HRB

PDB:2OLM

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC030592
Entry Clone Source:MGC clone AT27-B10
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

gsSAKRKQEEKHLKMLRDMTGLPHNRKCFDCDQRGPTYVNMTVGSFVCTSCSGSLRGLNPPHRVKSISMTTFTQQEIEFLQKHGNEV
CKQIWLGLFDDRSSAIPDFRDPQKVKEFLQEKYEKKRWYVPPEQAKVVASVHA

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and chloramphenicol at 37 °C. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15 °C, and the cells were allowed to grow overnight. Cultures were harvested by centrifugation and the cell pellets were flash frozen and stored at -80 °C .

Purification

Procedure
The supernatant was loaded onto 5 mL HiTrap Ni-NTA column (Pharmacia Amersham) equilibrated with the same binding buffer at 4 ºC using a peristaltic pump. The Ni-NTA column was connected to an FPLC instrument and washed with 25 mL of wash buffer. A linear gradient from 50 to 500mM imidazole (in 50 mL) was applied and the protein was eluted at around 175mM imidazole. Collected fractions were digested with thrombin (Sigma, ~ 7U/mg protein) overnight at 4 ºC and then passed through an open column filled with ~ 3mL Ni-NTA resin (Qiagen). The flowthrough was collected and 200 microL PMSF (100mM stock solution) was

added to inhibit residual thrombin protease activity. The protein were further purified and desalted using a gel filtration column, Superdex 75 (26/60), which was pre-equilibrated with the binding buffer. Collected fractions were concentrated using an Amicon Ultra centrifugal filter to a final concentration of around 50 mg/mL. Protein concentration was measured using Bradford assay and the purity was greater than 95% based on SDS-PAGE analysis.

Extraction

Procedure
The thawed 1.8 L cell pellets were resuspended in 100 mL of the binding buffer with a protease inhibitor cocktail (0.1 mM benzamidine-HCl, 0.1 mM phenylmethyl sulfonyl fluoride, and 2 microL benzonase [Sigma]), and 0.5% CHAPS. The cells were lysed by sonication for half a minute. The lysate was centrifuged at 38400 ×g for 60 min and collected the supernatant.
Concentration:20 mg/mL
Ligand
MassSpec:16130.94 D
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 2.27 M (NH4)2SO4, 0.09M BTP, pH 7.0. Crystals reached a size of about 50 microns within two to three days at 20 ºC.
NMR Spectroscopy:
Data Collection:
Data Processing: