SHMT2

PDB:2A7V

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:19923315
Entry Clone Source:MGC
SGC Clone Accession:SHMT2:APC005_8-E02:C16046
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

GSGQLVRMAIRAQHSNAAQTQTGEANRGWTGQESLSDSDPEMWELLQREKDRQCRGLELIASENFCSRAALEALGSCLNNKYSEGYP
GKRYYGGAEVVDEIELLCQRRALEAFDLDPAQWGVNVQPYSGSPANLAVYTALLQPHDRIMGLDLPDGGHLTHGYMSDVKRISATSI
FFESMPYKLNPKTGLIDYNQLALTARLFRPRLIIAGTSAYARLIDYARMREVCDEVKAHLLADMAHISGLVAAKVIPSPFKHADIVT
TTTHKTLRGARSGLIFYRKGVKAVDPKTGREIPYTFEDRINFAVFPSLQGGPHNHAIAAVAVALKQACTPMFREYSLQVLKNARAMA
DALLERGYSLVSGGTDNHLVLVDLRPKGLDGARAERVLELVSITANKNTCPGDRSAITPGGLRLGAPALTSRQFREDDFRRVVDFID
EGVNIGLEVKSKTAKLQDFKSFLLKDSETSQRLANLRQRVEQFARAFPMPGFDEH

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SHMT2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37oC to an OD600 of 1.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation and passing through a 20-ml DE52 column equilibrated in 20 mM HEPES, pH 7.4, containing 500 mM NaCl. The clarified lysate was loaded onto 10-ml Chelating Fastflow column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole). The fractions containing SHMT2 was pooled and dialyzed against buffer containing 20 mM PIPES, pH 6.5, 250 mM NaCl, 5% glycerol. Thrombin (Sigma) and 2 mM CaCl2 were added to the protein sample and incubated overnight at 4oC. Dialyzed protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM MES buffer, pH 6.0, and 500 mM Ammonium Acetate, at flow rate 4 ml/min. Purification yield was 72 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:61.6 mg/ml
Ligand
MassSpec: Enzymatic treatment: thrombin. Expected MW is 54152.75, measured MW is 54190.28.
Crystallization: Purified SHMT2 was complexed with tetrahydrofolic acid (2 mM final concentration) and glycine (10 mM final concentration) and crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 5% PEG8000, 0.1 M Tris-HCl, pH 8.0. Crystals were frozen in reservoir solution containing 14% PEG8000, 20% glycerol as cryo protectant.
NMR Spectroscopy:
Data Collection:
Data Processing: