KRASB

PDB:3GFT

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC013572
Entry Clone Source:MGC clone AT9-E8
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with Tev cleavage site: mhhhhhhssgrenlyfqg
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

```
mhhhhhhssgrenlyfqgMTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGHEEYSAMRD
QYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAF
YTLVREIRKHKEK
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Vector:pET28a-mhl

Growth

Medium:TB
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 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 before harvesting and flash frozen.

Purification

Procedure
Column 1: HiTrap Ni-NTA column (Pharmacia Amersham)
Column 2: superdex 75.
The lysate was centrifuged at 15000 rpm for 45 min and the supernatant was loaded onto 5 mL HiTrap Ni-NTA column (Pharmacia Amersham) equilibrated with the same binding buffer at 4 ºC using peristaltic pump. The HiTrap Ni-NTA column was steply washed with 25 mL of binding buffer, 25 mL of binding buffer with 30 mM imidazole, and 25 mL of binding buffer with 50 mM imidazole. The His-tagged protein was eluted by linear gradient of imidazole from 50 mM to 500

mM in 50 mL. The eluted protein peak fractions detected by UV280 nm were combined and further purified by gel filtration column superdex 75 with a buffer containing Gel filtration buffer. Protein peak fractions were combined, and DTT was added to a final concentration of 10 mM. Five times molarity of GppNHp is added before concentrating.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 150 mL the binding buffer with 0.5% CHAPS (Sigma) and 1 mM phenylmethyl sulfonyl fluoride (PMSF) and lysed with microfluidizer.
Concentration: The protein was concentrated using an Amicon Ultra centrifugal filter and the concentration for protein stock solution was estimated by Bradford to be 45.9 mg/mL.
Ligand
MassSpec:21448.50 Da, expected: 21439.09 Da. The template encodes a mutation Q61H. The measured mass matches with a sequence with H61. This residue is not visible in electron density map.
Crystallization: Crystallization trials were set up using the hanging drop vapor diffusion method at room temperature. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 20% PEG 3350, 0.2M Lithium Citrate pH 4.0. Crystals reached a size of about 20 microns within one week.
NMR Spectroscopy:
Data Collection:
Data Processing: