PLAA

PDB:3EBB
Entry Clone Accession:NP_001026859
Entry Clone Source:plap.BC032551.MGC.AT56D1.pCMVSPORT6
SGC Clone Accession:plap.454.738.75G03 (SDC75G03)
Tag:N-terminal: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)
Vector:pET28-LIC

Sequence:

mgsshhhhhhssglvprgsMAGVDPFTGNSAYRSAASKTMNIYFPKKEAVTFDQANPTQILGKLKELNGTAPEEKKLTEDDLILLEK
ILSLICNSSSEKPTVQQLQILWKAINCPEDIVFPALDILRLSIKHPSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLALRTFCN
CFVGQAGQKLMMSQRESLMSHAIELKSGSNKNIHIALATLALNYSVCFHKDHNIEGKAQCLSLISTILEVVQDLEATFRLLVALGTL
ISDDSNAVQLAKSLGVDSQIKKYSSVSEPAKVSECCRFILNLL

Growth

Procedure: A 250 ml flask containing LB (Sigma L7658) supplemented with 50 microgram/milliliter kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 degC.

Using the Lex system, a 2 liter bottle (VWR 89000-242) containing 1800 milliliter of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 microgram/milliliter kanamycin and 600 microliter antifoam 204 (Sigma A-8311) was inoculated with 50 milliliter overnight LB culture, and incubated at 37 degC. The temperature of the media was reduced to 15 degree Celsius one hour prior to induction and induced at OD(600)= 6 with 100 micromolar isopropyl-thio-beta-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 degC, and cell pellets collected by centrifugation and frozen at -80 degC.

Purification

Procedure: Cleared lysate was loaded onto TALON metal-affinity resin (BD Biosciences; 1.5 ml settled beads per L cell culture) at 4 degC. The column was washed with 5 column volumes of Wash buffer A, 5 column volumes of Wash buffer B, and 5 column volumes of Wash buffer A. The protein was eluted with 2 column volumes of Elution buffer. The His tag was cut with thrombin (2 unit per miligram protein) overnight at 4 degC.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 5 kD cutoff.

The yield of the protein was approximately 10 mg per liter of bacterial culture.

Extraction

Procedure: Cell pellets were resuspended in Lysis buffer (30 mL per liter culture), lysed using a Microfluidizer (18,000 pounds per square inch), and cleared by centrifugation (40,000 xg for 30 minutes).

Concentration:40 mg/mL

Structure Determination

MassSpec: Mass-spectroscopy by LCMS shows that the product was pure and molecular weight is 15 Da greater than the calculated molecular weight. One possible explanation for the mass difference is modification of a lysine to delta-hydroxy-allysine.
Crystallization: Purified protein (40 mg/mL) was mixed in 1:1 molar ratio with P97 peptide (TEDNDDDLYG). Crystals were grown at 298 degrees Kelvin using the hanging drop method by mixing 1 volume of the protein-peptide mixture with 1 volume of well solution consisting of of 30% PEG-4000, 0.2 M MgCl2, 0.1 M Tris buffer, pH 8.5. The crystals were cryoprotected by emersion in the well solution supplemented with 25% (volume/volume) glycerol.