PHGDH

PDB:2G76

Revision

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Entry Clone Accession:PHGDHA-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:
Host:BL-21(DE3)R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMANLRKV LISDSLDPCCRKILQDGGLQVVEKQNLSK EELIAELQDCEGLIVRSATKVTADVIN
AA EKLQVVGRAGTGVDNVDLEAATRKGILVM NTPNGNSLSAAELTCGMIMCLARQIPQAT ASMKDGKWERKKFMGTELNGKTLG
ILGLG RIGREVATRMQSFGMKTIGYDPIISPEVS ASFGVQQLPLEEIWPLCDFITVHTPLLPS TTGLLNDNTFAQCKKGVRVVN
CARGGIVD EGALLRALQSGQCAGAALDVFTEEPPRDR ALVDHENVISCPHLGASTKEAQSRCGEEI AVQFVDMVKGKSLTGV

Vector:pNIC28-Bsa4.

Growth

Medium:
Antibiotics:
Procedure: Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 50 mg/ml kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 ml TB + 50 mg/ml kanamycin. These were grown overnight and glycerol stocks prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, mixing and then storing in a -80°C freezer.
The glycerol stock was used to innoculate 10 mls of TB + 50 mg/ml kanamycin which was grown overnight at 37°C as a starter culture for a 1 litre growth. The large scale growth was grown at 37°C until approximately 30 mins before induction when the temperature was lowered to 25°C. Protein production was induced with the addition of 1mM IPTG. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The pellet was then stored in the -80°C freezer.

Purification

Procedure

Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham)
The cell extract was loaded on the column at 0.8 ml/min on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Affinity Binding Buffer, 10 column volumes of Affinity Wash Buffer, and then eluted with Affinity Elution Buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected

Column 2: Gel filtration, Hiload 16/60, S75 16/60 - 120 ml
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions. Using a Centricon 10 K cutoff, The concentration of PHGDHA-c012 was measured and concentrated to a 15 mg/ml. The concentrated protein was aliquoted into 100 µl volumes before freezing in the -80°C freezer.

Extraction

Procedure
The pellet (14.73 gms) was resuspended with Extraction Buffer (approximately 120 mls final) by intermitently placing the pellet in a 37 o C water bath and vortexing. Once resuspended the cells were (1) broken by one passage through the Constant Systems cell breaker; (2) sonicating; (3) DNA precipitation with the addition of PEI to a final concentration of 0.15 % for 30 mins on ice followed by a 17,000 rpm at 4°C to remove precipitation; (4) the supernatant was filtered through 0.45 µm serum acrodiscs.
Concentration:
Ligand
MassSpec: The expected mass of PHGDHA-c012 with histidine is 36060. The experimentally determined mass was 36060.
Crystallization:5 mM of NAD+ were added to the protein. Crystals grew from a 1:2 ratio mix of protein-to-reservoir (0.1M MMT, 7.0, 30 % PEG 1k). Crystal x014, used for data collection, was stabilized by adding a solution containing 20 % EtGly + stock solution.
NMR Spectroscopy:
Data Collection: Resolution: 1.7Å; X-ray source: Synchrotron SLS -X10SA, single wavelength (0.99806A)
Data Processing: