Acta Crystallographica Section F
**Structural Biology and Crystallization Communications**
ISSN 1744-3091

# Expression, purification, crystallization and preliminary crystallographic analysis of the proliferation-associated protein Ebp1

**Eva Kowalinski,‡ Gert Bange, Klemens Wild and Irmgard Sinning***

Heidelberg University Biochemistry Center, INF 328, D-69120 Heidelberg, Germany

‡ Current address: European Molecular Biology Laboratory, Grenoble Outstation, F-38042 Grenoble CEDEX 9, France.

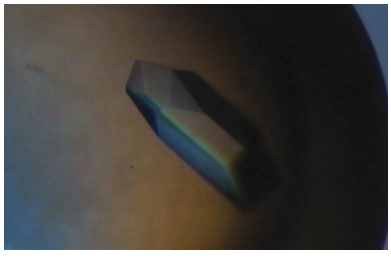
Correspondence e-mail: irmi.sinning@bzh.uni-heidelberg.de

Received 25 June 2007
Accepted 7 August 2007

ErbB-3-binding protein 1 (Ebp1) is a member of the family of proliferation-associated 2G4 proteins (PA2G4s) and plays a role in cellular growth and differentiation. Ligand-induced activation of the transmembrane receptor ErbB3 leads to dissociation of Ebp1 from the receptor in a phosphorylation-dependent manner. The non-associated protein is involved in transcriptional and translational regulation in the cell. Here, the overexpression, purification, crystallization and preliminary crystallographic studies of Ebp1 from *Homo sapiens* are reported. Initially observed crystals were improved by serial seeding to single crystals suitable for data collection. The optimized crystals belong to the tetragonal space group $P4_12_12$ or $P4_32_12$ and diffracted to a resolution of 1.6 Å.

## 1. Introduction

The proliferation-associated 2G4 proteins (PA2G4s) are conserved in eukaryotes. The members of this family are involved in cell proliferation and differentiation (Squatrito *et al.*, 2004; Horvath *et al.*, 2006; Liu *et al.*, 2006). The human PA2G4 member, Ebp1, was initially identified as a protein binding to the ErbB-3 receptor by yeast two-hybrid screening (Yoo *et al.*, 2000). Subsequently, Ebp1 was found to induce differentiation of human cancer cell lines (Lessor *et al.*, 2000). The protein shows cell-cycle-dependent cytoplasmic or nuclear localization (Radomski & Jost, 1995). The shuttling of Ebp1 seems to depend on the phosphorylation of Ser360, which is located in the C-terminal portion of the protein (Ahn *et al.*, 2006). The nuclear fraction of Ebp1 interacts with a number of factors involved in transcriptional regulation: (i) the androgen receptor (AR; Zhang & Hamburger, 2004), (ii) retinoblastoma protein (Xia *et al.*, 2001) and (iii) Sin3A (Zhang *et al.*, 2005). It acts as a corepressor at E2F1- and AR-regulated promoters involved in the recruitment of histone deacetylase 2 (HDAC2). Moreover, murine PA2G4, also known as p38-2G4 or Mpp1, acts as an IRES (internal ribosome-entry site) specific cellular *trans*-acting factor (ITAF) required for translation initiation (Pilipenko *et al.*, 2000). The observation that Ebp1 co-purified with ribosomes, ribosomal subunits and rRNA could be related to this regulatory activity (Squatrito *et al.*, 2004, 2006). Moreover, Ebp1 is a component of cytoplasmic bcl-2 rRNPs (Bose *et al.*, 2006). Here, we describe the purification, crystallization and preliminary crystallographic analysis of the Ebp1 protein from *Homo sapiens*.





## 2. Experimental procedures

### 2.1. Cloning and protein expression

A gene fragment encoding human Ebp1 (gi:13632817; Lamartine *et al.*, 1997) was amplified by polymerase chain reaction (PCR) using the Expand High Fidelity PCR system (Roche) and cloned *via* the *Nco*I/*Bam*HI restriction sites into a pET24d vector (Novagen). The forward and reverse primer compositions were 5′-AATTCCATGG-

GCCACCATCACCATCACCATTCGGGCGAGGACGAGCAAC and 5′-TTAAGGATCCTTAGTCCCCAGCTTCATTTTCTTC, respectively. The construct contained an N-terminal hexahistidine tag, introduced by the forward primer, for purification purposes. The calculated molecular weight of the expressed protein is 44.6 kDa. The huPA2G4 protein was overexpressed in *Escherichia coli* strain C43 (DE3) (Miroux & Walker, 1996). Cells were grown at 310 K in lysogeny broth medium (LB) complemented with 50 µg ml$^{-1}$ kanamycin. Protein expression was induced by 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) at an $OD_{600}$ of 0.7 and took place overnight at 310 K.

### 2.2. Protein purification

Cells were resuspended in buffer *A* [20 m*M* HEPES, 250 m*M* NaCl, 10 m*M* KCl, 10 m*M* $MgCl_2$, 2.5%(*v*/*v*) glycerol, 40 m*M* imidazole pH 8.0] containing protease-inhibitor mix (Serva) and passed twice through an M1-10L Microfluidizer (Microfludix). The cell lysate was cleared of cell debris by ultracentrifugation (125 000*g* for 15 min at 277 K). Nucleic acid contaminations were then precipitated with 20 mg ml$^{-1}$ protamine sulfate (Sigma–Aldrich) and removed by another ultracentrifugation step. The supernatant was applied onto a 1 ml HiTrap Chelating HP column (GE Healthcare) equilibrated with buffer *A*. The protein was eluted in buffer *B* [20 m*M* HEPES, 250 m*M* NaCl, 10 m*M* KCl, 10 m*M* $MgCl_2$, 2.5%(*v*/*v*) glycerol, 500 m*M* imidazole pH 8.0]. The eluate was concentrated and purified by gel-filtration chromatography (Sephadex 75/26–60; GE Healthcare) equilibrated in buffer *C* (20 m*M* Na HEPES pH 7.5, 200 m*M* NaCl, 10 m*M* KCl, 10 m*M* $MgCl_2$). Protein-containing fractions were analyzed by 12.5% SDS–PAGE and pooled.

### 2.3. Dynamic light-scattering measurements

The homogeneity of the protein was analyzed by dynamic light scattering (DLS; DynaPro, Protein Solutions). Prior to the measurements, sediment was removed by ultracentrifugation at 40 000*g* for 30 min at 277 K. The protein concentration was adjusted to 5 000 000 counts min$^{-1}$ (or 4 mg ml$^{-1}$). Measurements were performed at temperatures of 277, 284, 291 and 298 K.

### 2.4. Crystallization, data collection and processing

The protein was concentrated using Amicon Ultracell-30K concentrators (Millipore). Initial screening was performed using sparse-matrix screening. The hanging-drop vapour-diffusion method was applied using Greiner 24-well plates with 400 µl reservoir solution. Drops were set up with a 2:1 ratio of protein solution (80 mg ml$^{-1}$) to reservoir solution. The crystallization plates were stored at 298 K. Crystals were optimized by subsequent rounds of seeding (serial seeding). New crystallization drops were set up in 24-well plates and an existing crystal was gently touched with a cat whisker, which was then immediately moved through a new drop. The crystals were cryoprotected by transferring them from the drop into cryoprotectant solutions containing the reservoir conditions and stepwise increasing concentrations [5, 10, 15, 10, 25%(*v*/*v*)] of glycerol. The crystal remained in each solution for approximately 1 min. The crystal was then flash-cooled in liquid nitrogen for data collection. Diffraction data were collected at beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF) under cryogenic conditions (100 K, Oxford Cryosystems Cryostream). Data were recorded with a Q315r ADSC CCD detector, indexed and integrated with *DENZO* and scaled with *SCALEPACK* (Borek *et al.*, 2003).

**Table 1**
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

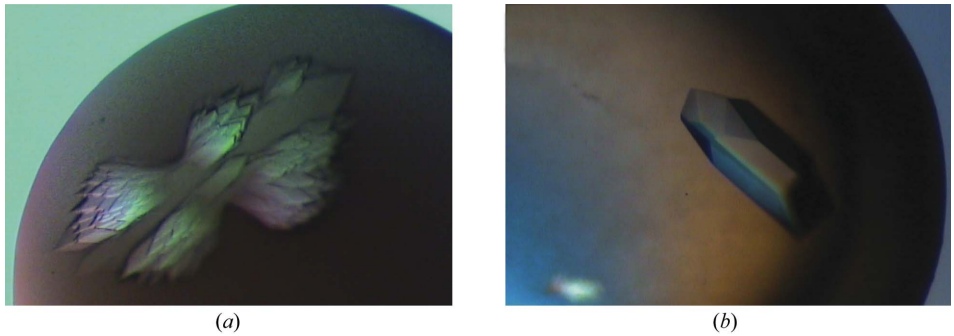
| | |
|---|---|
| Wavelength (Å) | 0.980 |
| Space group | $P4_12_12$ or $P4_32_12$ |
| Unit-cell parameters (Å) | $a = b = 73.9$, $c = 183.3$ |
| Resolution range (Å) | 68.0–1.6 (1.69–1.6) |
| Completeness (%) | 99.8 (100) |
| Mosaicity (°) | 0.2 |
| Average $I/\sigma(I)$ | 12.9 (2.8) |
| $R_{sym}$† (%) | 6.1 (40.7) |
| Solvent content (%) | 56 |
| Redundancy | 3.8 |
| Molecules in ASU‡ | 1 |

† $R_{sym} = \sum I - \langle I \rangle / \sum I$, where $I$ is the intensity measurement for a given reflection and $\langle I \rangle$ is the average intensity for multiple measurements of this reflection. ‡ As suggested by the Matthews parameter (Matthews, 1968).

## 3. Results and discussion

Human Ebp1 was expressed with a yield of 30 mg of protein per litre of *E. coli* C43 (DE3) and was purified according to standard protocols as described above. Protein purity was >>95% and its molecular weight was approximately 45 kDa as judged from Coomassie-stained SDS–PAGE (data not shown). Western blot analysis using an anti-penta-His antibody (Qiagen) was used to prove the identity of purified Ebp1 (data not shown). In gel-filtration chromatography, the protein showed an apparent molecular weight corresponding to a monomer (data not shown). Dynamic light-scattering (DLS) measurements at various temperatures revealed a homogenous solution of Ebp1 (data not shown).

Initially, an epitaxically twinned crystal was observed in a buffer containing 2.3 *M* ammonium sulfate, 0.1 *M* citric acid pH 5 at 291 K after approximately four weeks (Fig. 1*a*). A portion was cut out from



(*a*) (*b*)

**Figure 1**
Crystals of Ebp1. (*a*) Initially, epitaxically twinned crystals were observed. (*b*) Single crystals were obtained by serial seeding. The crystals were typically 200–500 µm in size.

this crystal and X-ray crystallographic experiments were performed. The crystal diffracted to a resolution of 3.2 Å and a complete data set was collected. Crystallization was improved by serial seeding as described above. Single crystals were observed within 7 d after two rounds of seeding (Fig. 1*b*). These crystals diffracted to a resolution of 1.6 Å and data were collected using an oscillation increment of 0.5° (over a total of 180°). Crystal data and data-collection statistics are shown in Table 1. The crystals belong to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 73.8$, $c = 183.1$ Å. The asymmetric unit contains one molecule, with a Matthews coefficient of 2.8 $Å^3$ $Da^{-1}$, corresponding to a solvent content of 56% (Matthews, 1968). Members of the PA2G4 family exhibit homology to the methionine aminopeptidases (EC 3.4.11.18; Squatrito *et al.*, 2004). We therefore expect the structure to be solvable by molecular replacement using the structure of a methionine aminopeptidase as a search model. The three-dimensional structure should provide a basis for understanding the multiple roles of Ebp1 in cell proliferation, differentiation and tumorigenesis.

The DNA encoding Ebp1 was a gift from the laboratory of Giulio Draetta, European Institute of Oncology, Milan, Italy to Professor Ed Hurt, BZH. This work was performed in collaboration with the group of Ed Hurt and was supported by a grant fom the SFB638 to IS. We acknowledge access to beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF) in Grenoble and the support by the beamline scientists.

## References

Ahn, J. Y., Liu, X., Liu, Z., Pereira, L., Cheng, D., Peng, J., Wade, P. A., Hamburger, A. W. & Ye, K. (2006). *EMBO J.* **25**, 2083–2095.
Borek, D., Minor, W. & Otwinowski, Z. (2003). *Acta Cryst.* D**59**, 2031–2038.
Bose, S. K., Sengupta, T. K., Bandyopadhyay, S. & Spicer, E. K. (2006). *Biochem. J.* **396**, 99–107.
Horvath, B. M., Magyar, Z., Zhang, Y., Hamburger, A. W., Bakó, L., Visser, R. G., Bachem, C. W. & Bögre, L. (2006). *EMBO J.* **25**, 4909–4920.
Lamartine, J., Seri, M., Cinti, R., Heitzmann, F., Creaven, M., Radomski, N., Jost, E., Lenoir, G. M., Romeo, G. & Sylla, B. S. (1997). *Cytogenet. Cell Genet.* **78**, 31–35.
Lessor, T. J., Yoo, J. Y., Xia, X., Woodford, N. & Hamburger, A. W. (2000). *J. Cell Physiol.* **183**, 321–329.
Liu, Z., Ahn, J. Y., Liu, X. & Ye, K. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 10917–10922.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Miroux, B. & Walker, J. E. (1996). *J. Mol. Biol.* **260**, 289–298.
Pilipenko, E. V., Pestova, T. V., Kolupaeva, V. G., Khitrina, E. V., Poperechnaya, A. N., Agol, V. I. & Hellen, C. U. (2000). *Genes Dev.* **14**, 2028–2045.
Radomski, N. & Jost, E. (1995). *Exp. Cell Res.* **220**, 434–445.
Squatrito, M., Mancino, M., Donzelli, M., Areces, L. B. & Draetta, G. F. (2004). *Oncogene*, **23**, 4454–4465.
Squatrito, M., Mancino, M., Sala, L. & Draetta, G. F. (2006). *Biochem. Biophys. Res. Commun.* **344**, 859–868.
Xia, X., Cheng, A., Lessor, T., Zhang, Y. & Hamburger, A. W. (2001). *J. Cell Physiol.* **187**, 209–217.
Yoo, J. Y., Wang, X. W., Rishi, A. K., Lessor, T., Xia, X. M., Gustafson, T. A. & Hamburger, A. W. (2000). *Br. J. Cancer*, **82**, 683–690.
Zhang, Y., Akinmade, D. & Hamburger, A. W. (2005). *Nucleic Acids Res.* **33**, 6024–6033.
Zhang, Y. & Hamburger, A. W. (2004). *J. Biol. Chem.* **279**, 26126–26133.