Expression, Purification, Crystallization and Preliminary X-Ray Analysis of Human Spindlin1, an Ovarian Cancer-Related Protein

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Abstract: Human spindlin1 is a newly screened and identified gene product related to ovarian carcinomas and is highly homologous to mouse spindlin. It is an abundant maternal transcript expressed in the mouse during the transition from oocyte to embryo. Here, the recombinant human spindlin1 has been overexpressed in *Escherichia coli* BL21, purified and crystallized using the hanging-drop vapour-diffusion method. Crystals diffracting to 2.25 Å resolution were obtained using ammonium sulfate as precipitant. The crystals belong to the space group $P2_12_12_1$, with unit-cell parameters a =40.7 Å, b =84.4 Å, c =136.4 Å, $\alpha=\beta=\gamma=90°$. Assuming two molecules per asymmetric unit, the solvent content is calculated to be 42.4%.

1. INTRODUCTION

Spindle is a specific substructure in the cell during meiosis and gamete maturity division. Its structure and assembly style are different in somatic, embryo and even in maturity divisions of male and female gametes [1]. Since its structure and function directly influence the development, differentiation, growth and reproduction of individuals [2-3], studies on spindle's subunit assembly process as well as its regulation factors are helpful to reveal the biological behavior and molecular foundation of germ cells. The mouse spindlin (Spin) gene is an abundant maternal transcript present in the unfertilized egg and 2-cell, but not 8-cell stage embryo [4]. Spindlin exhibits high homology to a multicopy gene, Y-linked spermiogenesis-specific transcript (Ssty), and together they form a new gene family expressed during gametogenesis [5]. It is well known that the mammalian MOS protein, product of the proto-oncogene *c-mos*, may activate the MAP Kinase pathway to regulate the structure and function of oocyte spindle, which leads to meiosis Metaphase II arrest of mature egg cell and determines its development after fecundation [6-7]. Spindlin is largely synthesized in mouse oocytes and 2-cell stage embryo, then binds to and comigrates with spindle Mos/MAP Kinase pathway phosphorylation. Accordingly, mouse spindlin is supposed to play a role in cell-cycle regulation during the transition from gamete to embryo [8].

Ovarian cancer is the leading cause of death among gynecological malignancies. However, little is known to date about the specific tumor-suppressor genes or oncogenes involved in ovarian cancer genesis. Human spindlin1 is a newly cloned gene during the isolation and characterization of new candidate genes in the ovarian cancer genesis [9]. Modified mRNA differential display PCR and reverse Northern dot analysis were used to screen and identify different displayed genes between ovarian carcinoma tissue and normal ovarian tissue. Interestingly, the novel gene spindlin1 encoding a 27kDa ovarian cancer-related protein, with 96% sequence homology to mouse spindlin, a member of gene family specially expressed during gametogenesis, is highly expressed in 19 samples of ovarian cancer from a total of 36 cases, but is not expressed in control samples [9]. It is speculated that human spindlin1 may be the homologous gene of mouse spindlin and play roles during the early period of embryo development and tumorigenesis [10].

So far, the biochemical function of human spindlin1 is largely unknown. In the present study, the crystallization and preliminary crystallographic analysis of human spindlin1 are reported. The structure of this protein will be helpful in the illustration of the properties and function of human spindlin1.

2. MATERIALS AND METHODS

2.1 Protein Expression and Purification

Human spindlin1 gene encoding a putative ovarian cancer-related protein was amplified by the polymerase chain reaction and subcloned into the pGEX-6p-1 expression vector (Amersham Pharmacia Biotech). The complete nucleotide sequence of the insert was confirmed by dideoxy-DNA sequencing. The recombinant plasmid was transformed into *Escherichia coli* strain BL21(DE3) and transformants were selected on LB agar plates containing 50 μg ml^{-1} Ampicillin. The cells were cultured at 310 K in 2×YT medium containing 100 μg ml^{-1} Ampicillin. When the culture density reached 0.6~0.8 (OD_{600}), the culture was induced with 0.2 mM IPTG and grown for an additional 12 h at 289 K before the cells were harvested.

The bacterial cell pellet was resuspended in the lysis buffer (PBS, 10 mM sodium phosphate pH 7.3, 150 mM NaCl) and homogenized by sonication. Triton X-100 was

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then added to a final concentration of 1% and the lysate was incubated for 30 min at 273 K and subsequently clarified by centrifugation at 20,000 g for 30 min at 277 K. The supernatant was applied to a Glutathione Sepharose 4B disposable column (Amersham Pharmacia Biotech) pre-equilibrated with PBS. The GST-fusion protein bound column was then washed with 30 bed volumes of PBS and PreScission Protease was added to a final concentration of 80 U for each milliliter of Glutathione Sepharose bed volume in order to remove GST from the fusion protein. After incubation at 277 °K for 16 h, the eluate containing target protein was collected. This sample was subsequently loaded onto a 1 ml Resource Q anion-exchange column and eluted with a linear sodium chloride gradient. The fractions that eluted out at 400 mM salt concentration were collected and concentrated using an Ultrafree 10,000 NMWL filter unit (Millipore). Further purification was accomplished by gel-filtration chromatography on Superdex 75 in buffer A (20 mM Tris-HCl, pH 8.0, 100 mM NaCl). All pre-packed columns were purchased from Amersham Pharmacia Biotech and all steps were performed on an **ÄKTA** explorer system at 289 °K. Homogeneity was analyzed by SDS-PAGE.

2.2 Crystallization

The purified protein with the buffer A (20mM Tris-HCl pH 8.0, 100 mM NaCl) was concentrated to 20-30 mg ml^{-1} at 277 K. Crystallization trials were carried out by the hanging-drop vapour-diffusion method at 291 K in 16-well plates. Initial crystallization conditions were established using Hampton Research Crystal Screen Kits I and II. Conditions yielding small crystals were further optimized by variation of concentrations of protein and precipitant and the buffer pH.

2.3 X-Ray Crystallographic Studies

Preliminary diffraction data sets were collected at 100 K in house on a Rigaku Micro007 rotating Cu K_α anode X-ray generator operating at 40 kV and 20 mA (λ=1.5418 Å) with a Rigaku R-AXIS IV++ image plate detector. The beam was focused by Osmic mirror system. All intensity data were indexed, integrated and scaled with the *HKL*2000 suite [11].

3. RESULTS AND DISCUSSION

The recombinant human spindlin1 was soluble and stable in PBS or Tris-HCl buffers. The final purified protein was confirmed to be homogenous by SDS-PAGE analysis and to be suitable for use in crystallization trials. Small hexagonal rod-shaped crystals appeared after 2 days from the condition (2.0 M ammonium sulfate, 0.1 M Tris-HCl, pH 8.5), but did not diffract well. Further optimization was performed and better crystals were obtained using 1.9 M ammonium sulfate as precipitant in 0.1 M Tris-HCl buffer (pH 8.7). Drops containing 2 µl protein solution and 2 µl of reservoir solution were equilibrated against 300 µl of reservoir solution (Fig. **1**). The best crystals grew to dimensions of 1.5×0.5×0.3 mm at 291 K in two weeks and diffracted to 2.25 Å. A set of data was subsequently collected from this crystal, which belonged to the space group $P2_12_12_1$, with unit-cell parameters a=40.7 Å, b=84.4 Å, c =136.4 Å, α=β=γ=90°. R_{merge} for the data set was 8.8% overall and 48.5% for the outermost (2.33-2.25Å) shell. Using the molecular weight of 27 kDa and assuming two molecules per asymmetric unit, the Matthews coefficient (V_M) was calculated to be 2.2$Å^3$ Da^{-1} [12], corresponding to a solvent content of 42.4% [13]. The crystal parameters and data collection statistics are shown in Table **1**.

Determination of the structure of spindlin1 is presently under way. The structure of human spindlin1 will be helpful to elucidate the function of the ovarian cancer-related protein in the early period of embryo development and tumorigenesis.

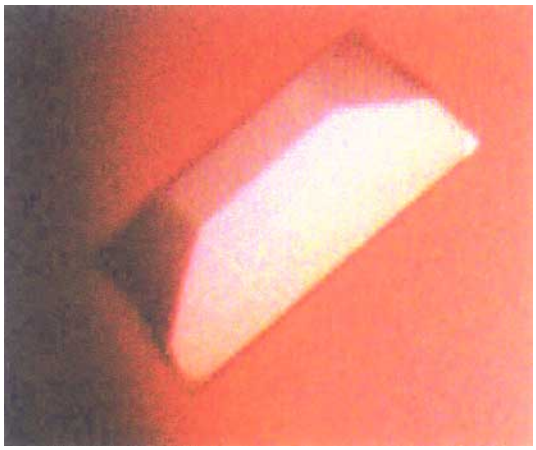

Figure 1. The crystal of human spindlin1. The size of the crystal is approximately 1.5×0.5×0.3mm.

Table 1. Data collection and processing statistics. Values in parentheses correspond to the highest resolution shell (2.33-2.25Å)

Space Group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 40.7, b = 84.4, c = 136.4
Matthews coefficient ($Å^3$ Da^{-1})	2.2
Resolution limits (Å)	50.0-2.25
Total observations	98234
Unique reflections	21335
Redundancy	4.7(4.5)
Average I/σ(I)	13.1(3.3)
Rmerge (%)	8.8(48.5)
Data compleness(%)	92.2(81.9)

R_{merge} =100$\sum|I_i-\langle I\rangle|/\sum I_i$, where I_i is the intensity of the observation.

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