CDYL

PDB:2GTR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:25777617
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 (Stratagene).

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsAYRYRDIVVRKQDGFTHILLSTKSSENNSLNPEVMREVQSALSTAAADDSKLVLLSAVGSVFCCGLDF
IYFIRRLTDDRKRESTKMAEAIRNFVNTFIQFKKPIIVAVNGPAIGLGASILPLCDVVWANEKAWFQTPYTTFGQSPDGCSTVMFPK
IMGGASANEMLLSGRKLTAQEACGKGLVSQVFWPGTFTQEVMVRIKELASCNPVVLEESKALVRCNMKMELEQANERECEVLKKIWG
SAQGMDSMLKYLQRKIDEF

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:CDYL was expressed in E.coli BL21 (DE3) codon plus RIL in TB medium in the presence of 50 µg/ml of kanamycin. Cells were grown at 37oC to an OD600 of 0.8 and 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 20-ml DE52 column equilibrated in 20 mM Hepes, pH 7.5, containing 500 mM NaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Hepes pH 7.5, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Hepes pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM Hepes, pH 7.5,

500mM Ammonuim Acetate, 5% glycerol, 5mM ß-mercaptoethanol. Purification yield was 2.6 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 (1xPBS pH 7.4, 0.5 M NaCl, 5% glycerol, 0.1% Igepal) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:6.9 mg/ml (uncut)
Ligand
MassSpec: Expected MW is 31144.9, measured mass is 31013.92.
Crystallization: Purified CDYL was crystallized using hanging drop vapor diffusion method drop at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 12% Isopropanol, 0.2M NaCitrate, 0.1M NaCacodylate pH 5.0.
NMR Spectroscopy:
Data Collection:
Data Processing: