TM0561 corA

PDB:2BBJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:
Host:

Construct

Prelude:
Sequence:

Vector:modified pET15b (Novagen) vector in which the thrombin cleavage site (LVPR^GS) had been replaced with a TEV protease recognition site (ENLYFQ^G)

Growth

Medium:
Antibiotics:
Procedure: CorA was expressed in E.coli BL21 (DE3) in Luria Broth (LB) in the presence of 50 µg/mL of kanamycin and 100 µg/mL of ampicillin. Cells were grown at 37oC to an OD_{600} of 0.6-0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 16oC.

Purification

Procedure
All procedures were carried out at 4ºC unless otherwise specified. The suspension was centrifuged for 30 minutes at 100,000 x g. The pellets were then solubilized in 250 mL Binding Buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 10mM imidazole, 1% n-dodecyl-?-D-maltopyranoside (DDM, Anatrace), protease inhibitors) and stirred gently for 12 hours. The sample was then centrifuged for 30 minutes at 100 000 x g, and the supernatant loaded onto a 1 x 10 cm Ni-NTA gravity column equilibrated with Binding Buffer. The column was washed with 20 column volumes of Wash Buffer (50 mM Hepes, pH 7.5, 500 mM NaCl, 5% glycerol, 35mM imidazole, 0.02% DDM). Bound protein was eluted with Elution Buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 200mM imidazole, 0.02% DDM) and dialyzed overnight against Dialysis Buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol). During dialysis, removal

of the hexahistidine tag was facilitated by the addition of histidine-tagged TEV protease (Invitrogen), according to the TEV protease technical manual (Invitrogen). Digestions were monitored by SDS-polyacrylamide gel electrophoresis. The resulting proteins contained three additional residues at the N terminus (Gly-Ser-His). TEV protease and the histidine tag were separated from CorA by collecting the flow-through from a second Ni-NTA column purification, as described earlier.

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 purification, the cell paste was thawed and resuspended in ice-cold Lysis Buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, protease inhibitors (Complete Protease Inhibitor Cocktail (Roche), according to the technical manual)) and lysed using a French press
Concentration:
Ligand
MassSpec:
Crystallization: Protein solutions were used immediately after purification or after storage at -78°C. Crystals were grown by the hanging drop method at 22°C, at a concentration of 2-4 mg/mL. 2 µl protein was mixed with 2 µl reservoir solution containing 20% (w/v) PEG 2000 (Fluka), 0.3M Mg(NO3)2 and 0.1M Tris pH 8.0. Needle-like crystals appeared after 3-5 days and matured to full size within 2-3 weeks.
NMR Spectroscopy:
Data Collection:
Data Processing: