Biochemical and Biophysical Research Communications 414 (2011) 390–396

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The translation initiation factor, PeIF5B, from *Pisum sativum* displays chaperone activity

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ARTICLE INFO

Article history:
Received 6 September 2011
Available online 21 September 2011

Keywords:
eIF5B
Pea translation initiation factor
Chaperone
Thermo-stability
Light scattering

ABSTRACT

We earlier documented the structural and functional characterization of PeIF5B factor from *Pisum sativum* that shows strong homology to the universal translation initiation factor eIF5B (Rasheedi et al., 2007, 2010 [12,13]). We now show that PeIF5B is an unusually thermo-stable protein resisting temperatures up to 95 °C. PeIF5B prevents thermal aggregation of heat labile proteins, such as citrate synthase (CS) and *Nde*I, under heat stress or chemical denaturation conditions and promotes their functional folding. It also prevents the aggregation of DTT induced insulin reduction. GTP appears to stimulate PeIF5B-mediated chaperone activity. *In-vivo*, PeIF5B over expression significantly enhances, the viability of *Escherichia coli* cells after heat stress (50 °C). These observations lead us to conclude that PeIF5B, in addition to its role in protein translation, has chaperone like activity and could be likely involved in protein folding and protection from stress.

1. Introduction

The process of translation initiation, the rate-limiting step of protein synthesis, has been conserved in all living forms: bacteria, archea and eukaryotes [1,2]. Protein synthesis in prokaryotes mainly requires three initiation factors (IF1, IF2, IF3), whereas eukaryotes use several initiation factors (eIFs), many of which consist of multiple subunits. Currently >12 eIFs, composed of at least 29 distinct subunits, have been identified [3]. eIF5B is one of the few translation initiation factors, which is considered as a universal translation factor with homologs in bacteria (IF2) and archea (aIF2) [2,4]. eIF5B has been characterized so far from *Drosophila melanogaster* [5], *Homo sapiens* [2] and *Sacharomyces cerevisea* [6]. eIF5B stabilizes the binding of methionyl tRNA to the ribosomal P-site [4], assists in ribosome subunit joining and also harbors GTPase activity to facilitate its dissociation for recycling [4]. eIF5B deletion in yeast leads to growth retardation due to impaired translation initiation thereby demonstrating its significance *in vivo* [6]. Although IF2 and eIF5B are similar in their structures, they differ greatly in their functions [7–11].

We earlier demonstrated the presence of a novel 96 kDa eIF5B-like factor from plant kingdom (*Pisum sativum*) with homology to human eIF5B and bacterial IF2 initiation factors [12]. The recombinant 96 kDa PeIF5B was characterized in terms of its GTP binding, physico-chemical properties, followed by homology modeling [13]. We now describe the chaperone-like activity of PeIF5B and show that it is an unusually thermo-stable protein that prevents heat and chemical stress mediated aggregation and loss of functional activity and also helps to maintain viability of *Escherichia coli* cells under high temperatures.

2. Materials and methods

2.1. Materials

Citrate synthase (CS) from porcine heart, IPTG, imidazole, 8-anilino-1-naphthalene-sulfonic acid (ANS), insulin and DTT were obtained from Sigma, *Nde*I was from NEB. All other chemicals were of high analytical grade.

Abbreviations: rPeIF5B, recombinant PeIF5B; CS, citrate synthase; GnHCl, guanidine hydrochloride.

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doi:10.1016/j.bbrc.2011.09.085

2.2. Expression and purification of rPeIF5B

PeIF5B was expressed in *E. coli* as described [13]. Recombinant PeIF5B (rPeIF5B) protein was purified by affinity chromatography. The unfolded purified protein was folded back to its native conformation by dialysis against 20 mM Tris–HCl (pH 8.0) and 100 mM NaCl and used for further study. Protein concentration was determined using Bradford assay.

2.3. Fluorescence spectroscopy

Fluorescence measurements were carried out on Perkin Elmer LS55 luminescence Spectrometer equipped with a Peltier device. Protein sample was excited at 280 nm for intrinsic fluorescence and emission spectrum was collected in the range from 300–400 nm with 10 and 5 nm slit widths for excitation and emission, respectively. For ANS binding, excitation was carried out at 390 nm whereas emission spectra were recorded in the range 450–550 nm with 10 nm slit width. The molar ratio of PeIF5B and ANS was 1:50. The spectra were corrected with appropriate protein and buffer blanks [14].

2.4. Circular dichroism

CD measurements were recorded on Jasco J-810 spectropolarimeter. Mean residual ellipticity (MRE) in deg cm^2 $dmol^{-1}$ was calculated using the following equation [15]:

$$\text{MRE} = \theta_{obs}/n \times l \times c \times 10$$

where θ_{obs} is the observed ellipticity (millidegrees), n is the number of amino acid residues, l is the path length (cm) and c is the concentration (moles/liter). Each spectrum is an average of five scans and base line recorded for the buffer under similar conditions was subtracted from the values obtained. Secondary structure was monitored in the range 200–250 nm in 1 mm path length cuvette with a protein concentration of 1 μM as measured using Bradford's method.

2.5. Thermo-stability studies

Protein samples were incubated in a thermostatically controlled cell holder fitted with the Julabo-F25 water bath and changes were monitored at regular intervals of temperature by fluorescence, CD, light scattering or enzyme assay. Light scattering measurements of 1 μM of rPeIF5B and CS and 5 units of *Nde*I in 50 mM HEPES–KOH (pH 8.0) were recorded for 45 min at 45 °C. Thermal inactivation of the restriction enzyme was carried out at 60 °C for 20 min for *Nde*I in absence and presence of 1 and 2 μg of rPeIF5B.

2.6. Light scattering analysis

Light scattering measurements were recorded on Cary 100 spectrophotometer at 320 nm for heat induced aggregation study [16,17], or at 360 nm for aggregation caused by disulfide bond reduction [14].

2.7. Aggregation by reduction of disulfide bonds

0.4 mg/ml of insulin was treated with 20 mM DTT in 50 mM sodium phosphate buffer (pH 7.5) in presence of different concentrations of rPeIF5B. Stability of insulin, as a function of time, was measured by light scattering at 360 nm. BSA was used as a control.

2.8. Restriction enzyme activity assay

Ten units of *Nde*I were used to digest 150 ng of pUC18 plasmid DNA in appropriate buffer in a final volume of 10 μl. The reaction mixtures were incubated at 37 °C. The digested products were fractionated on 1% agarose gel.

2.9. Refolding of GnHCl induced denatured protein

Denaturation, refolding and activity assay of CS were performed as described previously [14]. Briefly, CS was denatured at room temperature for 30 min with 6 M guanidine hydrochloride (GnHCl). The denatured CS was refolded by 100× dilution with refolding buffer (0.02 M potassium phosphate buffer, pH 7.5, 0.01 M $MgCl_2$, 2 mM ATP, 1 mM oxaloacetic acid (OAA), in presence or absence of rPeIF5B at 37 °C for 1 h.

2.10. CS activity assay

Activity assay was performed by adding 15 μM of enzyme to the assay mixture containing OAA (1 mM), acetyl-CoA (0.16 mM) and 20 mM Tris (pH 7.4). Enzyme activity was measured by decrease in absorbance at 233 nm.

2.11. Cell viability assay

E. coli cells, carrying either pET23a vector or pET*PeIF5B*, were grown at 37 °C till log phase. Induction of *PeIF5B* gene expression was achieved by adding IPTG (1 mM) and the culture further incubated for 20 min at 37 °C. All cultures were diluted to attain an OD of 0.4 at 600 nm. Following induction, one set of culture was continued at 37 °C while the other set of culture was transferred to

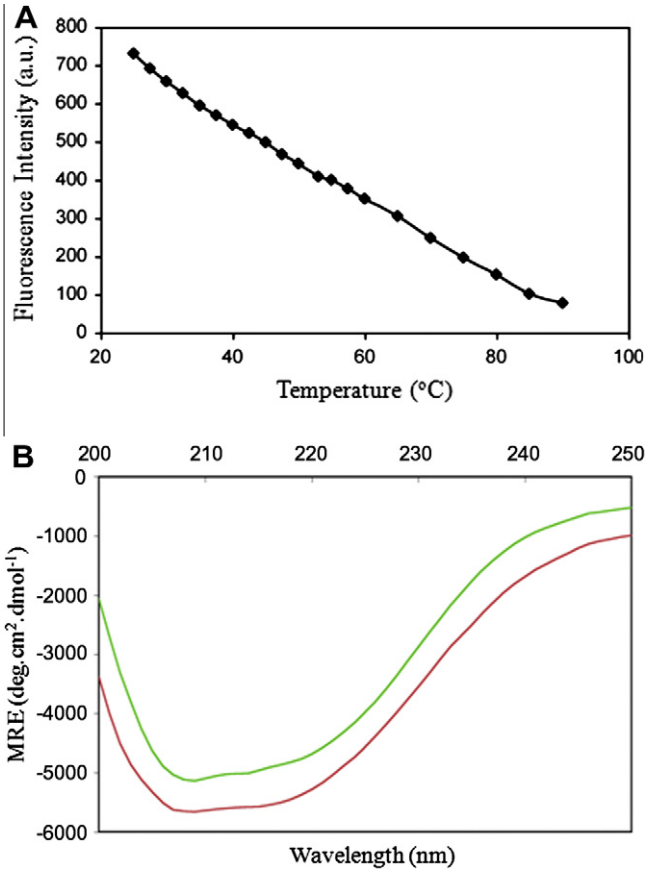

Fig. 1. Temperature induced denaturation of rPeIF5B. (A) Changes in intrinsic fluorescence at 345 nm on thermal denaturation of rPeIF5B (25–95 °C) when excited at 280 nm. (B) Far-UV CD spectra of rPeIF5B at 25 °C (green) and 95 °C (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)