GFPT1

PDB:2V4M

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_002047
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:GFPT1A-k001
Tag:N-terminal hexahistidine tag: mghhhhhh
Host:*Epicurian Coli* BL21(DE3) (Stratagene)

Construct

Prelude:
Sequence:

mghhhhhhQQIMKGNFSSFMQKEIFEQPESVVNTMRGRVNFDDYTVNLGGLKDHIKEIQRCRRLILIACGTSYHAGVATRQVLEELT
ELPVMVELASDFLDRNTPVFRDDVCFFLSQSGETATLMGLRYCKERGALTVGITNTVGSSISRETDCGVHINAGPEIGVASTKAYTS
QFVSLVMFALMMCDDRISMQERRKEIMLGLKRLPDLIKEVLSMDDEIQKLATELYHQKSVLIMGRGYHYATCLEGALKIKEITYMHS
EGILAGELKHGPLALVDKLMPVIMIIMRDHTYAKCQNALQQVVARQGRPVVICDKEDTETIKNTKRTIKVPHSVDCLQGILSVIPLQ
LLAFHLAVLRGYDVDFPRNLAKSVTVE

Vector:pET-28 (Novagen)

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 3 ml TB supplemented with 50 µg/ml kanamycin at 30 °C overnight. The overnight culture was used to inoculate 2 l TB supplemented with 25 µg/ml kanamycin. The culture was grown at 37 °C until OD600 reached ~0.5. The culture was then down-tempered to 18 °C and target expression was induced by addition of 1 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (6000 x *g*, 10 min, 4 °C). The resulting cell pellet was resuspended in 300 ml lysis buffer (5 ml/g cell pellet). The cell suspension was stored at -80 °C.

Purification

Procedure

Columns
IMAC: Ni-charged 5 ml HiTrap Chelating HP (GE Healthcare)
IEX column: 1 ml RESOURCE Q column (GE Healthcare)

Procedure
Purification of the protein was performed as a two step process. The cell lysate was loaded onto the 5 ml HiTrap Ni-sepharose column. A stepwise elution procedure was used with 5, 8 and 50% IMAC elution buffer. The protein was identified as the major component in the 50% elution buffer fraction. The eluted protein was subjected to dialysis against IEX binding buffer. After buffer exchange, the sample was loaded onto a 1 ml RESOURCE Q column and eluted around 40% using a linear gradient of 0-100% IEX elution buffer.

Extraction

Procedure
The cell suspension was quickly thawed in water. Cells were disrupted by sonication under continuous flow and cell debris was removed by centrifugation (20,000 x *g*, 60 min, 4 ºC).
Concentration:
Ligand
Fructose 6-phosphate**MassSpec:**
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method in a 24-well plate. The well solution consisted of 100 mM Tris pH 8.0, 3 % PEG 6000, 10 mM CaCl2, 3% MPD. The plate was incubated at 4 ºC. The crystals were transferred to a cryo solution containing 0.1 M Tris pH 8.0, 5% PEG 6000, 5 mM CaCl2, 1 mM TCEP, 1 mM F6P, 25% MPD for 5 minutes and then flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at MAXLAB (I711)
Data Processing: The structure was solved by MR in MOLREP using 1MOQ from *Escherichia coli* as search model. Twinning of the dataset was recognised running PHENIX xtriage. Refinement was performed in REFMAC5.5 and model building in COOT. Refinement was initiated without TLS parameters or twinning operator until convergence, then refinement continued with TLS parameters without twinning operator again until convergence. Finally TLS parameters was removed again and refinement including the twin operator was performed in REFMAC5.5. Taking the twin operator into account improved the maps significantly and small structural errors in rotamer conformations and pep-flips was suddenly visible in the fo-fc difference map. During the refinement process the possibility to introduce an Rfree set taking the twin operator into account in PHENIX was discovered. The new test set was introduced (too) late during twin refinement and likely the small difference in R-values between the working set and the test set originate from the late introduction of the new test set.