Molecular Biology

Entry Clone Accession: MGC:9490 IMAGE:3922902; note this clone harbours the p.Asn314Asp polymorphism
Entry Clone Source: MGC
SGC Construct ID: GALTA-c023 (A21Y:A22T:T23P:R25L mutation of GALTA-c001)
Protein Region: M1-A379
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMSRSGTDPQQRQQASEADAAYTPFLANDHQHIRYNP
LQDEWVLVSAHRMKRPWQGQVEPQLLKTVPRHDPLNPLCPGAIRANGEVNPQYDSTFL
FDNDFPALQPDAPSPGPSDHPLFQAKSARGVCKVMCFHPWSDVTLPLMSVPEIRAVVDA
WASVTEELGAQYPWVQIFENKGAMMGCSNPHPHCQVWASSFLPDIAQREERSQQAYKS
QHGEPLLMEYSRQELLRKERLVLTSEHWLVLVPFWATWPYQTLLLPRRHVRRLPELTPAE
RDDLASIMKKLLTKYDNLFETSFPYSMGWHGAPTGSEAGANWDHWQLHAHYYPPLLRS
ATVRKFMVGYEMLAQAQRDLTPEQAAERLRALPEVHYHLGQKDRETATIA
Sequence after tag cleavage:
SMSRSGTDPQQRQQASEADAAYTPFLANDHQHIRYNPLQDEWVLVSAHRMKRPWQGQ
VEPQLLKTVPRHDPLNPLCPGAIRANGEVNPQYDSTFLFDNDFPALQPDAPSPGPSDHPLF
QAKSARGVCKVMCFHPWSDVTLPLMSVPEIRAVVDAWASVTEELGAQYPWVQIFENKG
AMMGCSNPHPHCQVWASSFLPDIAQREERSQQAYKSQHGEPLLMEYSRQELLRKERLVL
TSEHWLVLVPFWATWPYQTLLLPRRHVRRLPELTPAERDDLASIMKKLLTKYDNLFETSF
PYSMGWHGAPTGSEAGANWDHWQLHAHYYPPLLRSATVRKFMVGYEMLAQAQRDLT
PEQAAERLRALPEVHYHLGQKDRETATIA

Note: this sequence represents a crystal epitope variant (A21Y:A22T:T23P:R25L) of the GALT protein

DNA Sequence:
ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGTCGCGCAGTGGAACCGATCCTCAGCAACGCCAGCAGGCGTCAGAGGC
GGACGCCGCATACACCCCTTTCCTGGCAAACGACCATCAGCATATCCGCTACAACCCG
CTGCAGGATGAGTGGGTGCTGGTGTCAGCTCACCGCATGAAGCGGCCCTGGCAGGGT
CAAGTGGAGCCCCAGCTTCTGAAGACAGTGCCCCGCCATGACCCTCTCAACCCTCTG
TGTCCTGGGGCCATCCGAGCCAACGGAGAGGTGAATCCCCAGTACGATAGCACCTTC
CTGTTTGACAACGACTTCCCAGCTCTGCAGCCTGATGCCCCCAGTCCAGGACCCAGT
GATCATCCCCTTTTCCAAGCAAAGTCTGCTCGAGGAGTCTGTAAGGTCATGTGCTTCC
ACCCCTGGTCGGATGTAACGCTGCCACTCATGTCGGTCCCTGAGATCCGGGCTGTTGT
TGATGCATGGGCCTCAGTCACAGAGGAGCTGGGTGCCCAGTACCCTTGGGTGCAGAT
CTTTGAAAACAAAGGTGCCATGATGGGCTGTTCTAACCCCCACCCCCACTGCCAGGTA
TGGGCCAGCAGTTTCCTGCCAGATATTGCCCAGCGTGAGGAGCGATCTCAGCAGGCC
TATAAGAGTCAGCATGGAGAGCCCCTGCTAATGGAGTACAGCCGCCAGGAGCTACTC
AGGAAGGAACGTCTGGTCCTAACCAGTGAGCACTGGTTAGTACTGGTCCCCTTCTGG
GCAACATGGCCCTACCAGACACTGCTGCTGCCCCGTCGGCATGTGCGGCGGCTACCTG
AGCTGACCCCTGCTGAGCGTGATGATCTAGCCTCCATCATGAAGAAGCTCTTGACCAA
GTATGACAACCTCTTTGAGACGTCCTTTCCCTACTCCATGGGCTGGCATGGGGCTCCC
ACAGGATCAGAGGCTGGGGCCAACTGGGACCATTGGCAGCTGCACGCTCATTACTAC
CCTCCGCTCCTGCGCTCTGCCACTGTCCGGAAATTCATGGTTGGCTACGAAATGCTTG
CTCAGGCTCAGAGGGACCTCACCCCTGAGCAGGCTGCAGAGAGACTAAGGGCACTT
CCTGAGGTTCATTACCACCTGGGGCAGAAGGACAGGGAGACAGCAACCATCGCCTGA

Protein Expression

Medium: Auto induction Terrific Broth (50 g ForMedium AIM-TB, 20 g glycerol and water to 1 L) was autoclaved. After autoclaving 1 mL of 10 % Antifoam 204 (in ethanol) was added.
Antibiotics: Kanamycin (50 µg/mL) and Chloramphenicol (35 µg/mL)
Procedure: A 10 mL LB o/n culture was grown and used to inoculate 1 L of AIM-TB in a 2.5 L Ultra Yield baffled flask. Cells were grown for 4 h at 37 °C 250 rpm shaking and then for a further 40 h at 18 °C. Cells were harvested by centrifugation at 4,000 g for 20 minutes.

Protein Purification

Procedure: 3 mL of Base Buffer was added per g of cell pellet (10 mM HEPES, 500 mM NaCl, 5 % Glycerol, 0.5 mM TCEP, pH 7.5) + 0.5 mg/mL lysozyme, 1 µg/mL benzonase, 1 % Triton X-100, 30 mM imidazole. After stirring for 30 minutes at room temperature cells were frozen at -80 °C for 1-2 h. Cells were thawed at RT for 30 minutes and subsequently centrifuged at 4,000 g for 1 h at 4 °C. 40 mL of SN was then applied to a 1 mL His GraviTrap column (GE healthcare) fitted with a LabMate extender. The column was then washed twice with 10 mL of Base Buffer + 30 mM imidazole. The His GraviTrap column was then slotted into a PD-10 column fitted with a LabMate extender and the protein eluted with 2.5 mL of Base Buffer + 500 mM imidazole. The His GraviTrap columne was then removed and protein eluted from the PD-10 with 3.5 mL of Base Buffer + 30 mM imidazole. 1 part TEV was then added to 10 parts GALTA and the sample incubated o/n at 4 °C. . Cleaved protein was then run back over the His GraviTrap column to remove TEV and any un-cleaved protein. Protein was then concentrated to 20-40 mg/mL and run on a Bio-Rad SEC 650 gel filtration column using Base Buffer as the mobile phase. Peak fractions were pooled and concentrated to 15.1 mg/mL.
Concentration: 15.1 mg/ml
Mass-spec Verification: Yes, Expected 43526.5 Da, Observed 43526.1

Structure Determination

Data Collection: X-ray collection (SAD); *Beamline:* Dmnd I04-1; *Resolution:* 1.52 Å

Crystallization and Data Processing: Crystals were obtained after 2 weeks at 4 ^{o}C in reservoir solution containing 0.2M ammonium sulphate and 30% PEG4000. Crystals were cryo-protected with reservoir solution supplemented with 25% (v/v) ethylene glycol and flash-cooled in liquid nitrogen. The structures were solved by molecular replacement with PHASER, using the structure of *E. coli* GALT (1HXP) as template. Modelling and refinement were carried out using Refmac and Coot.