Molecular Biology

Entry Clone Accession: IMAGE:5744956
Entry Clone Source: MGC
SGC Construct ID: HMGCS2A-c010
Protein Region: P51-V508
Vector: pNH-TrxT
Tag: N-6HIS;N-TRX;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGMSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADE
YQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLA
GTENLYFQSMPKDVGILALEVYFPAQYVDQTDLEKYNNVEAGKYTVGLGQTRMGFCSV
QEDINSLCLTVVQRLMERIQLPWDSVGRLEVGTETIIDKSKAVKTVLMELFQDSGNTDIE
GIDTTNACYGGTASLFNAANWMESSSWDGRYAMVVCGDIAVYPSGNARPTGGAGAVA
MLIGPKAPLALERGLRGTHMENVYDFYKPNLASEYPIVDGKLSIQCYLRALDRCYTSYR
KKIQNQWKQAGSDRPFTLDDLQYMIFHTPFCKMVQKSLARLMFNDFLSASSDTQTSLYK
GLEAFGGLKLEDTYTNKDLDKALLKASQDMFDKKTKASLYLSTHNGNMYTSSLYGCLA
SLLSHHSAQELAGSRIGAFSYGSGLAASFFSFRVSQDAAPGSPLDKLVSSTSDLPKRLASR
KCVSPEEFTEIMNQREQFYHKVNFSPPGDTNSLFPGTWYLERVDEQHRRKYARRPV
Sequence after tag cleavage:
SMPKDVGILALEVYFPAQYVDQTDLEKYNNVEAGKYTVGLGQTRMGFCSVQEDINSLC
LTVVQRLMERIQLPWDSVGRLEVGTETIIDKSKAVKTVLMELFQDSGNTDIEGIDTTNAC
YGGTASLFNAANWMESSSWDGRYAMVVCGDIAVYPSGNARPTGGAGAVAMLIGPKAPL
ALERGLRGTHMENVYDFYKPNLASEYPIVDGKLSIQCYLRALDRCYTSYRKKIQNQWK
QAGSDRPFTLDDLQYMIFHTPFCKMVQKSLARLMFNDFLSASSDTQTSLYKGLEAFGGL
KLEDTYTNKDLDKALLKASQDMFDKKTKASLYLSTHNGNMYTSSLYGCLASLLSHHSA
QELAGSRIGAFSYGSGLAASFFSFRVSQDAAPGSPLDKLVSSTSDLPKRLASRKCVSPEEF
TEIMNQREQFYHKVNFSPPGDTNSLFPGTWYLERVDEQHRRKYARRPV
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTATGAGCGATAAAATTATTCACCTGACT
GACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTCGATTTCT
GGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTG
ACGAATATCAGGGCAAACTGACCGTTGCAAAACTGAACATCGATCAAAACCCTGGCA
CTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGCTGCTGTTCAAAAACGGTGA
AGTGGCGGCAACCAAAGTGGGCGCACTGTCTAAAGGTCAGTTGAAAGAGTTCCTCG
ACGCTAACCTGGCCGGTACCGAGAACTTGTACTTCCAATCCATGCCAAAGGACGTGG
GCATCCTGGCCCTGGAGGTCTACTTCCCAGCCCAATATGTGGACCAAACTGACCTGGA
GAAGTATAACAATGTGGAAGCAGGAAAGTATACAGTGGGCTTGGGCCAGACCCGTAT
GGGCTTCTGCTCAGTCCAAGAGGACATCAACTCCCTGTGCCTGACGGTGGTGCAACG
GCTGATGGAGCGCATACAGCTCCCATGGGACTCTGTGGGCAGGCTGGAAGTAGGCAC
TGAGACCATCATTGACAAGTCCAAAGCTGTCAAAACAGTGCTCATGGAACTCTTCCA
GGATTCAGGCAATACTGATATTGAGGGCATAGATACCACCAATGCCTGCTACGGTGGTA
CTGCCTCCCTCTTCAATGCTGCCAACTGGATGGAGTCCAGTTCCTGGGATGGTCGTTAT
GCCATGGTGGTCTGTGGAGACATTGCCGTCTATCCCAGTGGTAATGCTCGTCCCACAG
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CTCGGAGTACCCAATAGTGGATGGGAAGCTTTCCATCCAGTGCTACTTGCGGGCCTTG
GATCGATGTTACACATCATACCGTAAAAAAATCCAGAATCAGTGGAAGCAAGCTGGCA
GCGATCGACCCTTCACCCTTGACGATTTACAGTACATGATCTTTCATACACCCTTTTGC
AAGATGGTCCAGAAGTCTCTGGCTCGCCTGATGTTCAATGACTTCCTGTCAGCCAGCA
GTGACACACAAACCAGCTTATATAAGGGGCTGGAGGCTTTCGGGGGGCTAAAGCTGG

AAGACACCTACACCAACAAGGACCTGGATAAAGCACTTCTAAAGGCCTCTCAGGACA
TGTTCGACAAGAAAACCAAGGCTTCCCTTTACCTCTCCACTCACAATGGGAACATGTA
CACCTCATCCCTGTACGGGTGCCTGGCCTCGCTTCTGTCCCACCACTCTGCCCAAGAA
CTGGCTGGCTCCAGGATTGGTGCCTTCTCTTATGGCTCTGGTTTAGCAGCAAGTTTCTT
TTCATTTCGAGTATCCCAGGATGCTGCTCCAGGCTCTCCCCTGGACAAGTTGGTGTCC
AGCACATCAGACCTGCCAAAACGCCTAGCCTCCCGAAAGTGTGTGTCTCCTGAGGAG
TTCACAGAAATAATGAACCAAAGAGAGCAATTCTACCATAAGGTGAATTTCTCCCCAC
CTGGTGACACAAACAGCCTTTTCCCAGGTACTTGGTACCTGGAGCGAGTGGACGAGC
AGCATCGCCGAAAGTATGCCCGGCGTCCCGTCTAACAGTAAAGGTGGATACGGATCCG
AATTCGAGCTCCGTCGACAAGCTT

Protein Expression

Medium: TB
Antibiotics: Kanamycin
Procedure: The plasmid was transformed into E. coli BL21(DE3)-R3, cultured in 1 L Terrific Broth at 37°C to OD600 ~1.5, and induced with 0.5 mM IPTG overnight at 18oC. Harvested cells were homogenized in buffer A (50 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 20 mM imidazole) and centrifuged.

Protein Purification

Procedure: The supernatant was applied to Ni-sepharose resin pre-equilibrated with buffer A, and eluted with buffer B (buffer A + 230 mM imidazole). The eluant was applied to Superdex S200 column pre-equilibrated with buffer GF (10 mM Hepes pH 7.5, 500 mM NaCl, 5% (w/v) glycerol, 0.5 mM TCEP). To remove fusion tag, purified protein was treated with His-tagged TEV protease overnight at 4°C, and passed over Ni-sepharose resin equilibrated with buffer GF. Protein was concentrated to 12 mg/ml (*h*HMGCS2).
Concentration: 12.0 mg/ml
Mass-spec Verification: yes

Structure Determination

Crystallization: Protein was pre-incubated with 5 mM Ac-CoA and 5 mM AcAc-CoA prior to crystallization. Sitting drops containing 100 nL protein and 50 nL reservoir solution (0.2 M ammonium sulphate, 0.1 M Bis-Tris pH 6.5, 25% (*w/v*) PEG 3350) were equilibrated at 20°C. Crystals were cryo-protected using 25% glycerol and flash-cooled in liquid nitrogen. Diffraction data were collected at the SLS beamline X10S, and processed using the CCP4 program suite.

Data Collection: *Beamline:* SLS-X10; *Resolution:* 1.75 Å
The structure was solved by molecular replacement using the refined *h*HMGCS1 structure as search model. Automated model building was performed with ARP/wARP, followed by iterative cycles of restrained refinement with medium NCS restraints and model building using COOT and PHENIX.