Tg-CyPR

PDB:3BO7
SGC Clone Accession:55.m00007:I39-E531:B3 from plate MAC01T
Tag:N-terminal tag: mgsshhhhhhssgrenlyfq
Host:E. coli BL21-(DE3)-R3-pRARE2

Sequence:

gKLKKKGYLRIVTTQGSLNIELHADMAPRACDSFLRLCAVKYFDDTIFHRCIRNFMIQGGRAELRQPSKKKEVQQSPRSISGFPGGA
PFEDEFDNRLVHQGIGVLSMANDGKHSNLSEFFITFKSCEHLNNKHTIFGRVVGGLDVLRQWEKLETDKKDKPLKPPKVEEIIVFKN
PFEDARKEMEDEKREEEEKEKKKLENA

Growth

Medium:TB

Procedure: The protein was expressed in E. coli BL21-(DE3)-R3-pRARE2 in Terrific Broth (TB) in the presence of kanamycin/chloramphenicol (50 µg/mL and 25 µg/mL respectively). A single colony was inoculated into 10 mL of LB with of kanamycin/chloramphenicol (50 µg/mL and 25 µg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 50 mL of TB with 50 µg/mL kanamycin in a 250 mL shaking flask and incubated at 37 ºC for 3 hours. Then the culture was transfer into 1.8 L of TB with 50 µg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of 5, cooled to 15 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure: The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 1.0 Â 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1Â 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 Â 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. The protein was further purified by Size exclusion chromatography in a Superdex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 164 mL corresponding to tetrameric protein were pooled and the protein identity was evaluated by SDS-Page and mass spectroscopy.

Extraction

Procedure: The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 ºC were thawed overnight at 4 ºC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 ºC.

Structure Determination

Crystallization: The protein was crystallized at 20 ºC in 28% PEG 3350, 0.2 M LiSO4, 0.1 M Hepes pH 7.3, 2 mM TCEP, 2 mM Cyclosporin A, 20% ethylene glycol, temperature 293K using the sitting drop vapor diffusion method.