FEBS Letters 581 (2007) 4450–4454

The crystal structure of Ebp1 reveals a methionine aminopeptidase fold as binding platform for multiple interactions

Eva Kowalinski[1,2], Gert Bange[1], Bettina Bradatsch, Ed Hurt, Klemens Wild, Irmgard Sinning*

Heidelberg University Biochemistry Center (BZH), INF 328, D-69120 Heidelberg, Germany

Received 11 July 2007; revised 13 August 2007; accepted 13 August 2007

Available online 27 August 2007

Edited by Hans Eklund

Abstract The ErbB-3 receptor binding protein (Ebp1) is a member of the proliferation-associated 2G4 (PA2G4) family implicated in regulation of cell growth and differentiation. Here, we report the crystal structure of the human Ebp1 at 1.6 Å resolution. The protein has the conserved pita bread fold of methionine aminopeptidases, but without the characteristic enzymatic activity. Moreover, Ebp1 is known to interact with a number of proteins and RNAs involved in either transcription regulation or translation control. The structure provides insights in how Ebp1 discriminates between its different interaction partners.

Keywords: Structural biology; Methionine aminopeptidase; Cell cycle; RNA binding protein; IRES

1. Introduction

Proliferation-associated 2G4 proteins (PA2G4) are highly conserved in eukaryotes and involved in the regulation of cell growth and differentiation [1,2]. The human member of this family, ErbB3 binding protein 1 (Ebp1), is ubiquitously expressed and localizes in both nucleus and cytoplasm [3]. It was initially identified to bind to the juxtamembrane domain of non-phosphorylated ErbB3 receptors [4]. Ectopic expression inhibits the growth of human breast and prostate cancer cells and induces cellular differentiation [5]. Treatment of serum-starved human breast cancer cells with the ErbB3/4 ligand heregulin (HRG) induces translocation of Ebp1 from cytoplasm to nucleus [4]. In the nucleus Ebp1 is involved in transcriptional regulation by interaction with Sin3A [6], Akt [7], Rb and histone deacetylase 2 (HDAC2) on E2F promoters [8].

The protein binds structured RNAs [3,9–11] and was suggested to be involved in linking ribosome biosynthesis and cell proliferation [3]. The yeast member of the PA2G4 family, Arx1, which is associated with a late pre-60S particle, functions as a nuclear export receptor for the large ribosomal subunit [12]. Ebp1 was shown to act as a cellular inhibitor of the eukaryotic initiation factor (eIF) 2α phosphorylation suggesting a role in protein translation control [11]. The protein has been identified as an IRES-*trans*-acting factor (ITAF45) [10]. Internal ribosomal entry site (IRES) elements consist of highly structured RNA regions located at the 5′ UTR of viral or cellular mRNAs. Initiation of translation from IRES elements is mediated by a set of canonical eIFs and a series of message specific cellular ITAFs. Thus, the RNA binding activity of Ebp1 could be critical for its cellular function as a modulator of stability and translation of specific mRNAs [11]. To gain insight into the multiple roles of this conserved protein family, we determined the atomic structure of human Ebp1.

2. Materials and methods

2.1. Structure determination

Ebp1 was cloned, expressed, purified and crystallized as described elsewhere [13]. The structure was solved by molecular replacement with CCP4 implemented PHASER [14], using the structure of the methionine aminopeptidase from *Pyrococcus furiosus* (PDB code: 1wkm) [15] as a search model. The sequence identity between search model and Ebp1 is 24%. The asymmetric unit contains one molecule. The crystal solvent content was 56%. Model building was initially performed automatically with program wARP [16] and completed manually with COOT [17] and O [18]. The structure was refined with program REFMAC [19] and validated with programs PROCHECK [20] and WHATIF [21]. Refinement statistics are listed in Supplementary Table 1. Residues 83 and 274 appeared as outliers in the Ramachandran plot, however, they were well defined in the electron density. Illustrations were prepared with PYMOL [22] and GRASP [23].

2.2. Methionine amino peptidase activity assay

The coupled enzymatic assay for monitoring methionine aminopeptidase activity was performed as described [24]. The assay contained 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 50 μM Amplex Red (Molecular Probes), 5 U AAO (Worthington), 1 U Horse Radish Peroxidase (Fluka), 0.3 mM L-Met-Ala-Ser (Sigma). The concentrations of MAP1 and Ebp1 were 100 nM and 130 nM, respectively. Emission at 590 nm was measured in a Jasco FP-6500 spectrofluorometer (578 nm excitation).

2.3. Polysome purification

Ebp1 was expressed under the ADH1 promoter as a TAP-Flag tagged fusion protein from pYCplac111-PADH1-NTAPFlag-EBP1 in an ARX1 deletion *Saccharomyces cerevisiae* strain (arx1Δ DS1-2b [12]). Lysate preparation and sucrose density gradient centrifugation were performed as described [25]. The TCA-precipitated fractions were analyzed by SDS–PAGE and Western blot analysis using anti-Rpl3 antibody and anti-PAP antibody to detect tagged Ebp1.

*Corresponding author. Fax: +49 6221 544790.
E-mail address: irmi.sinning@bzh.uni-heidelberg.de (I. Sinning).

[1]These authors contribute equally to this work.
[2]Present address: European Molecular Biology Laboratory, Grenoble Outstation, F-38042 Grenoble Cedex 9, France.

Abbreviations: Ebp1, ErbB3 binding protein 1; PA2G4, proliferation associated 2G4; IRES, internal ribosomal entry site; MAP, methionine aminopeptidase; eIF, eukaryotic initiation factor; ITAF, IRES *trans* acting factor

0014-5793/$32.00
doi:10.1016/j.febslet.2007.08.024

2.4. RNA-electrophoresis mobility shift assay

RNA bandshift assays were carried out as described [26]. Yeast 5S rRNA was produced by in vitro transcription as described [26]. Total yeast tRNA was ordered from Sigma. The SRP-RNA construct (95 nucleotides) consisted of the highly conserved SRP core of *Sulfolobus solfataricus* (Bange & Sinning, unpublished). RNA was incubated with increasing concentrations of recombinant protein.

3. Results and discussion

3.1. Ebp1 shares the pita-bread fold of methionine aminopeptidases

The crystal structure of the human PA2G4 homologue, Ebp1, was determined at 1.6 Å resolution (Supplementary Table 1). The atomic coordinates and structure factors have been deposited in the Protein Data Bank under the accession code: 2Q8K. The structure reveals a pita-bread fold as conserved in methionine aminopeptidases (MAP), prolidase, creatinase and amino peptidase P [27] (Fig. 1). Human MAP2 was identified as the closest structural homologue of Ebp1 by the program DALI [28] with an root mean square deviation (r.m.s.d.) of 2.0 Å over 299 residues. Like MAP2, Ebp1 contains an insert domain (residues: 244–305) of unknown function. Despite the high degree of structural conservation, Ebp1 shows a rather low sequence identity (less than 25%) with members of the MAP family (see sequence alignment and superposition in Supplementary Figs. 1 and 2, respectively).

3.2. Ebp1 does not show methionine aminopeptidase activity

A major structural feature of pita-bread enzymes is a large cavity primarily formed by the inner surface of the central beta-barrel (β-sheets 1, 3, 4, 5, 7 and 13 in Ebp1, Fig. 1). In MAP2, this cavity harbors the active site and adjacent elements for substrate recognition/binding. In all enzymes of this family (except creatinase), a divalent metal centre within the active site is involved in the hydrolytic cleavage of the substrate [27]. The two metal ions in human MAP2 are bound by monodentate (His331, and Glu364) and bidentate (Asp251, Asp262, and Glu459) ligation [24] (Fig. 2A). In

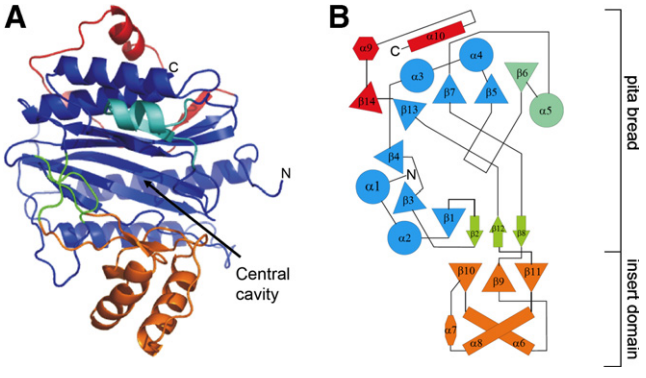

Fig. 1. Structure of Ebp1. (A) Ebp1 is shown in a ribbon representation, with the beta barrel forming the hydrophobic cavity in the center (indicated by an arrow). The pita-bread domain is coloured in blue, the insert domain in orange, the β-sheet connecting these domains in green, the Ebp1 specific helix at the entrance of the cavity in turquoise and the C-terminal elongation in red. (B) Topology diagram of Ebp1. For clarity the orientation is chosen 90° rotated compared to (A). β-Strands are represented by arrows and triangles, α-helices by cylinders and circles, and 3_{10} helices by hexagons.

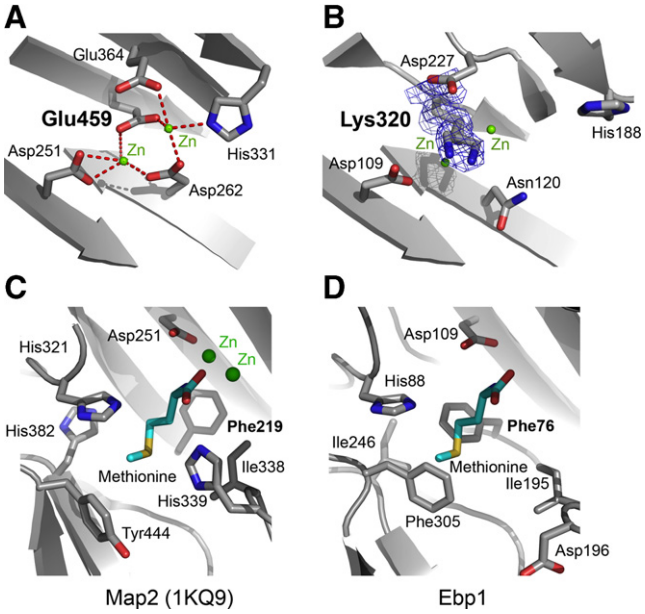

Fig. 2. Comparison of the central cavity in Ebp1 and MAP2. (A) Residues involved in metal binding and catalysis in MAP2 (pdb code: 1kq9) are shown. (B) The same region and orientation as in (A) is shown for Ebp1. For comparison the metal ions are included from a superposition with MAP2. The ε-amino group of Lys320 (adopting two alternative conformations) is occupying the metal binding site. Final $2F_O–F_C$ electron density for Lys320 is contoured at 1.5σ. (C) The substrate binding site of MAP2 is shown with bound Zn ions and methionine. (D) The hydrophobic binding site of Ebp1 with methionine placed from superposition with MAP2. Phe76 has a different conformation than the corresponding Phe219 in the MAP2/methionine complex and might block the binding site as suggested by the clash with methionine. In MAP2, residues involved in ligand binding are: Phe219 (Phe76), His231 (His88), Asp251 (Asp109), Ile338 (Ile195), His339 (Asp196), His382 (Ile246) and Tyr444 (Phe305) (corresponding residues in Ebp1 are given in brackets).

Ebp1, the metal ions are not present in the crystal structure and only two of the metal coordinating residues are conserved (Asp109 and His188) (Fig. 2A and B). Two negatively charged residues (Asp262, Glu364) in MAP2 are replaced by Asn120 and Asp227 in Ebp1. The most significant difference, however, is the change from Glu459 in MAP2 to Lys320 in Ebp1. The positive charge and the length of the Lys320 side chain hinder metal coordination both electrostatically and sterically (Fig. 2A and B). Thus, Ebp1 should not be able to perform hydrolytic substrate cleavage which was confirmed by a methionine aminopeptidase assay with a common MAP substrate (Fig. 3A).

3.3. The substrate binding pocket of methionine aminopeptidases is conserved in Ebp1

In MAP2, the substrate binding pocket is adjacent to the divalent metal ion binding site. The overall hydrophobic character of the methionine binding site as well as the electrostatic character of the cavity seems conserved in Ebp1 (Fig. 2C and D). This suggests that methionine or a small hydrophobic ligand should be able to bind. In MAP2, Phe219 and Ile338 are involved in hydrophobic interactions with the side chain of a bound methionine. In Ebp1 both residues are conserved (Phe76 and Ile195). However, the side chain of Phe76 has a different orientation with respect to Phe219 in the MAP2/methio-