I. Subcloning, purification and expression of sapB for Crystallization

Subcloning

Saposin B was subcloned into the expression vector pET28a by PCR using the forward primer (5'-AATTGCTAGCGGCGATGTTTGCCAGGATTG-3') and the reverse primer (5'-AATTGCGGCCGCTTATTCATCACAGAAGCCAACC-3'), which introduced *Nhe*1 and *Not*1 restriction sites (underlined), respectively. The PCR product was digested by the *Nhe*1 and *Not*1 restriction endonucleases and the product was purified by agarose gel and ligated into a modified pET28a vector using the *Nhe*1 and *Not*1 sites. The pET28a vector comprises an N-terminal His_6-MBP tag followed by a TEV protease cleavage site. The resulting construct containing the saposin B gene was confirmed by sequencing (Macrogen, Amsterdam).

Expression and purification

Shuffle Express E. coli cells (New England Biolabs) were transformed according to the manufacturer's protocol. Cells were grown overnight at 310 K inL^{-1} kanamycin. The temperature was lowered to 303 K and protein expression was induced by the addition of 0.5 mM IPTG. Cells were grown for 18 hr and harvested by centrifugation at 6000g for 20 min. The cell pellet was resuspended in binding buffer (50 mM Tris-HCl, pH 7.8, 150 mM NaCl) and the cells lysed by sonication on ice. After centrifugation at 20000 g for 40 min, the supernatant was collected and applied to a MBPTrap column attached to an AKTAprime system (GE Healthcare, Buckinghamshire, England). The protein was washed with 10 column volumes of binding buffer and eluted with five columns of elution buffer (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM L-maltose). TEV protease was added to the eluted protein and dialyzed overnight at 273 K against 25 mM Tris-HCl, pH 7.8 and 25 mM NaCl to remove the His_6-MBP tag. TEV protease and uncleaved protein was depleted over a 5 ml Ni-NTA column and the saposin B protein collected. The sample was then applied to a monoQ column (GE Healthcare, Buckinghamshire, England) and eluted with a salt concentration gradient from 25 to 500 mM. The pure protein eluted at approximately 200 mM NaCl. The sample was concentrated to ~10 mg/ml and applied to a Superdex 200 column (GE Healthcare, Buckinghamshire, England) pre-equilibrated with dialysis buffer. The protein eluted as a single peak with a molecular weight of ~18 kDa. Pure protein fractions were pooled and concentrated to ~15 mg mL^{-1} for crystallization screening.

Crystallization of sapB

Screening for suitable crystallization conditions was performed with the HTX crystallization robot (EMBL-Grenoble, France). All crystallization trials were done at 293 K using 2:1 (protein:reservoir) sitting drops. Rod-like crystals were obtained with 0.1 M MES buffer, pH 6, and 30% PEG 6000. For co-crystallization experiments, chloroquine diphosphate was dissolved in dialysis buffer (25 mM Tris buffer, pH 7.8, 25 mM NaCl) to a final concentration of 50 mM. Sitting drops containing 2 μL of

protein solution, 1 µL reservoir solution and 0.5 µL chloroquine diphosphate solution yielded well-diffracting crystals within 24-48 h with dimensions of 100 x 20 x 20 microns.

Data collection and processing

For cryoprotection, crystals were soaked for a few seconds in reservoir solution containing 15%(v/v) glycerol. The crystals were mounted in a cryo loop and subsequently flash-frozen in liquid nitrogen. X-ray diffraction data were collected at 100 K on beam line ID29 at the European Synchrotron Radiation Facility (Grenoble, France). The crystal diffracted to a resolution of 2.12 Å. The data was processed and integrated using the program XDS and scaled using XSCALE (Kabsch, 2010). The structure was phased using molecular replacement with the program *Phaser* (McCoy *et al*., 2007) and the structure of saposin B (PDB code 1N69) as the search model. Manual rebuilding was performed in COOT (Emsley *et al*., 2010). All refinements were performed using BUSTER (Bricogne 2011). Final Ramachandran statistics were 99.1% favoured and 0.9% allowed. The structure is deposited under PDB code 4V2O.

II. Subcloning, purification and expression of sapB E69A

Subcloning

E69A was subcloned into the expression vector pET27b+ by PCR using the forward primer (5′-GACTGGATCCATGGATGGCGATG-3′) and the reverse primer (5′-CGTCCTCGAGTTCATCACAGAAGCC-3′), which introduced *Nco*1 and *Xho*1 restriction sites (underlined), respectively. The PCR product was digested by the *Nco*1 and *Xho*1 restriction endonucleases and the product was purified by agarose gel and ligated into a modified pET27b+ vector using the *Nco*1 and *Xho*1 sites. The pET27b+ vector comprises an N-terminal His_6 tag. The resulting construct containing the E69A gene was confirmed by sequencing (Genewiz, New York).

Expression and purification

Chemically competent BL21 DE3(Gold) *E coli* cells (New England Biolabs) were transformed according to the manufacturer's protocol. Cells were grown overnight at 310 K in Luria-Bertani medium to an optical density at 600 nm (OD600) of 0.4 to 0.6 in the presence of 50 µg mL^{-1} kanamycin. The temperature was lowered to 303 K and protein expression was induced by the addition of 0.01 mM IPTG. Cells were grown for 18 hours and harvested by centrifugation at 4000g for 60 min. The medium was collected and filtered and applied to a Ni_NTA column attached to an

AKTAprime system (GE Healthcare). The pure protein eluted at approximately 100 mM Imidazole. The sample was dialyzed in pH 5.5 and pH 7.4 dialysis buffers respectively. The protein was then lyophilized and kept in -80 °C fridge for future analysis.

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