GAMT

PDB:1ZX0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4503909
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen)

Construct

Prelude:
Sequence:

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GSAPSATPIFAPGENCSPAWGAAPAAYDAADTHLRILGKPVMERWETPYMHALAAAASSKGGRVLEVGFGMAIAASKVQEAPIDEHW
IIECNDGVFQRLRDWAPRQTHKVIPLKGLWEDVAPTLPDGHFDGILYDTYPLSEETWHTHQFNFIKNHAFRLLKPGGVLTYCNLTSW
GELMKSKYSDITIMFEETQVPALLEAGFRRENIRTEVMALVPPADCRYYAFPQMITPLVTKG
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Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:GAMT 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. The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 mL/min. Thrombin (Sigma) was added to combined fractions containing GAMT and incubated overnight at 4oC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 70 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 (1 XPBS, 0.25 M NaCl, 5 mM imidazol, 2 mM ?-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:
Ligand
MassSpec:
Crystallization: Purified GAMT was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH 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 21% PEG3350, 0.1 M CaCl2,0.1 M BisTris, pH 6.5, 1 mM Guanidine HCl.
NMR Spectroscopy:
Data Collection:
Data Processing: