSDLIVCAWTAMEHISRNN
GCLVVLPGTHKGSLKPHDYPKWEGGVNKMFHGIQDYEENKARVHLVMEKGDTVFFHPLLIHGSGQNKTQGFRKAISCHFASADCHYI
DVKGTSQENIEKEVVGIAHKFFGAENSVNLKDIWMFRARLVKGERTNL

Vector:pET24a

Growth

Medium:
Antibiotics:
Procedure: Cells were grown at 37 degC in 2TY media containing 30 µg/mL kanamycin. When cells reached OD600 of 0.7, the temperature was reduced to 25degC and expression was induced by adding IPTG to a final concentration of 1 mM. Expression was allowed to continue for 6 hours.

Purification

Procedure
Column 1: CM-sepharose

Loading Buffer: 20 mM MOPS, 10 % glycerol, 1 mM benzidamine, 1 mM PMSF, 0.5 mM 0-phenathroline, 1 mM EDTA, pH 7.2. Elution Buffer: 20 mM MOPS, 700 mM NaCl, 10% (v/v) glycerol, 1 mM benzidamine, 1 mM PMSF, 0.5 mM 0-phenathroline, 1 mM EDTA, pH 7.2.

Procedure: The supernatant was loaded onto a pre-equilibrated CM-Sepharose column and washed with 1 liter of loading buffer. Protein was eluted with a NaCl gradient from 0-700 mM . The PHYHA-containing fractions (eluting at ~300-600 mM NaCl) were pooled, concentrated, and exchanged into 10 mM Tris, 10% (v/v) glycerol, 1 mM EDTA, pH 7.5.

Column 2: Superdex S75

Running Buffer : 100 mM Tris, 50 mM NaCl, 10 % (v/v) glycerol, 1 mM EDTA, pH 7.5.

Procedure: The protein was loaded onto a pre-equilibrated Superdex-75 column at 2 mL/min. Fractions containing PHYH were pooled, concentrated, and exchanged into 10 mM Tris, 10% (v/v) glycerol, 1 mM EDTA, pH 7.5.

Extraction

Procedure
Cell pellets were resuspended in 20 mM MOPS, 10% glycerol, 1 mM benzidamine, 1 mM PMSF, 0.5 mM 0-phenathroline, 1 mM EDTA, pH 7.2 and cells were disrupted by sonication. Polyethyleneimine (0.1 % w/v) and streptomycin sulfate (1 % w/v) were added to the cell lysate and the solution stirred for 10 minutes before the debris was pelleted by centrifugation.
Concentration:5.2 mg/mL
Ligand
MassSpec: Mass spec results were consistent with the loss of the N-terminal methionine. Observed mass: 35.436. Calculated (without initial methionine): 35,435.
Crystallization: FeSO4 (1 mM) and 2-oxoglutarate (2 mM) were added to the protein in a glove box under argon to prevent oxidation of the iron. Hanging drops of 2 µl protein and 2 µl well solution were suspended over well solution consisting of 21% PEG 3350 and 0.3 M tri-ammonium citrate pH 7.1. Crystals were transferred to a cryo-protectant consisting of 15% glycerol, 85 % well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.5 Å; X-ray source: Synchrotron SLS -X10, single wavelength.
Data Processing: