

11 β -HSD1

PDB:2BEL

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:gi:5031765

Entry Clone Source:synthetic

SGC Clone Accession:

Tag:N terminus: mgsshhhhhhssgrenlyfq*gh. C terminus: gs

Host:BL21(DE3)

Construct

Prelude:

Sequence:

Vector:p11

Growth

Medium:

Antibiotics:

Procedure:The 11 β -HSD1 construct was expressed in the E. coli expression strain BL21(DE3), in TB medium with a plasmid encoding the chaperone GroEL/ES, under control of the arabinose promoter (Takara, Japan). High-level soluble protein production was achieved by IPTG induction (3 mM) at 18°C for 12 hrs in the presence of 1% sucrose, 1% glucose, 0.05% arabinose and 100 μ M carbenoxolone (CBX, 3 β -hydroxy-11-oxoolean-12-en-30-oic acid, 3-hemisuccinate, Sigma).

Purification

Procedure

Extraction

Procedure

Collected/resuspended cells (50 mM Tris/Cl, 300 mM NaCl, 5 mM Imidazole, 2 mM TCEP, 0.5 μ M CBX, 5% (w/v) glycerol, pH8.0) were disrupted in a high-pressure homogenizer, the supernatant after centrifugation at 15000 g for 40 min of the cell lysate was subjected to immobilized metal affinity chromatography, followed by incubation overnight in 0.05% Triton X-

100 (Anapoe X-100; Anatrace, Maumee, OH), 20 μ M CBX, 50 mM Tris/Cl, pH 8.0, 300 mM NaCl, 2 mM TCEP, 0.5 mM MgCl₂, 2 mM ATP and a final gel filtration chromatographic step on a Superdex 200 HiLoad 16/60 column (GE Healthcare, Uppsala, Sweden). The protein was concentrated in 20 mM Tris-HCl, pH 8.0, containing 2mM TCEP, 5% (w/v) glycerol, 0.05 % (w/v) Triton X-100, and 2 μ M of the inhibitor to around 12 mg/mL using a 30000 kDa MW cutoff Amicon Ultra concentration device (Millipore, Bedford, MA).

Concentration: 12 mg/mL

Ligand

Carbenoxolone and NADP+**MassSpec:**observed mass 31064 Da, theoretical mass 31063.9 Da

Crystallization:Crystals were grown using the hanging-drop vapor diffusion technique. 2 mL drops containing equal volumes of protein solution and a buffer solution composed of 200 mM MgCl₂, 17% PEG 3350, 100 mM Bis-Tris, pH 5.5 were equilibrated against a well containing 300 μ l of the buffer solution. Crystals reached a size of ~200 microns over a period of 3-7 days.

NMR Spectroscopy:

Data Collection:

Data Processing: