

FASN: Human cytosolic fatty acid synthase - malonyl/acetyl-ACP transferase domain

PDB:2JFD

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:FASNA-s002 (BC063242)

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence

mhhhhhssgvdlgtenlyfq*s

Host:BL21(DE3)-R3

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfqSMRLLRAS GRTPEAVQKLLEQGLRHSQDLAFLSMLND IAAVPATAMPFRGYAVLGGERGGPEVQ
QV PAGERPLWFICSGMGQTQWRGMGLSLMRLD RFRDSILRSDEAVKPFGLKVSQLLSTDE STFDDIVHSFVSLTAIQIGLIDLL
SCMGL RPDGIVGHSLGEVACGYADGCLSQEEAVL AAYWRGQCIKEAHLPPGAMAAGLSWEEC KQRCPPGVVPACHNSKDTVTI
SGPQAPVF EFVEQLRKEGVFAKEVRTGGMAFHSYFME AIAPLLQELKKVIREPKPR SARWLSTSI PEAQWHSSLARTSSAEYN
VNNLVSPVLFQ EALWHVPEHAVVLEIAPHALLQAVLKRGL KPSCTIIPLMKKDHRDNLEFFLAGIGRLH LSGIDANPNALFPPV
EFPA

Vector:pNIC28-Bsa4

Growth

Medium:

Antibiotics:

Procedure:An overnight culture (10 ml) was used to inoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin and 34 µg/ml Chloramphenicol). The cells were cultured in 2 litres at 37°C with vigorous shaking (160 rpm) until the culture reached an OD600 of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultivated further for 16 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet was stored at -20°C until further use.

Purification

Procedure

Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

Procedure: AKTA Xpress Affinity/Gel Filtration. The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)
Procedure: AKTA Xpress Affinity/Gel Filtration. The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: The protein was concentrated to 13.7 mg/ml by using Vivaspin, 10K concentrator.

Extraction

Procedure

The frozen cell pellet was resuspended in 25 ml of extraction buffer. The sample was homogenised by using Emulsiflex-05 homogenizer (Glen Creston) and then centrifuged at 17000 rpm for 45 min.

Concentration:

Ligand

MassSpec: The experimentally determined mass of FASNA was 46682 Da, which corresponds to the theoretical mass.

Crystallization: Initial screens set up with native FASN yielded several hits. However, to decrease crystal degradation by crystal handling a condition was selected that provided sufficient cryoprotection. After optimisation, diffraction quality rod-like crystals were obtained from 20% PEG10K, 0.04 M Na/KPO₄ and 25% glycerol with average dimensions 0.6*0.2*0.2 mm³, and were mounted with a loop and flash-frozen by plunging into liquid nitrogen. Datasets were collected at the PXII beamline at the Swiss Light Source using a MAR225 detector at 0.9793 Å. Selenomethionine-labelled protein could be crystallised using the same crystallisation condition, and crystals were analogous to the native in morphology and size but degraded after one week and had to be mounted immediately after they appeared. A SAD dataset was collected at the PXII beamline at the SLS, using the selenium peak wavelength (0.9789 Å, determined from a fluorescence scan).

NMR Spectroscopy:

Data Collection:

Data Processing: The diffraction pattern for both the native and selenomethionine-labelled protein was highly anisotropic, but images could be processed with XDS. Different scans were scaled together using XSCALE.

Due to severe anisotropy the anomalous signal obtained from the selenomethionine-labelled crystal was significant to 4.5 Å only. However, this proved to be sufficient for SHELXD to locate 40 selenium positions. These were refined by SOLVE and solvent-flattened by RESOLVE to yield an interpretable map. NCS operators could be derived from selenium positions, but NCS averaging did not improve maps, possibly due to translational-only symmetry. Although an initial model could be built, the sequence could not be assigned and refinement stalled.

Phases obtained from this model were therefore used as external phase information in SHARP, which allowed the completion of the anomalous substructure by finding minor selenium sites. Phases were solvent-flattened and the model was rebuilt using the new map. Phase restraints (solvent-flattened phases expressed as Hendrickson-Lattman coefficients) proved very useful to keep refinement stable and improved maps significantly. After several rounds of rebuilding and refinement, the new model was used to solve the higher resolution native dataset, and was refined to convergence.