FEBS Letters 585 (2011) 630–634

FEBS Letters

journal homepage: www.FEBSLetters.org

Both plant and animal LEA proteins act as kinetic stabilisers of polyglutamine-dependent protein aggregation

Yun Liu [a,1], Sohini Chakrabortee [b,1], Ranhui Li [a], Yizhi Zheng [a,*], Alan Tunnacliffe [b,*]

[a] School of Life Science, Shenzhen University, Nanhai Ave 3688, Shenzhen City, Guangdong Province, 518060, PR China
[b] Department of Chemical Engineering and Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, UK

ARTICLE INFO

Article history:
Received 12 November 2010
Revised 10 January 2011
Accepted 12 January 2011
Available online 18 January 2011

Edited by Julian Schroeder

Keywords:
Molecular shield
Protein homeostasis
Proteostasis
Desiccation tolerance
Anhydrobiosis
Cold tolerance

ABSTRACT

LEA (late embryogenesis abundant) proteins are intrinsically disordered proteins that contribute to stress tolerance in plants and invertebrates. Here we show that, when both plant and animal LEA proteins are co-expressed in mammalian cells with self-aggregating polyglutamine (polyQ) proteins, they reduce aggregation in a time-dependent fashion, showing more protection at early time points. A similar effect was also observed in vitro, where recombinant LEA proteins were able to slow the rate of polyQ aggregation, but not abolish it altogether. Thus, LEA proteins act as kinetic stabilisers of aggregating proteins, a novel function in protein homeostasis consistent with a proposed role as molecular shields.

1. Introduction

Work on water stress in plants and animals has highlighted a role for the late embryogenesis abundant (LEA) proteins in tolerance to desiccation and cold stress [1,2]. The LEA proteins, originally termed LEA proteins due to their discovery in maturing plant seeds, are also expressed in plant tissues, both in response to stress and constitutively. Various invertebrates that undergo anhydrobiosis, including nematodes, rotifers, tardigrades and brine shrimps, are known to express LEA proteins. There are three main LEA protein groups (i.e. groups 1, 2 and 3), each differentiated by sequence motifs and peptide profiles, but sharing properties of high hydrophilicity and lack of secondary structure: they are intrinsically disordered proteins [3]. While plants express a large diversity of members of all groups, with over 50 LEA proteins known in *Arabidopsis* [4,5], for example, invertebrates contain only group 3, and in some cases group 1, proteins, and their LEA proteomes are of lower complexity.

Recent research has focused on the molecular functions of LEA proteins, in particular the protection of proteins, membranes and nucleic acids during water stress [1]. An animal group 3 LEA protein, AavLEA1, from the anhydrobiotic nematode *Aphelenchus avenae*, and a plant group 1 LEA protein, Em, from wheat, reduce water stress-induced protein aggregation in vitro [6,7]. Other LEA proteins have also been shown to reduce aggregation of target proteins under stress conditions [8–11]. Strikingly, anti-aggregation activity has also been demonstrated for AavLEA1 under hydrated conditions in cells, where the nematode protein is able to reduce formation of aggregates in proteins containing extended polyglutamine (polyQ) and polyalanine sequences [7].

This protein protection activity is reminiscent of that of molecular chaperones involved in protein homeostasis (or proteostasis), i.e. the facilitation and maintenance of correct protein folding and assembly. However, there are significant points of difference, since LEA proteins are essentially unstructured and their genes are generally not upregulated in response to heat stress, in contrast to those of many of the classical molecular chaperones, still often known as "heat shock proteins". In addition, many chaperones function by forming transient complexes with their target proteins, often through hydrophobic interfaces. This is unlikely for LEA proteins, which are entropic chains with very low hydrophobicity and

Abbreviations: LEA, late embryogenesis abundant; PBS, phosphate-buffered saline; IDP, intrinsically disordered protein; EGFP, enhanced green fluorescent protein; GST, glutathione transferase; ThT, thioflavin T; polyQ, polyglutamine; FRET, Förster resonance energy transfer; DAPI, 4′,6-diamidino-2-phenylindole

* Corresponding authors. Fax: +86 755 26534274 (Y. Zheng), +44 1223 334162 (A. Tunnacliffe).

E-mail addresses: yzzheng@szu.edu.cn (Y. Zheng), at10004@cam.ac.uk, at10004@biotech.cam.ac.uk (A. Tunnacliffe).

[1] These authors contributed equally.

0014-5793/$36.00
doi:10.1016/j.febslet.2011.01.020

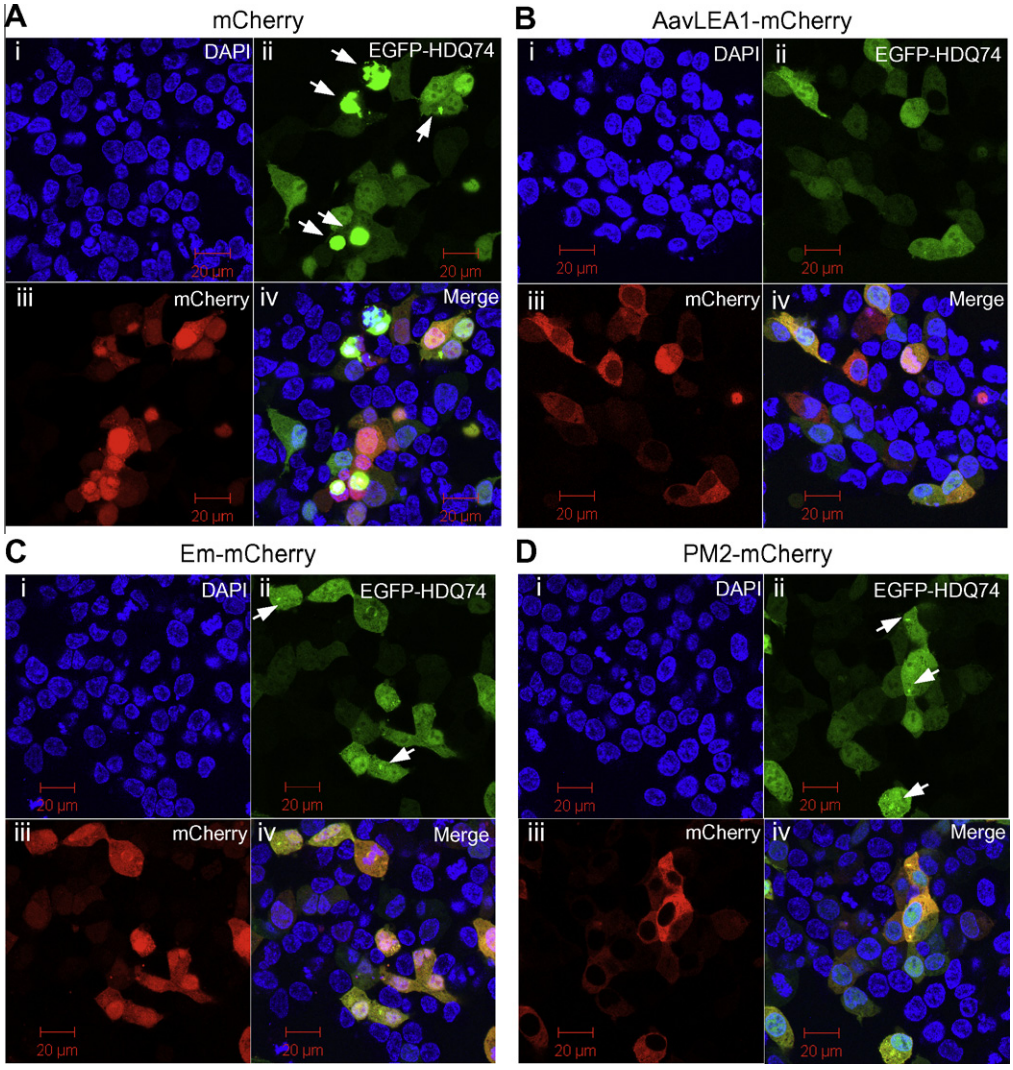

Fig. 1. Confocal microscopy of T-REx293 cells co-expressing EGFP-HDQ74 and mCherry tagged LEA proteins. Images were captured 24 h after co-transfection of constructs encoding EGFP-HDQ74 and (A) mCherry, (B) AavLEA1-mCherry, (C) Em-mCherry, and (D) PM2-mCherry. Nuclei are stained blue with DAPI (panels labelled i) EGFP-HDQ74 is green (ii) and mCherry proteins are red (iii); a merged image is also shown (iv). Indicative EGFP-HDQ74 aggregates are shown by arrowheads. Scale bar: 20 µm.

structure content; instead, we have proposed they behave more like "molecular shields", exerting an excluded volume effect that decreases interaction between polypeptides with the potential to aggregate. One implication of this hypothesis is that molecular shield proteins will decrease the collision frequency between aggregating species, but will not abolish such interactions entirely. Therefore, over sufficient time, protein aggregates will still form, but at a reduced rate. We set out to test this idea here with LEA proteins from plant and animal sources, and show that, both in living cells and in the test tube, polyQ-dependent protein aggregation is slowed but not abolished, consistent with a molecular shield function.

2. Materials and methods

2.1. Constructs

EGFP-HDQ74. constructs have been described [7,12]. cDNA sequences of AavLEA1 [13], Em (Accession No. AAB71224) and PM2 [14] were cloned into the pmCherry vector (Clontech) using *Kpn*I and *Bam*HI sites for AavLEA1 and PM2, and *Hin*dIII and *Bam*HI sites for Em. PolyQ sequences (Q23 or Q37) were obtained by PCR using HDQ23/HDQ74 as the template, and products were cloned into the pGEX5a vector (Pharmacia Biotech) using the restriction sites *Bam*HI and *Eco*RI. All clones were validated by sequencing.

2.2. Mammalian cell aggregation assay

T-REx293 (Invitrogen) cells were grown, and transfected as described [7] and analysed at the indicated time points post-transfection. Approximately 200 EGFP-positive T-REx293 cells co-expressing mCherry, AavLEA1-mCherry, Em-mCherry or PM2-mCherry were counted for each sample in triplicate, and each experiment was repeated at least twice. Images were acquired with a Zeiss LSM510 META confocal microscope (63x, 1.4NA PlanApochromat objective; v3.2 software). Aggregate-containing cells were scored as percentages and *P* values were determined by logistic regression analysis (estimated odds ratio and 95% confidence intervals) using SPSS software (SPSS, Chicago) as described [12].

2.3. Recombinant proteins

Expression and purification of recombinant His-tagged versions of AavLEA1 and PM2 were performed as described [14,15]. The Em sequence was cloned into the pET28a+ vector (Novagen; at the