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# Crystallization and preliminary X-ray crystallographic analysis of SEDL

**Se Bok Jang,[a] Yong-Soon Cho,[a] Soo-Jung Eom,[b] Eui-Ju Choi,[b] Kyung-Hwa Kim,[c] Pann-Ghill Suh[a] and Byung-Ha Oh[a,c]***

[a]National Creative Research Initiative Center for Biomolecular Recognition and Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, South Korea, [b]National Creative Research Initiative Center for Cell Death, Graduate School of Biotechnology, Korea University, Seoul 136-701, South Korea, and [c]Pohang Accelerator Laboratory, Pohang, Kyungbuk 790-784, South Korea

Correspondence e-mail: bhoh@postech.ac.kr

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SEDL (known also as sedlin) is a 140 amino-acid protein with a putative role in endoplasmic reticulum-to-Golgi transport. Several missense mutations and deletion mutations in the SEDL gene, which result in protein truncation by frame shift, are responsible for spondyloepiphyseal dysplasia tarda, a progressive skeletal disorder. The protein is identical to MIP-2A, which was shown to interact physically with c-*myc* promotor-binding protein 1 (MBP-1) and relieve the regulatory role of MBP-1 as a general transcription repressor. In order to gain insights into the function of SEDL by structural analysis, the protein was overexpressed and crystallized as a first step. SEDL was overexpressed in *Escherichia coli* and crystallized using the hanging-drop vapour-diffusion method at 298 K. The crystals belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 46.69$, $b = 101.30$, $c = 66.15$ Å. The unit cell is likely to contain one molecule of SEDL, with a crystal volume per protein mass ($V_M$) of 2.36 Å$^3$ Da$^{-1}$ and a solvent content of about 47.9% by volume. A native data set to 2.8 Å resolution was obtained from a flash-cooled crystal using synchrotron radiation.

## 1. Introduction

X-linked spondyloepiphyseal dysplasia tarda (SEDT) is a genetic disease characterized by disproportionately short trunk, short stature and an absence of systemic complications (Bannerman *et al.*, 1971; MacKenzie *et al.*, 1996). SEDT is caused by the production of a probably non-functional form of SEDL caused by mutations in the SEDL gene located at human chromosome Xp22 (Gedeon *et al.*, 1999). Analysis of a spectrum of SEDL mutations in unrelated cases of SEDT ascertained from different ethnic populations showed that frameshift mutations leading to a premature termination of translation account for >43% of SEDT cases, with half of these arising from dinucleotide deletions (Gedeon *et al.*, 2001). An analysis of a Japanese family showed that such a deletion mutation was present in a proband (diagnosed as having SEDT) and his unaffected mother (a heterozygote), but not in an unaffected sister and an unaffected uncle (Matsui *et al.*, 2001), indicating that loss of SEDL caused SEDT. A total of seven SEDL pseudogenes were detected in the human genome. Among these, *SEDLP1*, a transcribed retropseudogene on chromosome 19, encodes a protein identical to that of the SEDL gene on chromosome Xp22 as described below (Gecz *et al.*, 2000). It is not yet clear whether a compensatory effect of SEDLP1 may contribute to the absence of a phenotypic effect in most tissues that are under control of a different promoter (Gedeon *et al.*, 1999).

SEDL appears to be an evolutionary conserved protein, as its homologues have been identified in yeast, *Drosophila melanogaster*, *Caenorhabditis elegans*, mouse and rat (Gedeon *et al.*, 1999). The yeast SEDL homologue (ORF YBR254C or p20) was recently characterized as a member of a transport protein particle known as TRAPP (Sacher & Ferro-Novick, 2001), which is a large protein complex required in the endoplasmic reticulum-to-Golgi vesicle transport (Lowe, 2000). The yeast gene was shown to be an essential gene by tetrad analysis following sporulation of heterozygous disruptants (Sanjuan *et al.*, 1999).

A protein identical to SEDL was found to physically interact with and antagonize the functional activity of c-*myc* promotor-binding protein 1 (MBP-1; Ghosh *et al.*, 2001), which transcriptionally down-regulates c-*myc* promotor activity by directly binding to the c-*myc* P2 promotor sequence (Ray & Miller, 1991). The protein was named MBP-1 interacting protein (MIP-2A) because of the observation of a direct interaction between the two in a yeast two-hybrid analysis and in an *in vitro* glutathione S-transferase pull-down experiment (Ghosh *et al.*, 2001). Functional analysis indicates that MIP-2A relieves the transcriptional repression of MBP-1 and antagonizes MBP-1-mediated cell death in

fibroblasts (Ghosh *et al.*, 2001). Although the coding sequences of the MIP-2A and SEDL genes are identical, their untranslated regions are significantly different and the MIP-2A gene (described above as *SEDLP1*) is located at human chromosome 19, while the SEDL gene is located at chromosome Xp22. Further characterization of *MIP-2A*/*SEDLP1*, *SEDL* and *SEDL* pseudogenes are necessary in order to understand the functional relationships between these genes.

A database search for sequence homology with SEDL does not yield any protein whose biochemical function or three-dimensional structure is known. Structural information on SEDL could lead to the identification of the biochemical function of the protein. The structure of SEDL could serve as a stepping stone for probing its seemingly completely different roles in antagonizing MBP-1 and for deciphering the potential complexity of the biochemical roles of SEDL. In this report, we describe preliminary X-ray crystallographic analyses of mouse SEDL, which differs from human SEDL by the presence of seven conservative mutations out of a total of 140 amino acids.

## 2. Protein expression and purification

The *SEDL* gene was amplified by the polymerase chain reaction (PCR) technique from a mouse cDNA library. The PCR products were purified, digested with *Xho*I and *Blp*I and then ligated into the pET15b vector (Novagen). The resulting vector was introduced into *Escherichia coli* BL21 (DE3) RIL strain. The vector construction resulted in SEDL fused to a $His_6$ tag at the N-terminus. The expression of the recombinant SEDL protein was induced by 1 m*M* isopropyl-D-thiogalactopyranoside at an optical density of 0.5–0.6 at 298 K for 8 h. Bacterial lysates were prepared by sonication in buffer *A* (50 m*M* Tris–HCl pH 7.6 and 200 m*M* NaCl).

SEDL was obtained after cleavage by thrombin. The $His_6$-tagged fusion protein bound to an Ni–NTA column (Qiagen) was eluted with buffer *A* containing 200 m*M* imidazole after washing the column with the same buffer. The eluted fractions were dialyzed against buffer *A* and reacted with thrombin (Roche Molecular Biomedicals) at a fusion protein:thrombin weight ratio of 20:1 for 12 h at 277 K. The reaction mixture was loaded onto an Ni–NTA column and unbound fractions were collected. The fractions were concentrated to 0.5 mg ml$^{-1}$ and loaded onto a Resource Q anion-exchange column (Amersham-Pharmacia) pre-equilibrated with buffer *A*. Unbound fractions were collected and concentrated and subsequently loaded onto a Superdex 75 column (Amercham-Pharmacia) pre-equilibrated with a buffer composed of 50 m*M* Tris–HCl pH 7.6 and 250 m*M* NaCl. The protein solution was concentrated to about 10 mg ml$^{-1}$ using a Vivaspin 20 (Satorius). The purified SEDL was at least 95% pure as judged on a polyacrylamide gel.

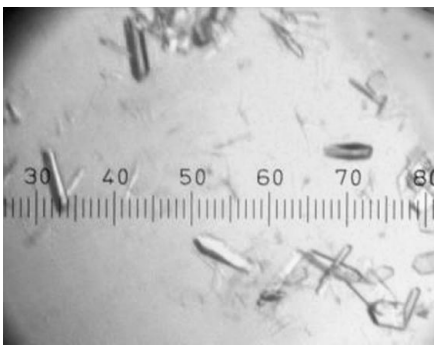


**Figure 1**
Crystals of mouse SEDL, grown larger by macroseeding (see text). Eight divisions on the ruler indicate 0.1 mm.

## 3. Crystallization of SEDL

Crystals of SEDL were obtained by the hanging-drop vapour-diffusion method at 298 K using 24-well Linbro plates (Hampton Research). A hanging drop was prepared by mixing equal volumes (1.5 µl each) of the protein solution and the reservoir solution. Each hanging drop was placed over 0.5 ml of reservoir solution. The first crystallization screening was performed with Crystal Screen (Hampton Research), a sparse-matrix screening kit (Jancarik & Kim, 1991). SEDL crystallized as clusters of microcrystals with a precipitant containing 1.4 *M* sodium acetate and 0.1 *M* sodium cacodylate pH 6.5. Under these conditions, crystals appeared after 3–7 d and grew to a maximum size within two weeks. Microseed crystals were transferred to a 1:1 mixture of the same protein sample and a precipitant containing 1.0 *M* sodium acetate, 0.1 *M* sodium cacodylate pH 6.5 and 5–10% glycerol to obtain larger crystals; crystals were touched on the surface using the tip of a human hair. The crystals thus obtained were larger than the original ones, but still were small thin plates with a largest dimension of approximately 0.1 mm (Fig. 1).

## 4. Data collection and analysis

Diffraction data were collected from a flash-cooled crystal at 100 K using synchrotron radiation from beamline 6B at Pohang Accelerator Laboratory, Korea. Before data collection, the crystal was soaked briefly in a cryoprotectant solution which was the precipitant solution containing 15% glycerol. Diffraction data (Table 1) were obtained and processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The native diffraction data were collected to 2.8 Å. The crystals belong to the orthorhombic space group $C222_1$. We calculated that the crystal volume per molecular weight ($V_M$) is 2.361 Å$^3$ Da$^{-1}$ with a solvent content of 47.9% by volume (Matthews, 1968) when one unit cell was assumed to contain eight molecules of SEDL. This corresponds to one molecule of SEDL per asymmetric unit. A search for suitable heavy-atom derivative crystals is in progress in parallel with an effort to obtain crystals of selenomethionine-substituted protein.

**Table 1**
Crystal information and data-collection statistics.

The numbers in parentheses are statistics for the highest resolution shell, 2.9–2.8 Å.

| | |
|---|---|
| Source | 6B, PAL |
| Wavelength (Å) | 1.1270 |
| Space group | $C222_1$ |
| Unit-cell parameters (Å) | $a$ = 46.69, $b$ = 101.30, $c$ = 66.15 |
| Resolution range (Å) | 30–2.8 |
| No. of observed reflections | 29601 |
| No. of unique reflections | 3911 |
| Data completeness (>1$\sigma$) (%) | 92.3 (86.1) |
| $R_{sym}$† (%) | 6.9 (23.5) |
| $I/\sigma(I)$ | 15.26 (2.1) |

† $R_{sym} = \sum|I_{obs} - I_{avg}|/I_{obs}$, where $I_{obs}$ is the observed intensity of an individual reflection and $I_{avg}$ is the average over symmetry equivalents.

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