Pk-SOD: Plasmodium knowlesi superoxide dismutase

PDB:2AWP

Revision

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Entry Clone Accession:PKH_142350
Entry Clone Source:*P. knowlesi* strain H gDNA
SGC Clone Accession:PKN-PF08_0071; plate MAC008:D1
Tag:N-terminal His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

gMAIILPKLKYALNAMAIILPKLKYALNALSPHISEETLNFHYNKHHAGYVNKLNGLIKDTPFATKSLVEIMKESTGAIFNNAAQIW
NHSFYWDSMGPNCGGEPHGEIKEKIQEDFGSFNNFKNEFSNVLCGHFGSGWGWLVLNNNNKLVILQTHDAGNPIKDNTGIPILTCDI
WEHAYYIDYRNDRPSYVKAWWNLVNWNFANENLKKALQK

Vector:p15TvL

Growth

Medium: Terrific Broth (TB)
Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~2.5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared cell lysate was loaded onto a column containing 10 g DE-52 resin (Whatman) anion exchangeresin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer), and then directly onto a 3 mL Ni-NTA (Qiagen) column at approximately 1.5 mL/min. When all the lysate was loaded, both columns were washed with 20 mL Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer After washing, the protein was eluted from the Ni-

NTA column with 15-20 mL of Elution Buffer.EDTA was added immediately to 1 mM.DTT was added to 1 mM 15 minutes later.The eluted protein was put in a dialysis cassette (Pierce) for overnight dialysis in Crystal Buffer. The following day they were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Protein concentration was estimated by taking absorbance at OD280. Finally aliquots of the purified protein were labeled and stored at -80degC.

The His-tag was cleaved with TEV protease overnight at 4degC in the presence of 1mM DTT. The cleaved sample was applied to a 1ml Ni-NTA column pre-equilibrated with Binding buffer. The flow-through was collected; and the column was rinsed with an additional 5 mL of Binding buffer. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The cleaved, concentrated protein was flash frozen and stored at -80 ºC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18,000 psi. The cell lysate was centrifuged at ~75,000 x g for 20 minutes at 10 degC.
Concentration:30 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a 24-well Linbro plate. The plate was set with 1.5 microL uncleaved protein (27 mg/mL) with MnCl2 added to 5 mM and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals grew overnight in 30% PEG 4000, 0.2M NH4AC, 0.1M NaCaco and pH 5.1 at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: