NRAS

PDB:3CON

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC005219
Entry Clone Source:MGC AU41-B12
SGC Clone Accession:HPC073-B11
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRD
QYMRTGEGFLCVFAINNSKSFADINLYREQIKRVKDSDDVPMVLVGNKCDLPTRTVDTKQAHELAKSYGIPFIETSAKTRQGVEDAF
YTLVREIRQYRMKKLN

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target protein 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 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Ni-NTA beads and incubated at 4°reeC on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-trough was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions were collected and concentrated with

Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 95%.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:44.14 mg/mL
Ligand
GDP, Mg2+**MassSpec:**Native: 21740.43, expected 21740.58
In a separate experiment, His-tag removal was tried with TEV protease, yet only 10% protein had tag successfully removed.
Crystallization:GDP were added to the concentrated protein to a final concentration of 5mM. Crystallization were set up using SGC-I and Red Wings screen with 1:100 chymotrypsin. Crystals were seen for the following conditions:
Red Wings: RD09 RE06 RH08 RG10

Crystals of 50-100 micron were seen within 2 days after plate setup
Two types of xtals were seen in the same drop for RD09 condition: hollow rod and hexagnol plate.

Crystal used for structure determination were grown in Red Wings initial screen RD09: 30.0% PEG3350, 0.2M MgCl2, 0.1 M Tris pH 8.5. Sitting drop vaporization. No cryo used.

In a separate experiment, crystallization were tried without adding chymotrypsin protease, hexagnoal plate like crystals were seen at conditions: SD08 and RA08, yet best crystal diffracts to only 7A.

Last updated by ytong 20080410
NMR Spectroscopy:
Data Collection:
Data Processing: