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Crystal Structure Analysis of a Putative Oxidoreductase from *Klebsiella pneumoniae*

Mohammad Baig, Ann Brown, S. Eswaramoorthy and S. Swaminathan

ABSTRACT

Klebsiella pneumoniae, a gram-negative enteric bacterium, is found in nosocomial infections which are acquired during hospital stays for about 10% of hospital patients in the United States. The crystal structure of a putative oxidoreductase from *K. pneumoniae* has been determined. The structural information of this *K. pneumoniae* protein was used to understand its function. Crystals of the putative oxidoreductase enzyme were obtained by the sitting drop vapor diffusion method using Polyethylene glycol (PEG) 3350, Bis-Tris buffer, pH 5.5 as precipitant. These crystals were used to collect X-ray data at beam line X12C of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL). The crystal structure was determined using the SHELX program and refined with CNS 1.1. This protein, which is involved in the catalysis of an oxidation-reduction (redox) reaction, has an alpha/beta structure. It utilizes nicotinamide adenine dinucleotide phosphate (NADP) or nicotine adenine dinucleotide (NAD) to perform its function. This structure could be used to determine the active and co-factor binding sites of the protein, information that could help pharmaceutical companies in drug design and in determining the protein's relationship to disease treatment such as that for pneumonia and other related pathologies.

Introduction

Brookhaven National Laboratory (BNL), a U.S. Department of Energy (DOE)-sponsored research center located in Long Island, NY, provides research opportunities for scientists who conduct advanced research. A major interest of study at the BNL Structural Biology Laboratory is the determination of the crystallographic structure of bio-medically important target-proteins obtained from the New York GenomiX Research Consortium (NYSGXRC), a collaborative initiative with several major universities and research institutes. The research focus area at this laboratory is to use the three-dimensional structure of the target proteins to determine their functions [1]. The three-dimensional structure of a protein follows the theory of evolution, which predicts the homology of specific proteins. Proteins that show similar amino acid sequences have evolutionary commonalities and form large families of proteins with common characteristics. "By studying protein folds and families, scientists are able to reconstruct the evolutionary relationship between two species and to estimate the time of divergence between two organisms, since they last shared a common ancestry" [2]. Once the three-dimensional structure is determined, researchers can decipher the family of that specific protein based on the atomic configuration.

Klebsiella pneumoniae is a gram-negative bacterium and produces an enzyme, a putative oxidoreductase, which belongs to a family of oxidoreductases that catalyze the transfer of electrons

from one molecule to another [3]. In this study, we first produced the required crystals of the enzyme and then used them to collect X-ray diffraction data at BNL's National Synchrotron Light Source (NSLS). The data were used to determine the three-dimensional structure of the protein.

Materials and Methods

Purified protein was provided by the NYSGXRC consortium. It was crystallized using the sitting drop vapor diffusion method as follows. A droplet containing equal volumes (2 µl each) of purified protein and a precipitant was allowed to equilibrate against a large reservoir (100 µl) containing the same precipitant. Initially the droplet of protein solution contained an insufficient concentration of precipitant for crystallization, but as water vaporized from the drop and transferred to the reservoir, the precipitant concentration in the droplet increased to a level optimal for crystallization. Once the system was in equilibrium, these optimal conditions were maintained until the crystallization was complete [4]. After obtaining the initial crystallization condition, crystals of this protein were obtained using the precipitant containing 0.1 M Bis-Tris pH 5.5; 25% w/v polyethylene glycol (PEG) 3350, mixed with 10 mg/ml of protein sample at a 1:1 ratio (1:1 µl) in the crystallization well and equilibrated against 100 µl of the precipitant. The protein crystals obtained are shown in Figure 1. Crystals of the protein were then bombarded with X-rays to obtain diffraction intensity data used in structure determination.

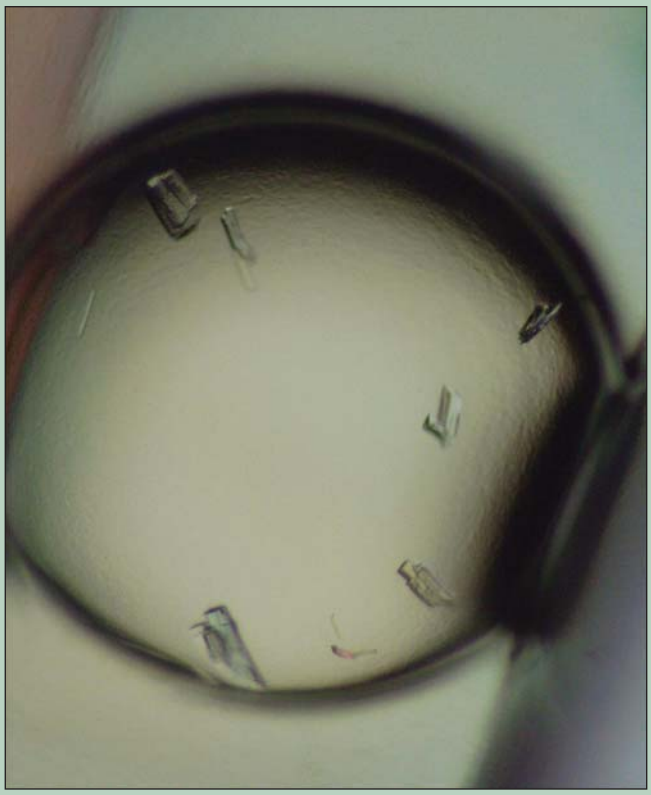

Figure 1. Crystals of the target protein. Crystals were formed in a solution containing 0.1 M Bis-Tris pH 5.5; 25% w/v polyethylene glycol (PEG) 3350 and 10 mg/ml of protein sample.

Crystals were flash frozen in liquid nitrogen using glycerol as cryoprotectant. Diffraction data at liquid N_2 temperature were collected at beam line X12C using a single wavelength (0.9795Å) X-ray beam. The crystal diffracted to a resolution of 2.0 Å. The CBASS program was used to collect data and processed with HKL2000. Crystal parameters and data collection details are shown in Table 2. The selenium position of the protein was obtained by Single Anomalous Dispersion (SAD) method using the SHELX program and phase calculation and refinement was carried out with SHARP as shown in Table 1. The protein model was built using a model building program, ARP/wARP, and a graphics program, O. Coordinates for this structure are deposited to the Protein Data Bank (Id. code: 3E82).

Protein Purification
Crystallization → Sitting drop method
Data Collection → NSLS beam line X12C
Data Processing → HKL2000
Structure Solution → SHELX
Phase Refinement → SHARP
Protein Model Building
Structure Refinement and Analysis

Table 1. Chart of protein structure determination procedure.

Number of crystals	1
X-ray source	Synchrotron (Beamline X12C)
Wavelength	0.9795 Å
Space group	$P2_1$
Unit cell parameter	a= 52.910Å α = 90° b= 79.750 Å β = 93.99° c= 209.144Å γ = 90°
Resolution	2.04Å
R-merge	0.096
Unique number of reflections collected	109371
Percentage of completeness	98.8
Redundancy	7.5
Protein molecular weight	40 kDa
Number of residues of the protein	364
R value (working set)	0.209
Free R value	0.232
RMS deviations from ideal values	
Bond lengths (Å)	0.006
Bond angles (°)	1.30

Table 2. X-ray diffraction data.

Results and Discussion

The crystal structure of the protein has been determined by the SAD method. This protein has selenium-substituted methionine (Se-Met) to facilitate anomalous dispersion data collection at the Se edge that is essential to determine the crystal structure. The crystal belongs to a monoclinic system ($P2_1$) and has two dimers in the crystallographic asymmetric unit, shown in Figure 2. The protein structure shown in Figure 3 represents the monomer. The molecule has a TIM barrel-like fold with eight helices at the outer circle and six strands inside. Sequence comparison of this 40 kDa protein shows more than 50% identity with many oxidoreducatase proteins. These proteins should utilize NADP or NAD as cofactors, however this structure showed none. Still, when the dimers were examined carefully, a groove was observed on one side which is visible in Figure 4. This groove is a potential site where a molecule like NAD could reside. Further experiments with a complex of this protein with NAD or NADP may reveal the mode of cofactor binding. In addition, a cavity exists on the other side of the observed groove that is covered with charged residues such as Arg128, Glu322 and Arg137. This site may accommodate a substrate. More work has to be done to characterize the functional mechanism of this protein.

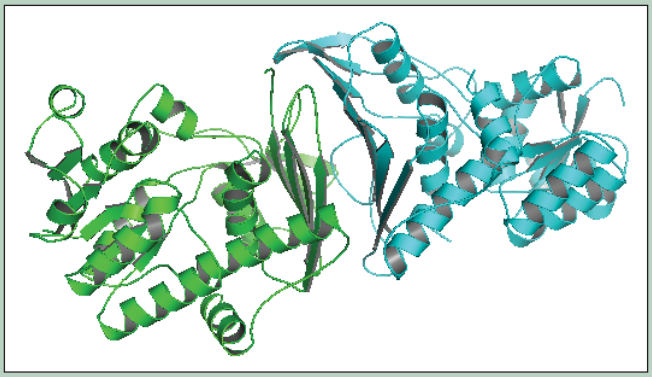

Figure 2. Ribbons representation of the dimeric association of the protein.

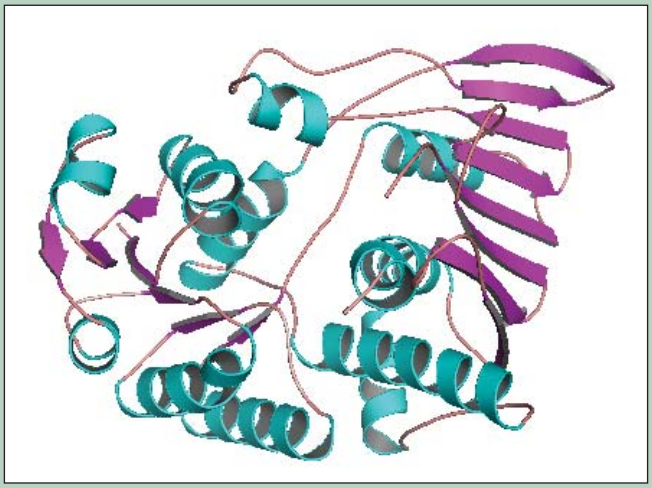

Figure 3. Ribbons representation of the fold of the putative oxidoreductase: The α-helices are represented in cyan and β-strands in purple.

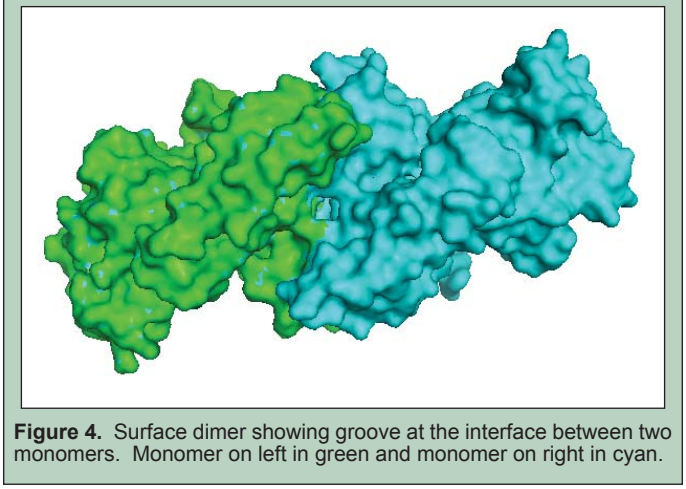

Figure 4. Surface dimer showing groove at the interface between two monomers. Monomer on left in green and monomer on right in cyan.

Conclusion

K. pneumoniae is a gram-negative bacteria that causes infection in the human intestinal tract, especially in hospital patients. The crystal structure of a putative oxidoreductase from this bacterium showed a TIM barrel fold and revealed the active site and co-factor binding sites. The three-dimensional structure of the protein plays a vital role in the protein's interaction with other molecules. This information can be used by pharmaceutical companies to design drugs that can be used to inhibit the activity of this particular protein.

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References

[1] M. Arshad, J. Levia, A. Brown, D. Kumaran and S. Swaminathan, "Crystallization and Crystallography Analysis of *Amidohydrolase* Enzyme from the Structure Genomic Project," Brookhaven National Laboratory, Tech. Rep., 2007.

[2] National Center for Biotechnology Information, "Just the Facts: A Basic Introduction to the Science Underlying NCBI Resources," 2004, http://www.ncbi.nlm.nih.gov/About/primer/bioinformatics.html.

[3] UniProt, An accessible resource of protein sequence and functional information, 2009, http://www.uniprot.org/uniprot/A6T882.

[4] J. Kogoy, Undergraduate Coursework, Davidson College, "Protein Crystallization," 2003, http://www.bio.davidson.edu/courses/MolBio/MolStudents/spring2003/Kogoy/protein.html.

[5] S. Eswaramoothy, S. Gerchman, V. Graziano, H. Kycia, F.W. Studier and S. Swaminathan, "Structure of a yeast hypothetical protein selected by a structure genomics approach," Acta crystallographic. Section D, Biological Crystallography, vol. 59,pp. 127–135, 2003.

[6] R. Agarwal, S. Eswaramoorthy, D. Kumran, T. Binz and S. Swaminathan, "Structure analysis of botulinum neurotoxin type E catalytic domain and its mutant Glu212→Glu reveals the pivotal role of the Glu212 carboxylate in the catalytic pathway," *Biochemistry*, vol. 43, pp. 6637–6644, 2004.