FTHFD + 6-Formylpteridin

PDB:2CFI

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:BC027241
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli BL21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMKIAVIGQSLFGQEVYCHLRKEGHEVVGVFTVPDKDGKADPLGLEAEKDGVPVFKYSRWRAKGQA
LPDVVAKYQALGAELNVLPFCSQFIPMEIISAPRHGSIIYHPSLLPRHRGASAINWTLIHGDKKGGFSIFWADDGLDTGDLLLQKEC
EVLPDDTVSTLYNRFLFPEGIKGMVQAVRLIAEGKAPRLPQPEEGATYEGIQKKETAKINWDQPAEAIHNWIRGNDKVPGAWTEACE
QKLTFFNSTLNTSGLVPEGDALPIPGAHRPGVVTKAGLILFGNDDKMLLVKNIQLEDGKMILASNFFK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:20 µL competent BL-21 cells were transformed with 1 µL plasmid. 10min on ice, 45sec at 42°C. Added 100µL SOC then 30min at 37°C. Cells were plated on Kanamycin. Colonies were grown in 50mL of TB + 4% glycerol at 37°C, until OD600 of 0.5 then diluted into 400mL TB + 4% glycerol. Growth at 37°C until OD600 of 1.2 - 1.5. Then at 18 °C for 1h. Induction with 0.5mM IPTG at OD600 of 1.4 - 1.8. Left culture at 18 °C over night.

Purification

Procedure
Columns: HisTrap HP 1 mL (IMAC); HiLoadÂ□ 16/60 Superdex 75 Prep Grade (Gel filtration)

Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software at a flow of 0.8 mL/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HisTrap HP) and Gel filtration buffer (Superdex 75). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed

by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 mL of IMAC Elution Buffer and loaded in the Gel filtration column. The chromatogram from Gel filtration showed one major protein peak that mainly consisted of FTHFDB-c203 of high purity as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein was concentrated to 33 mg/mL and stored at -80 degC.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, 50µg/mL of Lysosyme was added as well as 1 tablet Complete EDTA-free protease inhibitor tablet per cell pellet. Cells were disrupted by sonication (60%, 1s-1s pulse on-off) for three minutes. DNA precipitation was performed by addition of PEI to a final concentration of 0.15%. The sample was incubated on ice for 30 minutes and centrifuged for one hour at 40000×g. The soluble fraction was filtered through 0.22 µm and subjected to further purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using sitting drop method at 20°C.Drops were prepared using 900 nL of protein (16.5 mg/mL concentration) and 900 nl of the well solution (1.4 M Ammonium Sulfate, 50 mM HEPES pH 7.8). Crystals diffracted to 1.7 Å.

A cryo-solution containing:2.3 M NH4SO4, 20% Glyserol, 0.2M NaCl, 2 mM TCEP and 20mM HEPES pH 7.5 was used. The crystal was soaked in the cryosolution containing 5 mM Tetrahydrofolate and 20 mM Formate before it was flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: