RECQL

PDB:2V1X

Revision

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Entry Clone Accession:gi|14591904
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SGC Clone Accession:RECQLA-c533
Tag:C-terminal, TEV-cleavable(*) Histidine tag + Flag epitope: aenlyfq*shhhhhhdykddddk
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

MCLEDSDAGASNEYDSSPAAWNKEDFPWSGKVKDILQNVFKLEKFRPLQLETINVTMAGKEVFLVMPTGGGKSLCYQLPALCSDGFT
LVICPLISLMEDQLMVLKQLGISATMLNASSSKEHVKWVHAEMVNKNSELKLIYVTPEKIAKSKMFMSRLEKAYEARRFTRIAVDEV
HCCSQWGHDFRPDYKALGILKRQFPNASL IGLTATATNHVLTDAQKILCIEKCFTFTASFNRPNLYYEVRQKPSNTEDFIEDIVKL
INGRYKGQSGIIYCFSQKDSEQVTVSLQNLGIHAGAYHANLEPEDKTTVHRKWSANEIQVVVATVAFGMGIDKPDVRFVIHHSMSKS
MENYYQESGRAGRDDMKADCILYYGFGDIFRISSMVVMENVGQQKLYEMVSYCQNISKCRRVLMAQHFDEVWNSEACNKMCDNCCKD
SAFERKNITEYCRDLIKILKQAEELNEKLTPLKLIDSWMGKGAAKLRVAGVVAPTLPREDLEKIIAHFLIQQYLKEDYSFTAYATIS
YLKIGPKANLLNNEAHAITMQVTKSTQNSFRAESSQTCHSEQGDKKMEaenlyfq*shhhhhhdykddddk

Vector:pNIC-CTHF

Growth

Medium:TB
Antibiotics:
Procedure: The expression plasmid was transformed into the host strain and plated on LB-agar containing 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. Several colonies were combined to inoculate a 1-ml culture in TB (+ 50 µg/ml kanamycin, 35 µg/ml chloramphenicol). The culture was grown overnight, glycerol was added to 15% v/v (from a 60% stock), and the resulting glycerol stock was frozen at -80°C in 100 µl aliquots. A loopful of cells from the glycerol stock was inoculated into 2x 10-ml of TB medium containing 50 µg/ml kanamycin and 35 µg/ml chloramphenicol and grown overnight at 37°C. 1-L of TB medium (+ 50 µg/ml kanamycin) in each 2.5L UltraYield baffled flask (2L total) was inoculated with the overnight cultures. The culture was grown at 37°C until OD600 of 2-3 and then shifted to 25°C. After 30 minutes, IPTG was added to 0.1 mM, and growth continued overnight. The cells were collected by centrifugation then the pellets were scraped out and transferred to 50-ml Falcon tubes and frozen at -80°C.

Purification

Procedure
Column 1: Ni-affinity, HisTrap Crude FF, 1 ml (GE Healthcare)
Column 2: Gel filtration, Hiload 16/60 Superdex S75 prep grade, 120 ml (GE Healthcare)

The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE Healthcare). The column was washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

The eluted fractions from the Ni-affinity Histrap column was loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2-ml fractions and analyzed on SDS-PAGE.

Extraction

Procedure
Frozen cell pellets (28g) were thawed briefly in a bath of warm water (20 - 37°C) then transferred to ice. One volume (i.e. 1 ml for every gram of cells) of 2x lysis buffer was added, followed by 1x lysis buffer to a total volume of 40 ml. The cells were resuspended by agitating and disrupted by high pressure homogenization (20 kpsi). Nucleic acids and cell debris were removed by adding 0.15% PEI (polyethyleneimine) from a 5% (w/v, pH 7.5) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 25,000 x g . The supernatant was then further clarified by filtration (Acrodisc filters, 0.2 μm).
Concentration: The protein was concentrated in Amicon (30 K) to 9.8 mg/ml and stored at 4°C. The protein concentration was determined spectrophotometrically using ε280 = 72770 .
Ligand
MassSpec: Observed mass 67222, calculated mass 67294; (unexplained mass difference of -75). This may partly arise from cleavage of the N-terminal methionine.
Crystallization: Crystals were grown by vapour diffusion from nanolitre sitting drops at 4°C. Prior to setting up the crystallization trials, ATPγS and a 3'-tailed double-stranded oligonucleotide (5'-AAGCACAATTACCCACGC : 5'-GCGTGGGTAATTGTGCTTCAATGGACTGAC) were added to the protein solution, at a two-fold molar excess. Nanodrops comprising 75nl protein solution (10mg/ml) and 75nl reservoir solution were equilibrated against reservoir solution (0.2M sodium bromide, 20% PEG3350, 10% ethylene glycol, 0.1M bis-tris propane pH 7.5). Large brick-shaped crystals appeared within 3-4 days. Prior to flash freezing in liquid nitrogen, crystals were briefly soaked in stabilising solution (0.2M sodium bromide, 22% PEG3350, 0.1M bis-tris propane pH 7.5) containing 25% (v/v) ethylene glycol.
NMR Spectroscopy:
Data Collection: Resolution: 2.0Å; Beamline: X10SA (λ=1.03Å) at the Swiss Light Source (SLS).
Data Processing: Crystals belong to monoclinic space group P21 and contain two RECQ monomers per asymmetric unit. Poor initial phases were obtained using molecular replacement with the coordinates of E. coli RECQ (PDB: 1OYY) as a search model in PHASER. These phases were improved substantially by multi-crystal averaging with an orthorhombic crystal form. Despite the addition of a DNA oligonucleotide during crystallization, the crystals were found to contain RECQL alone. The final model, comprising residues 63-592, was refined with REFMAC5 to an R cryst / R free of 23.2 / 27.8 respectively.