Available online at www.sciencedirect.com
SCIENCE DIRECT
Protein Expression and Purification 48 (2006) 90–97
Protein Expression & Purification
www.elsevier.com/locate/yprep

Cloning, high yield over-expression, purification, and characterization of CG18594, a new PEBP/RKIP family member from *Drosophila melanogaster*

Gilles Rautureau, Laurence Jouvensal, Martine Decoville, Daniel Locker, Françoise Vovelle, Françoise Schoentgen *

Centre de Biophysique Moléculaire, UPR 4301 CNRS affiliated to Orléans University and to INSERM, Rue Charles Sadron, 45071 Orléans cedex 2, France

Received 24 November 2005, and in revised form 18 January 2006
Available online 20 February 2006

Abstract

The phosphatidylethanolamine-binding protein (PEBP) family is widely distributed in various species, from bacteria to mammals. These proteins seem to modulate important cell mechanisms: they control heterotrimeric G-proteins, inhibit the MAP-kinase and NFκB signaling pathways, and also serine proteases (thrombin, neuropsin, and chymotrypsin). In order to establish structure–function relationships for this family of proteins, our study focuses on PEBPs expressed within a single organism: *Drosophila melanogaster*, which constitutes a model system that lends itself well to establishing links between genes' expression and the corresponding proteins' functions, and to studying physiological mechanisms such as development. Here, we describe an optimized protocol for high level over-expression and high yield/high purity production of CG18594, one of *Drosophila* six putative PEBPs, for biophysical studies. The yield of the purified ^{15}N labeled protein is estimated to be 60 mg/L of M9 minimal medium. Analysis of the secondary structure using circular dichroism indicates that the protein comprises mainly β-sheets at pH 7. The good dispersion of the crosspeaks on the ^{1}H–^{15}N HSQC spectrum provides evidence of a proper folding of the purified protein, though its time evolution suggests a tendency to denature. Taken together, these data are consistent with the assumption that the CG18594 protein belongs to the PEPB family.

Keywords: RKIP; Phosphatidylethanolamine-binding protein; *Drosophila*; CG18594; CG7054; CG10298; CG17917; CG17919; CG6180; Purification; Isotope labeling; NMR

The phosphatidylethanolamine-binding protein (PEBP)[1] family is a highly conserved group of proteins found in a variety of tissues from a wide range of organisms, from bacteria to mammals and plants. PEBPs are proteins of about 20 kDa that were originally purified as cytosolic proteins from bovine brain [1]. Binding studies have shown that PEBPs have an affinity for anionic ligands, such as phosphatidylethanolamine, opioids, nucleotides such as GTP or GDP, and small GTP-binding proteins [2]. A number of biological functions have been proposed for PEBPs from various organisms, including inhibition of kinases of the MAP kinase [3] and NFκB signaling pathways [4], modulation of heterotrimeric G proteins [5], and also inhibition of serine proteases (thrombin, neuropsin, chymotrypsin) [6]. In humans, PEBP has been renamed Raf-1 kinase inhibitor protein (RKIP) [3]. Human PEBP/RKIP is implicated in cancer, and acts as a suppressor of metastases [7]. During cancer treatment, it sensitizes the cells to chemotherapy and immunotherapy [8]. Human PEBP/RKIP is also implicated in other pathologies: Alzheimer's disease [9], infertility [10,11], diabetes [12], etc. However, the molecular mechanisms by which PEBPs act remain obscure.

* Corresponding author. Fax: +33 (0) 238 63 15 17.
E-mail address: schoent@cnrs-orleans.fr (F. Schoentgen).

[1] *Abbreviations used:* PEBP, phosphatidylethanolamine-binding protein; RKIP, Raf-1 kinase inhibitor protein; ESTs, expressed sequence tags; LPS, lipopolysaccharides; SDS, sodium dodecyl sulfate; PCR, polymerase-chain reaction.

1046-5928/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2006.01.020

Crystal structures have been determined for a few members of the PEBP family (human (PDB code: 1BD9) [13], bovine (PDB code: 1B7A) [14], murine (PDB code: 1KN3) [15], yeast (PDB code: 1WPX) [16], *Antirrhinum* (PDB code: 1QOU) [17], *Arabidopsis* (PDB code: 1WKP) [18], and *Escherichia coli* (PDB code: 1FJJ) [19]), all of which show extensive fold conservation. The structure consists of a large central β-sheet flanked by a smaller one on one side, and by an α-helix on the other side. Sequence alignments show a conserved central region, D-P-D-x-P-x(11)-H-x(28,30)-H-R, which forms a consensus signature for the PEBP family [14]. This region forms part of a ligand-binding site, which can accommodate various anionic groups. The N- and C-terminal regions of the protein are the least conserved, and may be involved in interactions with different protein partners. In humans and rats, the N-terminal residues 2–12 form the natural cleavage peptide HCNP involved in neuronal development [20,21]. The C-terminal region is deleted in plant and bacterial PEBP homologues while it seems to help control accessibility toward the active site.

To establish structure–function relationships for this family of proteins, our study focuses on PEBPs expressed within a single organism: *Drosophila melanogaster*, which is one of the most intensively studied organisms in biology. Owing to its easy-to-manipulate genetic system, relatively low cost, and biological complexity comparable to that of a mammal, it is used as a model system for the investigation of many developmental and cellular processes common to higher eukaryotes, including humans. Indeed, many organ systems in mammals have well-conserved homologs in *Drosophila*, and research on *Drosophila* has already provided new insights into cancer, neurodegenerative diseases, behavior, immunity, aging, multigenic inheritance, and development. The study of the fruit fly genome (http://www.fruitfly.org/) has led to the identification of six PEBP paralogs. These six putative genes are transcribed since: full length cDNAs are commercially available for five of them (http://dgrc.cgb.indiana.edu/) (CG6180, CG17919, CG10298, CG7054 from adult head mRNAs, and CG18594 from larvae/pupae mRNA), and the last one, CG17917, has been detected as expressed sequence tags (ESTs) (http://flybase.bio.indiana.edu/). Moreover, CG18594 has been recently identified in the hemolymph of *D. melanogaster* [22], where it was proven to be over-expressed in larvae induced by *E. coli* lipopolysaccharides (LPS) [23] or by a suspension of Gram-positive bacteria or yeasts [24].

A sequence alignment of the six PEBPs from *D. melanogaster* is shown in Fig. 1. The six proteins show sequence differences (12% of identity for the six proteins) and their pI range from 5.0 to 9.7 for CG7054 and CG17917, respectively, indicating major differences in their physicochemical properties and probably in their modes of action. Understanding the reason why six different PEBPs have been maintained through evolution in *D. melanogaster* should be critical in deciphering the role of the PEBP family.

We have initiated a biophysical study on one of *Drosophila* putative PEBPs. In this paper, we present an improved method for high yield/high purity purification of CG18594, as well as an initial characterization of the purified protein.

Materials and methods

Cloning of cg18594 ORF into pET31b vector to produce untagged CG18594 and N- or C-terminally 6-His-tagged CG18594

Since the *cg18594* gene is intron free, purified genomic DNA from *D. melanogaster* Oregon R was used as a direct template for PCR instead of mRNA. Oligonucleotide primers corresponding to the *cg18594* ORF were designed and synthesized by MWG Biotech AG, based on the published genome sequence of *D. melanogaster* (http://flybase.bio.indiana.edu/). For the untagged protein, the forward primer (5′-CATATGGACACCGCCGGCATTAT-3′) included a *NdeI* restriction site at the 5′ end of the ORF (underlined) while the reverse primer (5′-CTCGAG TTACTGGAC CGTCTCG-3′) added a *XhoI* restriction site at the 3′ end of the ORF (underlined). The N-terminally 6-His-tagged protein

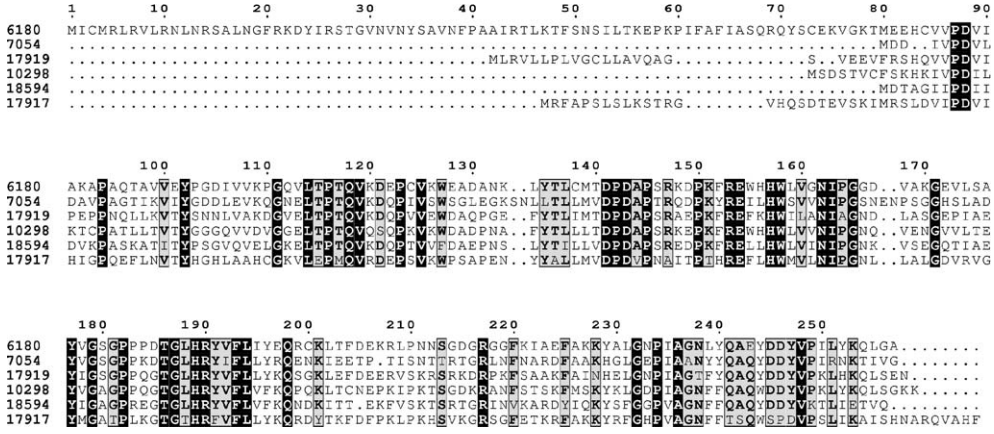

Fig. 1. Sequence alignment of the six PEBPs from *D. melanogaster*. Multiple sequence alignments were carried out using the sequence analysis program CLUSTALW. Black boxes indicate identities and clear boxes homologies.