Biochimica et Biophysica Acta 1864 (2016) 1237–1243

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Group 3 LEA protein model peptides protect enzymes against desiccation stress

Takao Furuki, Minoru Sakurai *

Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259-B-62, Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 23 March 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Group 3 LEA protein model peptide
Trehalose
Enzyme
Desiccation tolerance
Dry preservation

ABSTRACT

We tested whether model peptides for group 3 late embryogenesis abundant (G3LEA) proteins, which we developed previously, are capable of maintaining the catalytic activities of enzymes dried in their presence. Three different peptides were compared: 1) PvLEA-22, which consists of two tandem repeats of the 11-mer motif found in G3LEA proteins from an African sleeping chironomid; 2) PvLEA-44, which is made of four tandem repeats of the same 11-mer motif; and 3) a peptide whose amino acid composition is the same as that of PvLEA-22, but whose sequence is scrambled. We selected two enzymes, lactate dehydrogenase (LDH) and β-D-galactosidase (BDG), as targets because they have different isoelectric point (*pI*) values, in the alkaline and acidic range, respectively. While these enzymes were almost inactivated when dried alone, their catalytic activity was preserved at ≥70% of native levels in the presence of any of the above three peptides. This degree of protection is comparable to that conferred by several full-length G3LEA proteins, as reported previously for LDH. Interestingly, the protective activity of the peptides was enhanced slightly when they were mixed with trehalose, especially when the molar content of the peptides was low. On the basis of these results, the G3LEA model peptides show promise as protectants for the dry preservation of enzymes/proteins with a wide range of *pI* values.

1. Introduction

Late embryogenesis abundant (LEA) proteins were initially discovered in cotton seeds over 30 years ago [1,2]. They mainly accumulate to high concentrations in the late stages of embryo development in plant seeds [3–12]. In many plant species, LEA protein expression is associated with the acquisition of tolerance against drought, freezing, and salinity stresses [3–12]. Later, LEA proteins were also found in animals [11], including nematodes [13–17], bdelloid rotifers [18,19], an African sleeping chironomid, *Polypedilum vanderplanki* [20], crustaceans [21,22], tardigrades [23,24], and springtails [25]. LEA proteins are classified into several groups according to their gene expression pattern and amino acid sequence [3–6,8–11], or their amino acid usage and physicochemical properties [26]. Most LEA proteins discovered so far in animals are Group 3 LEA (G3LEA) proteins [11].

G3LEA proteins are characterized by several tandem repeats of an 11-mer motif, whose amino acid sequence is loosely conserved across all organisms mentioned above [8,11]. The 11-mer motifs are rich in polar residues, including Lys, Glu, and Asp, rendering G3LEA proteins very hydrophilic in nature [8,9,11]. G3LEA proteins are also intrinsically disordered proteins, i.e. they are disordered in aqueous solution, but they can develop predominantly α-helical structures when dried, as revealed by many in vitro FT-IR spectroscopic studies [19,27–31].

Multiple biological functions have been reported for G3LEA proteins [32–34]. In vitro experiments showed that a G3LEA protein from a nematode, *Aphelenchus avenae* (hereafter denoted as AavLEA1) suppresses desiccation-induced aggregation and inactivation of enzymes such as lactate dehydrogenase (LDH) and citrate synthase (CS) [35]. Similar results were obtained with water-soluble proteomes [36]. Interestingly, AavLEA1 is also able to inhibit the spontaneous aggregation of polyglutamine-containing proteins within cells [36–38]. Protection against desiccation-induced damage of enzymes is commonly observed for other G3LEA proteins, including PvLEA4 from African sleeping chironomid larvae (*P. vanderplanki*) [31,39–44]. It has been reported that some G3LEA proteins are also able to suppress the fusion of liposomes during desiccation [45,46] and others even to enhance desiccation tolerance in mammalian cells [47]. Thus, G3LEA proteins, as well as other LEA proteins, are associated with the ability of some invertebrates to undergo anhydrobiosis, or desiccation tolerance [8,11,12].

To elucidate the roles of the repeated 11-mer motifs in G3LEA proteins, we launched a series of investigations into the structural and functional properties of chemically synthesized 22-mer and 44-mer peptides representing two or four tandem repeats of the 11-mer consensus motif from several anhydrobiotic organisms [48–51]: for example, we synthesized 22-mer and 44-mer peptides (hereafter denoted

Abbreviations: G3LEA, Group 3 late embryogenesis abundant; PvLEA-22 and PvLEA-44, 22- and 44-mer peptides, respectively, derived from the G3LEA proteins of an African sleeping chironomid (*Polypedilum vanderplanki*); LDH, lactate dehydrogenase; BDG, β-D-galactosidase; *pI*, isoelectric point.

* Corresponding author.
E-mail address: msakurai@bio.titech.ac.jp (M. Sakurai).

http://dx.doi.org/10.1016/j.bbapap.2016.04.012
1570-9639/

as PvLEA-22 and PvLEA-44, respectively) derived from the consensus 11-mer motif of G3LEA proteins in an African sleeping chironomid. These G3LEA model peptides faithfully reproduce the conformational features of the parent G3LEA proteins in both the aqueous and dry states [48,49] and exhibit almost the same level of anti-aggregation activity with target proteins such as α-casein and lysozyme [50] in vitro. Recently, PvLEA-22 was also shown to inhibit desiccation-induced fusion of liposomes [51]. From these studies, it is evident that the G3LEA model peptides we designed can act as effective protectants against desiccation-induced aggregation and/or fusion of proteins and membranes. However, in the case of enzymes, we have not yet tested whether their catalytic activity is maintained by addition of G3LEA model peptides during the dehydration–rehydration process.

In this paper, to gain a more in-depth understanding of the functions of the repeated 11-mer motif region of G3LEA proteins and to further explore the potential utility of G3LEA model peptides as biological protectants, we examined to what extent the peptides can preserve the catalytic activity of target enzymes during dehydration–rehydration stress. Here, two different enzymes, LDH from rabbit muscle and β-D-galactosidase (BDG) from *Escherichia coli*, were selected as targets for the following reasons: (1) LDH from rabbit muscle has frequently been used in functional studies of several native G3LEA proteins [31,35,39,40,42–44], and (2) both LDH and BDG are tetrameric in their active state, but they have substantially different isoelectric points (*pI*s), which are 8.3 [52] and 4.6 [53], respectively. In addition to the above G3LEA model peptides, we also used a peptide with an identical amino acid composition to that of PvLEA-22, but whose sequence is scrambled [48–51]. This allowed us to examine whether amino acid sequence or composition is important for the biological functions of the 11-mer motif. Furthermore, because the disaccharide trehalose plays an important role in anhydrobiosis [54–61] and may cooperate with LEA proteins to preserve biological components in the dry state [31,35,48,62], we also examined the combined effect of trehalose and the G3LEA model peptides on the target enzymes during water stress.

2. Materials and methods

2.1. Reagents

PvLEA-22, PvLEA-44, and a peptide with scrambled sequence were purchased from Funakoshi Co. (Tokyo, Japan). PvLEA-22 and PvLEA-44 consist of two and four tandem repeats, respectively, of the 11-mer motif, AKDGTKEKAGE. The scrambled peptide has the sequence, AKEKEGTDKAGGAKDTGEKEKA, identical to that of the control peptide used in our previous studies [48–50]. Trehalose was kindly given as a gift by Hayashibara Co. (Okayama, Japan). LDH from rabbit muscle was purchased from Roche (Lewes, East Sussex, UK). BDG from *Escherichia coli* and anhydrous magnesium chloride were purchased from Wako Co. (Oosaka, Japan). Nicotinamide adenine dinucleotide (NADH), pyruvic acid, 2-nitrophenyl β-D-galactopyranoside (ONPG), and 2-mercaptoethanol were from Sigma-Aldrich Co. (St. Louis, USA).

2.2. Preparation of enzyme solutions

LDH, which was obtained in the form of ammonium sulfate suspensions, was subjected to gel filtration to remove the electrolytes before use. The LDH concentration in the eluted solution was determined by measuring the absorbance at 280 nm, using an extinction coefficient of 0.88 ml cm^{-1} mg^{-1} [63]. An LDH solution with a concentration of 10 μg/ml (7.4×10^{-11} mol/ml) was then prepared in 50 mM potassium phosphate buffer (pH 7.5). BDG in the lyophilized form was used as received. A BDG solution with a concentration of 7 μg/ml (1.5×10^{-11} mol/ml) was prepared in 100 mM potassium phosphate buffer (pH 7.5). An extinction coefficient of 2.09 ml cm^{-1} mg^{-1} at 280 nm was used [64] to determine BDG concentration.

The protective activity of the G3LEA model peptides was tested by adding them individually to the above enzyme solutions before drying. The molar ratios of the model peptide relative to LDH or BDG ranged from 100 to 2000, or from 150 to 2400, respectively. For comparison, the effect of trehalose alone was tested for both the enzymes. Then, it was added at molar ratios relative to LDH from 1000 (0.075 mM) to 2×10^4 (1.5 mM), and relative to BDG from 3000 (0.05 mM) to 6×10^4 (1 mM). Furthermore, to examine the synergistic effect of trehalose with either PvLEA-22 or PvLEA-44, trehalose was added at a molar ratio of 2000 (0.15 mM) and 6000 (0.1 mM) relative to LDH or BDG, respectively. Since the molecular surface area of BDG is about three times that of LDH, as described below, a three-times greater amount of trehalose was added in the BDG activity test than for LDH. A 50 μl droplet of each enzyme solution was dried on a flat Teflon plate (1.5 cm × 1.5 cm) in a vacuum desiccator at ambient temperature for three days. Then, the dried sample was rehydrated with 50 μl of Milli-Q water and was left as it was for 10 min, followed by being slowly stirred. At this stage, the 1st stress treatment was completed. For BDG samples, the 2nd stress treatment was done according to almost the same drying–rehydration procedure.

2.3. Enzyme activity assay

The activity of LDH can be monitored as the catalyzed oxidation of NADH, which is accompanied by reduction of pyruvic acid. Thirty μl of the LDH solution prepared above was added to a solution composed of (a) and (b), where (a) is 2 ml of 0.2 mM NADH solution, and (b) is 50 μl of 40 mM pyruvate solution. Both solutions (a) and (b) were prepared in 100 mM potassium phosphate buffer (pH 7.5). The change in absorbance at 340 nm, A_{340}, due to oxidation of NADH, was measured every 15 s. Residual LDH activity (%) was defined as follows:

$$\text{Residual activity} = \frac{\Delta A_{340}^{post}}{\Delta A_{340}^{pre}} \times 100\%$$

where ΔA_{340}^{pre} and ΔA_{340}^{post} are the absorbance changes at 340 nm from time = 0 to 4 min and the superscripts "*pre*" and "*post*" indicate the measurements before drying and after drying–rehydration, respectively.

The activity of BDG can be monitored as the catalyzed hydrolysis of ONPG, which produces o-nitrophenol (ONP) and D-galactose. Thirty μl of the BDG solution prepared above was added to 1 ml of an assay buffer (pH 7.5) composed of 100 mM phosphate, 100 mM mercaptethanol, 1 mM $MgCl_2$, and 2.26 mM ONPG. The change in absorbance at 410 nm, A_{410}, due to hydrolysis of ONP, was measured every 30 s. Residual BDG activity (%) was defined as follows:

$$\text{Residual activity} = \frac{\Delta A_{410}^{post}}{\Delta A_{410}^{pre}} \times 100\%$$

where ΔA_{410}^{pre} and ΔA_{410}^{post} represent the absorbance changes of ONP from time = 0 to 8 min, measured before drying and after drying–rehydration, respectively.

2.4. Statistics

Each experiment for the residual activity of the target enzyme was performed with six replicates. Statistical relevance was determined by two-way ANOVA using Prism version 6 (GraphPad Software, La Jolla, CA). Its results will be given in supplemental Tables S1 to S6 for Figs. 2, 3(A), (B), 5, 6(A), and (B), respectively.

3. Results

Fig. 1(A) shows the time course of the absorbance at 340 nm for the LDH solution without protectant peptide. Before drying (squares), the absorbance decreased with time, indicating that the enzyme was active.