Online supplementary data for manuscript number 49299

Supplementary materials and methods

Protein purification and crystallization

Full length SelB (residues 1 to 468) was cloned from *M. maripaludis* strain JJ (DSM 2067), expressed as an N-terminal 6xHis-fusion protein in *E. coli* and purified by affinity chromatography. SelB was further purified by ion exchange chromatography and transferred into crystallization buffer [10 mM KCl, 20 mM Tris/HCl (pH 7.5), 40 mM $MgSO_4$, 2 mM DTT] by gel exclusion chromatography. After addition of 3 mM GDP, SelB:GDP crystals were grown at 20 ºC by sitting drop vapour diffusion against 100 mM sodium citrate (pH 6.0), 1.8 - 2.0 M ammonium sulfate and 1 mM deoxycholic acid, using a protein concentration of ~ 12 mg/ml. The addition of deoxycholic acid dramatically improved the crystal quality. The crystals were stabilized in a buffer containing 26 % (w/v) sucrose and 3 mM GDP.

For the cocrystallization of SelB with GppNHp, 3 mM GppNHp was added to the protein solution. SelB:GppHNp crystals grew under the same conditions as SelB:GDP crystals and were morphologically similar. They had similar crystallographic properties but diffracted to lower resolution (less than 3.5 Å). This problem could be overcome by soaking GDP-grown crystals with a high concentration of GppNHp (20 mM) during stabilization. The procedure was validated by comparing both SelB:GppNHp crystal forms: The data were isomorphous (8.7 % cross R factor) and the F_o-F_o fourier difference map did not indicate any differences. Furthermore, the F_o-F_o difference fourier maps with SelB:GDP revealed a similar GDP→GTP shift of the Switch 2 helix in molecule A for both SelB:GppNHp crystal forms. Therefore, the data of a GppNHp-soaked crystal was used for structure determination.

SelB-apo crystals were obtained under the same crystallization conditions as SelB:GDP crystals, but by omitting the nucleotide from the crystallization solution. Alternatively, GDP was omitted during stabilization of GDP-grown crystals. Both crystal forms displayed similar crystallographic properties (cross R factor = 9.4%), and the F_o-F_o fourier map did not indicate any structural differences. Likewise, the difference fourier map with the SelB:GDP data revealed the absence of GDP for both SelB-apo crystal types. As the GDP-grown, back-soaked crystals had better diffraction properties (3.1 Å vs 3.4 Å), this form was used for structure determination.

After stabilization of the different SelB crystal forms, they were derivatized by soaking overnight with 0.25 mM Ta_6Br_{12} (SelB:GDP) or saturated methyl mercury chloride (SelB-apo, SelB:GDP and SelB:GppNHp) and flash frozen in liquid propane.

Data collection and structure determination

Ta MAD data and Hg SAD data were collected at three X-ray wavelengths of the Ta L-III absorption edge (peak: λ_1; inflection: λ_2; remote: λ_3) or remote of the Hg L-III absorption edge on the X06SA-PX beamline at SLS (PSI Villingen, Switzerland) using a 165mm MarCCD detector. The data were processed with the HKL software package (Otwinowski and Minor, 1997). Crystals belong to space group $P3_112$ with unit cell dimensions of a = b = 145.5 Å and c = 295.5 Å (Ta_6Br_{12}:SelB:GDP), a = b = 146.8 Å and c = 297.3 Å (MeHg-SelB:GDP), a = b = 146.8 Å and c = 298.0 Å (MeHg-SelB-apo, cocrystallized form), a = b = 146.8 Å and c = 297.2 Å (MeHg-SelB-apo, back-soaked form), a = b = 146.4 Å and c = 296.8 Å (MeHg-SelB:GppNHp, cocrystallized form) and a = b = 146.7 Å and c = 297.0 Å (MeHg-SelB:GppNHp, soaked form). Treating the Ta_6Br_{12} cluster as a point scatterer at 5.0 Å resolution, six sites were found using the program SOLVE (Terwilliger,

2004). After preliminary Ta MAD phasing and solvent flipping with CNS to 4.3 Å (Brünger *et al*, 1998), the anomalous difference fourier map using the Ta phases and Hg amplitudes revealed 16 Hg positions, corresponding to four molecules in the asymmetric unit (molecules A, B, C and D). The best experimental Hg SAD phases were obtained to 3.0 Å (SelB:GDP) , 3.2 Å (SelB:GppNHp) and 3.1 Å (SelB-apo) using CNS for phasing and solvent flipping (71% solvent content). For SelB:GDP, ~75% of the polypeptide chains could be built manually into the electron density map using O (Jones *et al*, 1991). The partial model was refined and completed by the calculation of electron density maps with combined phases and difference Fourier syntheses using CNS. The current model of SelB:GDP consists of residues -1 - 29 and 50 - 468 (molecule A), 1 - 30 and 38 - 388 (molecule B), -4 - 468 (molecule C), and 5 - 31, 51 - 65, 71 - 317, 325-328, and 336 - 387 (molecule D), with residues from the N-terminal His-tag numbered negatively to zero. In addition, the model contains 3 GDP:Mg^{2+}, 7 deoxycholic acid and 8 SO_4^{2-} molecules. The analysis of the model with PROCHECK (Laskowski *et al*, 1993) revealed an excellent stereochemistry. In the Ramachandran plot, 96.1 % of the residues were in most favoured/additionally allowed regions and 3.9 % in generously allowed regions, with no outliers.

The SelB-apo structure was solved using the atomic coordinates of SelB:GDP as a starting model. The nearly perfect fit with the experimental SelB-apo Hg SAD electron density revealed a high degree of structural isomorpism. Using CNS, the model was refined by an initial cycle of rigid body refinement using individual domains of all four molecules. It was completed by alternating cycles of energy minimization and model rebuilding after calculation of electron density maps with combined phases and difference Fourier syntheses. The current model of SelB-apo includes residues -1 - 30 and 50 - 468 (molecule A), 1 - 30 and 38 - 388 (molecule B),

-4 - 468 (molecule C), and 5 - 31, 51 - 65, 71 - 317, 325 - 328 and 336 - 387 (molecule D), 7 deoxycholic acid and 9 SO_4^{2-} molecules. The Ramachandran plot reveals 96.8 % of the residues in most favoured/additionally allowed and 3.2 % in generously allowed regions, with no outliers.

The structure of SelB:GppNHp was determined using the atomic coordinates of SelB:GDP as a starting model. The initial fit into the experimental SelB:GppNHp Hg SAD electron density revealed slight domain shifts and a significant rearrangement of the Switch 2 region in molecule A. This region was rebuilt manually using O. Following rigid body refinement with the remaining parts of the molecules using individual SelB domains, the model was completed by several cycles of energy minimization and rebuilding after the calculation of electron density maps with combined phases and difference Fourier maps. It contains residues -1 - 29 and 49 - 468 (molecule A), 1 - 29 and 38 - 388 (molecule B), -4 - 468 (molecule C), and 5 - 31, 51 - 65, 71 - 317, 325 - 328 and 336 - 387 (molecule D), 2 GppNHp:Mg^{2+}, 7 deoxycholic acid and 6 SO_4^{2-} molecules. In molecules A and D, several stretches were traced only as Cα due to high B factors of the side chains. The Ramachandran plot reveals an excellent stereochemistry with 95.6 % of the residues in most favoured/additionally allowed and 4.4 % in generously allowed regions, with no outliers.

Mutational analysis of *E. coli* SelB

Point mutations were introduced into a constitutively expressed, 6xHis tagged *E. coli selB* allele (Thanbichler and Böck, 2003) located on plasmid pMB by inverse PCR, which resulted in pMB variants listed in Table II. After confirming the mutations by sequencing, the plasmids were transferred into strains PT91300 (Tormay

et al, 1996) and AF90422 (F^-, *araD139*, Δ*(argF-lac)U169*, *ptsF25, deoC1*, *relA1*, *flbB5301*, *strA1*, Δ*selAB*; A. Friebel, unpublished) bearing plasmid pWT, and the cells were grown to stationary phase. Then the cell suspensions were diluted 1:100 in TP medium (Heider *et al*, 1992) and cultivated aerobically at 37 ºC to an OD_{600} of about 1.5. After cooling the cultures on ice, β-galactosidase activities were determined as described by Miller (Miller, 1972). Parallel experiments were performed with the wild-type (pMB) and empty (pMT7) plasmids, which served as positive and negative controls, respectively.

For the SelB expression tests, cells were pelleted by centrifugation and lysed in SDS sample buffer by heating. After separating the proteins on an SDS-PAGE gel, they were transferred onto a nitrocellulose membrane (BioTrace NT; Pall Corporation, Ann Arbor, MI) and probed with affinity purified anti-SelB antibodies. Immunocomplexes were detected by chemiluminescence using horseradish peroxidase–protein A conjugate from Bio-Rad Laboratories (Munich, Germany) and the ECL system from Roche Biochemicals (Penzberg, Germany).

Supplementary references for materials and methods

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