Structural Study of DNA Binding Protein PDCD5 from *Sulfolobus solfataricus*

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*Running title: Crystallographic analysis of PDCD5*

*Key words: hyperthermophilic archea; DNA binding protein*
Abstract

The human programmed cell death 5 (PDCD5) protein is a novel protein which is related to the regulation of cell apoptosis. The homologous enzyme from the hyperthermophilic archaea Sulfolobus solfataricus P2 was expressed in E. coli and the recombinant proteins was purified and crystallized. Structural analysis experiments showed that this protein was all helical structure. The native Sso PDCD5 crystals belong to the space group C2 with unit-cell dimensions of $a = 79.71$, $b = 36.82$, $c = 36.99$ Å, $\beta = 94.45^\circ$. Diffraction data have been collected and the structure determination is under way. Its DNA binding activity was assessed by using gel retardation assay.

1. Introduction

Sulfolobus solfataricus is an aerobic crenarchaeon that grows optimally at 80 °C and pH 2- to 4, metabolizing sulfur. The sequenced P2 strain was isolated from a sulfur hot spring near Naples, Italy. It is the most widely studied organism of the crenarchaeal branch of the Archaea and is a model for research on mechanisms of DNA replication, the cell cycle, chromosomal integration, transcription, RNA processing, and translation.

We have been working on some DNA binding proteins from hyperthermophilic archea, such as Sso7d and Sso10b2, which were structurally determined and functionally assayed in our group (Gao et al. 1998; Robinson et al. 1998; Chou et al. 2003). The Sso0352 (PDCD5) gene was predicted to
encode a hypothetical DNA binding protein which is homologous to the apoptosis-related protein TfAR19 in human leukemia cell line TF-1. Some studies indicate that recombinant human PDCD5 could accelerate apoptosis of some tumor cells (Hela, TF-1, MCF-7) (Wang et al. 2004), and translocated rapidly from the cytoplasm into the nuclei of cells during apoptosis (Liu et al. 1999; Chen et al. 2001). In order to understand the exact role of PDCD5, three-dimensional structural information is necessary. One homology protein from the archaeon Methanobacterium thermoautotrophicum, named MTH1615 was structural determined using NMR spectroscopy (Christendat et al., 2000), and was found to have N-terminal 31 residues unstructured in solution. By using heteronuclear NMR method, Liu et al., (2004) showed that human PDCD5, which shares 32.4% sequence identity with archaeon MTH1615, had a core triple-helix bundle region and two dissociated terminal regions. And it was suggested that the N-terminal residues tend to form a stable α-helical structure. Liu et al. (2005) expressed and purified a fragment of human PDCD5 containing 1-26 residues. The structural features this of human PDCD5 (1-26) N-terminal peptide showed a stable α-helix independently of the core of the protein. There is still no crystal structure of PDCD5 protein determined yet.

Proteins from hyperthermophilic microorganisms, that grow at temperature above 80 °C, share some interesting properties. They normally have increased number of charged amino acids (salt bridges), increased compactness of the molecules (loop reduction) and the firmness of the chain termini (Vieille & Zeikus, 2001). In addition, thermal stability leads to lower degradation rate and
enhanced resistance against protease digestion, making these proteins prone to be crystallized (Hennig et al., 1997). To understand the structural differences of PDCD5 homologous proteins between the hyperthermophilic archaeon *S. solfataricus* and the archaeon *M. thermoautotrophicum* and from human, which are 36% and 31% identical in amino acid sequences respectively (Figure 1a), we expressed and crystallized the protein from *S. solfataricus* and are determining the structure of *S. solfataricus* PDCD5 using multiwavelength anomalous diffraction (MAD) and Multiple Isomorphous Replacement (MIR) method.

2. Materials and methods

2.1. Cloning and protein preparation

2.1.1. Native Sso PDCD5

The *S. solfataricus* PDCD5 gene that encodes 118 amino-acid residues was amplified from genomic DNA by the polymerase chain reaction (PCR) using two primers for upstream and downstream sequences: 5’-CATGCCATGCCCTCAACTCCAAACTCATATGATGATGAAGAATTAGAAGAA-3’ (upstream) and 3’-CGCGGATCCTTAGTGGTGGTGGTGGTGTTTCCATCCTCTCTCTC-3’ (downstream). The DNA was subcloned using NcoI and BamH1 restriction sites and inserted into the vector pET-28a (Novagen) under the control of the inducible T7 promotor. Six histidine tag was designed at C-terminal right after the full length
protein. The entire coding sequence was verified by DNA sequencing. The recombinant plasmid was then transformed into host *E. coli* BL21 (DE-3) (Novagen) for expression. The protein was purified from Ni-NTA column and concentrated by Amicon (Millipore), and was lyophilized and stocked after salt removal by HiPrep 26/10 desalting column (Amersham Biosciences). The purified native Sso PDCD5 was verified by mass spectroscopic analysis and its purity (>95%) was checked by SDS-PAGE. The molecular mass as determined by ESI mass spectrometry at the Core Facilities for Proteomics and Structural Biology Research in Academia Sinica, Taiwan was 14935. This value was in good agreement with the theoretical weight predicted from the gene sequence (14947 Da). The 6×His affinity tag was not removed prior to crystallization trials.

### 2.1.2. Heavy atom substituted Sso PDCD5

In order to prepare heavy-atom derivatives for phase determination by MAD, the PDCD5 L78M, L45M and V63C mutation construct was made by using QuikChange Site-Directed Mutagenesis kit (Stratagene) due to lacking of Met and Cys in PDCD5 gene product. Se-Met Sso PDCD5 L78M was prepared according to the protocols described previously (Guerrero *et al.*, 2001). Sso PDCD5 V63C is crystallizing and soaking with heavy atoms. The purification protocol was the same with native protein.

### 2.2. Crystallization
Initial crystallization screening was performed using Hampton Research Crystal Screens (Laguna Niguel, CA, USA) with the hanging-drop vapor-diffusion method. In general, 2 μl of solution containing Sso PDCD5 (20 mM Tris-HCl, pH 7.5) was mixed with 2 μl of reservoir solution and the mixture was maintained at 300 K. Crystallization was carried out with PDCD5 protein concentrations between 5 and 10 mg/ml.

2.3 Data collection and analysis

Preliminary X-ray diffraction experiments were carried out with an R-Axis IV++ image-plate detector (Molecular Structure Corporation, The Woodlands, TX, USA) using CuKα radiation generated by a Rigaku MicroMax002 rotating-anode generator. Data were processed using the software package of HKL (Otwinowski & Minor, 1997).

2.4 Circular dichroism

Native Sso PDCD5 protein was dialyzed into 20mM sodium phosphate buffer at different pH value. The far-UV CD spectra were analyzed at 0.4 mg/ml in a 1 mm path length quartz cell at 20 °C using a JASCO- J715 spectropolarimeter.

2.6 Gel retardation assay
Sso PDCD5 protein was mixed with a fragment of 35bp dsDNA sequence of 5’-
CTAATAACAATGCTGCCACCGTGCTACAACCCCTCTCCTC- 3’ and its complimentary
5’- GAGGAAGTTGTAGCAGCGTGGCAGCATTGTTATTAG- 3’ with different molar ratio for
30 minutes. When binding with ssDNA, the following DNA fragment was used: 5’-
GGCTAACAAATATAAAATTATGTAAGCCTGAATTTGCCGAATCG- 3’. Mixtures were
analyzed with 2% agarose gel and EtBr staining.

3. Results and Discussion

As shown in Figure 1b, large single Sso PDCD5 crystals were obtained in 0.1 M Bis-Tris, pH 4.4
and 25% PEG3350, 0.2M CaCl2. Prior to data collection at 100 K, the crystals were mounted in a
cryoloop and flash frozen in liquid nitrogen with the addition of glycerol to 20% as a cryoprotectant.
Crystals of *S. solfataricus* PDCD5 belong to the tetragonal space group C2 with unit-cell
parameters *a* = 79.71, *b* = 36.82, *c* = 36.99 Å, β =94.45°. Assuming 1 molecule per asymmetric
unit, the Matthews coefficient *Vm* (Matthews, 1968) is 1.8 Å³/Da, suggesting a solvent content of
30%. Some statistical numbers for data collection results of *S. solfataricus* PDCD5 are listed in
Table 1. We also analyzed the state of Sso PDCD5 oligomerization by diffusion ordered
spectroscopy experiment (data not shown), and the Sso PDCD5 protein existed as a monomer in
solution. The secondary structure was analyzed by circular dichroism. Sso PDCD5 showed as an all-helical structure and the structure folding tightly at low pH value (pH 3) rather than at high pH value (pH 9) (Figure 1c). Based on the standard values of the mean residue molar ellipticity at 222nm, 4000 deg cm$^2$ dmol$^{-1}$ for the random coil and -38000 deg cm$^2$ dmol$^{-1}$ for the $\alpha$-helix (Creighton, 1993), we estimated the following $\alpha$-helical content for PDCD5 protein at pH 3, 5, 7, 9, respectively: 87%, 73%, 51% and 57%. The pH value change did not affect the $\alpha$-helical-major structure of PDCD5, but the molar ellipticity showed a pH dependent change manner. It is reasonable that this protein structure is stable at low pH value because it was originally from the hyperthermophilic archaean.

The gel retardation assay showed that the Sso PDCD5 protein interacted with dsDNA in a sequence general manner, and it binds mildly with ssDNA (Figure 1d and data not shown). Although Sso PDCD5 showed no preference on DNA sequence, it binds mainly with dsDNAs and weakly binding with ssDNAs. When binding with dsDNA with increasing molar ratio from 1 to 10 (Figure 1d), Sso PDCD5 protein showed cooperativity and formed some protein/DNA aggregates observed from agarose gel. With this kind of DNA binding feature, the biological function of Sso PDCD5 in S. solfataricus might be histone-like DNA binding protein. How the Sso PDCD5 binds with dsDNA needs further investigation.

We are working on heavy atom derivatives and trying to solve the structure using the MAD and MIR method. In order to get Se-Met derivatives of Sso PDCD5, we have chosen some site for
mutation due to lacking of Met on native Sso PDCD5. And the Structure determination by molecular replacement is also in progress. In addition, we are preparing PDCD5 protein in complex with dsDNAs and searching for crystallization conditions.
4. References


Gao Y.-G., Su S.-Y., Robinson H., Padmanabhan S., Lim L., McCrary B. S., Edmondson S.-P.,


Table 1.
Data collection statistics of the native Sso PDCD5 crystal

<table>
<thead>
<tr>
<th>Data set</th>
<th>Sso PDCD5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>C2</td>
</tr>
<tr>
<td>Unit cell dimensions (a, b, c)</td>
<td>79.7, 36.82, 36.99, 94.45</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>30- 2.87</td>
</tr>
<tr>
<td>Number of observations</td>
<td>7393</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>2476</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>97.4 (98.8)</td>
</tr>
<tr>
<td>$R_{merge}$ (%)</td>
<td>0.08 (0.43)</td>
</tr>
<tr>
<td>Average I/σ(I)</td>
<td>22.9 (3.7)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are for the highest resolution shell.*
Figure legends

Figure 1.
(a) Sequence alignment of the *S. solfataricus* PDCD5 with MTH1615 from *M. thermoautotrophicum* and PDCD5 from human.

The identical amino acids are highlighted in colored boxes. The secondary structures obtained for human PDCD5 protein are labeled at the top of sequence alignment.

(b) Crystals of PDCD5 from *Sulfolobus solfataricus* P2

Photograph of the crystals used in data collection. The approximate dimensions of the crystals are 0.1 x 0.1 x 0.6 mm$^3$.

(c) The secondary structure analyzed in using circular dichroism.

(d) Gel retardation of 17.3 μg of dsDNA binding with Sso PDCD5 with different molar ratio: 0, 1, 2.5, 5, 7.5, 10 (Lane 2, 3, 4, 5, 6, 7) respectively. Lane 1: 100 bp DNA marker.
Figure 1
Gel retardation of 8.14 μg of 44 base ssDNA binding with Sso PDCD5 with different molar ratio: 0, 1, 2, 5, 10, 20, 40 (Lane 2, 3, 4, 5, 6, 7, 8) respectively. Lane 1: 100 bp DNA marker.