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Review

Conserved methionines in chloroplasts

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Abstract

Heat shock proteins counteract heat and oxidative stress. In chloroplasts, a small heat shock protein (Hsp21) contains a set of conserved methionines, which date back to early in the emergence of terrestrial plants. Methionines M49, M52, M55, M59, M62, M67 are located on one side of an amphipathic helix, which may fold back over two other conserved methionines (M97 and M101), to form a binding groove lined with methionines, for sequence-independent recognition of peptides with an overall hydrophobic character. The sHsps protect other proteins from aggregation by binding to their hydrophobic surfaces, which become exposed under stress. Data are presented showing that keeping the conserved methionines in Hsp21 in a reduced form is a prerequisite to maintain such binding. The chloroplast generates reactive oxygen species under both stress and unstressed conditions, but this organelle is also a highly reducing cellular compartment. Chloroplasts contain a specialized isoform of the enzyme, peptide methionine sulfoxide reductase, the expression of which is light-induced. Recombinant proteins were used to measure that this reductase can restore Hsp21 methionines after sulfoxidation. This paper also describes how methionine sulfoxidation–reduction can be directly assessed by mass spectrometry, how methionine-to-leucine substitution affects Hsp21, and discusses the possible role for an Hsp21 methionine sulfoxidation–reduction cycle in quenching reactive oxygen species.

Keywords: Chaperone; Heat stress; Oxidative stress; Photosynthesis; Protein mass spectrometry; Protein–protein interaction

1. Introduction

Heat shock proteins, or chaperones, participate in the proper folding of proteins under normal conditions, and especially under stress conditions. The so-called small heat shock proteins (sHsps) [1,2] actually form large oligomeric complexes. The name signifies that sHsps have monomeric subunits that are much smaller (approximately 20 kDa) than those of other classes of chaperones (Hsp60, 70, 90 and 100). The sHsps play an important role in stress protection. The large oligomeric complexes provide a means of rapidly exposing subunits, which can offer hydrophobic surfaces onto which hydrophobic regions of partially denatured substrate proteins can be bound, and thereby be rescued from aggregation (Fig. 1). When stress is over, bound substrate proteins can be released and refolded, assisted by other chaperones in an ATP-dependent manner [3–5]. Thus, sHsps have the ability to chaperone other proteins during stress. They can also interact with the membranes [6,7]. In mammalian cells, sHsps interact with the cytoskeleton [8,9], and are involved in regulation of the redox state of a cell [10,11]. Mammalian sHsps are especially important in fighting the "misfolded-protein" diseases, such as Parkinson's Disease, Creutzfeldt–Jakob Disease and several amyloidoses [12], where sHsps are detected in the malfunctioning protein aggregates [13–15].

In plants, sHsps show an unusual abundance and diversity [16], which may reflect the need for plants to quickly adapt to continuously changing physical conditions. Plants cannot escape from adverse stresses, either

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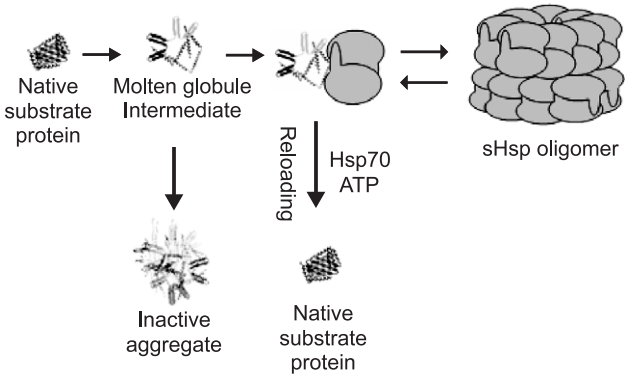

Fig. 1. Small heat shock proteins (sHsps) chaperone other proteins during transient stress. The large oligomeric sHsps complexes provide a means of rapidly exposing subunits with hydrophobic surfaces onto which hydrophobic regions of partially denatured substrate proteins can be bound, and thereby be rescued from aggregation. When stress is over, bound substrate proteins can be released and refolded, assisted by other chaperones, like Hsp70, and ATP [5].

biotic or abiotic, and are also incapable of any fine control, or homoeostasis, of their body temperature. Plants are therefore fully exposed to any variation in their ambient conditions, including often drastically changing temperatures, switching between low and high light intensities, drought stress, salinity stress, nutrient deficiency stress and oxidative stress. Many stress responses and regulatory pathways are regulated by transcription factors (TF) and these proteins play an especially important role in the life of higher plants. The crucial role of TFs is shown by their great abundance in the genome, for example in *Arabidopsis thaliana*, as much as 5.9% of the nuclear genome encodes TF genes, resulting in the expression of more than 1500 TF proteins [17,18].

The diversity of plant sHsps is reflected by the presence in the *Arabidopsis* genome of no fewer than 19 open reading frames that encode sHsp-related proteins [19]. Van Montfort et al. [20] have made important contributions to our understanding of plant sHsps. The first structure of a eukaryotic sHsp, a cytosolic Hsp16.9 from wheat, *Triticum aestivum*, was resolved to atomic resolution. This structure revealed a dodecameric double ring, each ring containing a trimer of dimers, each dimer formed by intertwined monomers, each monomer made of a β-sandwich of two antiparallel β-sheets. The C-terminal extensions tie the two rings together, in each dimer one C-terminal extension interacts with another dimer in the ring, the other with a dimer in the other ring. Another novel insight gained from this structure showed how the N-terminal arms, previously known to be important for sHsp oligomer stability [21], are located in the interior of the double ring and stabilize the oligomer by three loose knots formed by pairwise intertwining of the N-terminal α-helices. All sHsps are composed of a conserved C-terminal domain of approximately 90 amino acids ("the α-crystallin domain"), flanked by a variable N-terminal arm (24–94 amino acids) and a C-terminal extension (12–30 amino acids) [1]. The N-terminal region is probably important for binding of substrate proteins, as outlined below for Hsp21.

Lee and Vierling [22] and Mogk et al. [5] also have demonstrated the requirement of ATP plus other chaperones, Dna J, K, E and Hsp100, for release of substrate proteins bound to a sHsp, utilizing the Hsp16.6 in the model organism *Synechocystis*, a cyanobacterium. Furthermore, several endogenous substrate proteins which bind to this sHsp were recently identified in a rationale way [23], by combining wild-type *Synechocystis* and a deletion mutant, Hsp16.6 antiserum and preimmunized serum to avoid protein which bind unspecifically. By two different immunotechniques, immunoprecipitation and affinity chromatography, 13 substrate proteins were identified. All these Hsp16.6 substrates were heat-labile proteins, representing a wide range of cellular functions (transcription, translation, cell signalling, secondary metabolism), where they supposedly play key roles. These pioneering results pave the way for similar investigations on other sHsps, and open up new opportunities for more detailed investigations of these 13 sHsp substrate proteins that are proposed to play key roles in the life cycle of the photosynthetic cyanobacterium *Synechocystis*, and which may also serve as a starting point for a better understanding of sHsps in higher plants.

The small heat shock proteins comprise much of the protein induced during heat stress in plants. However, sHsps, and plant sHsps, can also be induced by specific developmental stimuli [16,24], such as embryo development, germination, pollen development, fruit maturation, or abscisic acid-induced ageing and leaf senescence. These conditions probably all involve an element of oxidative stress. There are five well-characterized plant sHsp gene families, of which two are expressed as soluble cytosolic proteins, one as an ER-associated protein, one in mitochondria and one in chloroplasts. Phylogenetic analyses show that the organellar sHsps have evolved from one nuclear-encoded cytosolic protein during land plant evolution, since the land plant sHsps in different cellular compartments are more closely related to each other than to bacterial or other eukaryotic sHsps [25]. Thus, the chloroplast localized Hsp21 is much more similar to its cytosolic counterparts within each plant, than to the cyanobacterial Hsp16.6.

2. The chloroplast-localized sHsp, Hsp21

The chloroplast-localized sHsp, Hsp21, contains a set of conserved methionines, which are the topic of this paper. Whereas all land plants have an amphipathic helix in the N-terminal arm of their chloroplast sHsps, it is only in higher plants that this helix contains such conserved methionines, which are not present in the moss *Funaria hygrometrica*