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Multidimensional significance of crystallin protein–protein interactions and their implications in various human diseases☆

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ABSTRACT

Background: Crystallins are the important structural and functional proteins in the eye lens responsible for refractive index. Post-translational modifications (PTMs) and mutations are major causative factors that affect crystallin structural conformation and functional characteristics thus playing a vital role in the etiology of cataractogenesis.
Scope of review: The significance of crystallin protein–protein interactions (PPIs) in the lens and non-lenticular tissues is summarized.
Major conclusions: Aberrancy of PPIs between crystallin, its associated protein and metal ions has been accomplished in various human diseases including cataract. A detailed account on multidimensional structural and functional significance of crystallin PPI in humans must be brought into limelight, in order to understand the biochemical and molecular basis augmenting the aberrancies of such interaction. In this scenario, the present review is focused to shed light on studies which will aid to expand our present understanding on disease pathogenesis related to loss of PPI thereby paving the way for putative future therapeutic targets to curb such diseases.
General significance: The interactions with α-crystallins always aid to protect their structural and functional characteristics. The up-regulation of αB-crystallin in the non-lenticular tissues always decodes as biomarker for various stress related disorders. For better understanding and treatment of various diseases, PPI studies provide overall outline about the structural and functional characteristics of the proteins. This information not only helps to find out the route of cataractogenesis but also aid to identify potential molecules to inhibit/prevent the further development of such complicated phenomenon. This article is part of a Special Issue entitled Crystallin Biochemistry in Health and Disease.

1. Introduction

Vertebrate eye lenses are densely packed with crystallin at a high concentration gradient of ~200–460 mg/ml [1] and constitute about 90% of the total lens proteins. Crystallin is broadly categorized as α-, β- and γ-crystallins based on the genetic organization and expression pattern, has a long half-life and is majorly expressed in the eye lens epithelium and fiber cells comprising of 40%, 35% and 25% respectively [2]. They are essential determinants for lens transparency, solubility, refractive index, chaperone activity, apoptosis and unfolded protein degradation. The heterogeneous oligomeric structure of α-crystallin is made up of αA- and αB-subunits and has a molecular mass ranging from ~300–1200 kDa, and an average oligomeric size of ~800 kDa, which effectively facilitate molecular chaperone activity [3].

Until the mid-1980s, αA-crystallin is thought to be a lens specific protein, playing a vital role in lens development, differentiation and maintenance. Later the expression of αA-crystallin was noticed in the number of non-lenticular tissues especially in the non-mitotic tissues including retina, heart, brain, spleen, and liver with undefined function. Recent *in vitro* and *in vivo* siRNA knockdown experiments proved that αA-crystallin has the ability to inhibit the pathologic neovacularization by both the exogeneous and endogeneous [4] pathways. Further studies also supported the protective role of αA-crystallin up-regulation in retina and diabetic retinopathy [5,6], autoimmune uveitis (EAU) [7], and negative regulation of pancreatic carcinogenesis [8]. It was observed that upregulation of crystallin in the cells negatively regulates the caspase-dependent apoptosis through positively regulating the Akt pathway (αA-crystallin) and negatively regulating the Raf/Mek/Erk pathways (αB-crystallin). This regulation leads to a significant increase in cell survival while facing environmental insults [9].

The overexpression of αB-crystallin outside the lens always correlates with various diseased states including neurodegenerative diseases, diabetics, retinopathy and cancer. It is a better molecular chaperone than αA-crystallin and proved to protect multiple cell types against various post-translational modifications (PTMs) including deamidation,

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glycation, phosphorylation, oxidation, isomerization, racemization, truncations, reactive oxygen species (ROS) and metabolic effects under stress conditions. However, αA-crystallin has higher thermal and pH stability than αB-crystallin which has been proved by ^{1}H and ^{31}P NMR studies [10].

Biophysical studies strongly justified the role of PPI and cell homeostasis. An amino acid substitution in crystallin by point mutation changes the PPI and stability significantly [11]. Based on the binding efficiency, PPIs are classified into two type's namely transient and stable interactions. Transient interactions are weak interactions happening in normal active state and most frequently in all the living cells but are exhibited for a short-period of time. On the other hand the stable interactions are very strong and occur in the partially unfolded amorphous proteins that mediate to prevent aggregation, precipitation and fibril formation by inviting degradation process. The stable interactions are inherent throughout the life time to prevent protein fibril formation. The best well known events are DNA replication, packaging of chromatin, cell cycle control, signal transduction, communication among cells, transcription, translation, intermediary metabolism, formation of cellular macrostructures, enzymatic complexes, gene expression, muscle contraction/relaxation, maintenance of lens transparency, *etc*. The loss of interaction is often associated with disease state. Hence, for the understanding of various cellular activities, identifying novel drug molecules, vaccine development, disease diagnosis and treatment, PPI studies are the basic instrumental tool.

Fig. 1 highlights the consequences of α-crystallin PPIs in lens during normal and diseased states. The key factors influencing the PPIs are structural conformation, increased hydrophobicity, the number and position of cysteine residues, the involvement of disulfide linkage formation, pH, temperature and binding potential of the microenvironment, Crystallins have a remarkable ability to interact with partially unfolded proteins in an ATP-independent way and maintain cell homeostasis, which is justified by their up-regulation under various stress conditions. For this process both the C-terminal and N-terminal domains of α-crystallin play a significant role and αA-crystallin has less binding subunits than the αB-crystallin. The interacting partners studied by microarray analysis evidenced that αA-crystallin has the ability to bind with 127 full length proteins out of 17,225 proteins analyzed [12].

2. Disease caused due to various structural modifications in crystallin and its associated proteins

The non-enzymatic PTMs of proteins are the common process of aging and any successful modification can enhance longevity. Obviously, the lens crystallins are also not exceptional to PTMs and have been reported to have 491 PTMs sites [13]. However, due to their chaperone activity they are protective when compared to other proteins within the eye lens or outside. PTMs have the ability to change the crystallin structural confirmation, PPI and induce aggregation during aging [13, 14]. The amino acids including lysine, serine, aspartic acid, phenylalanine and arginine are more prone to PTMs and environmental insults. Several studies have shown arginine as the most frequently mutated point mutation site in congenital cataract (CC) cases worldwide [15–18]. The mutation of arginine residue promotes the binding affinity to other associated proteins (eg. vimentin, α-lactalbumin, filensin, CP49, glial fibrillary acidic protein, simple epithelial keratins, nestin, synemin) thus increasing the heteroaggregation in the specific cell types. Due to the loss of positive charge (arginine deletion), the hydrophobic residues of crystallin are exposed on the surface of partially unfolded proteins, which increase the chance of oxidation, proteolysis, thermal and chemical denaturation and cross-link formations. The above factors ultimately reduce solubility and promote aggregation and complexation with other proteins. In addition, due to arginine point mutation causes structural alternations preventing their interaction with apoptotic protein such as Bcl-2, thus enabling activated caspase-3 formation in live cells. Our earlier studies illustrated that arginine mutation promoted protein aggregation, aggresome formation, changes in oligomerization, hydrophobicity, tryptophan fluorescence, bis-ANS binding ability, reduced solubility, stability,

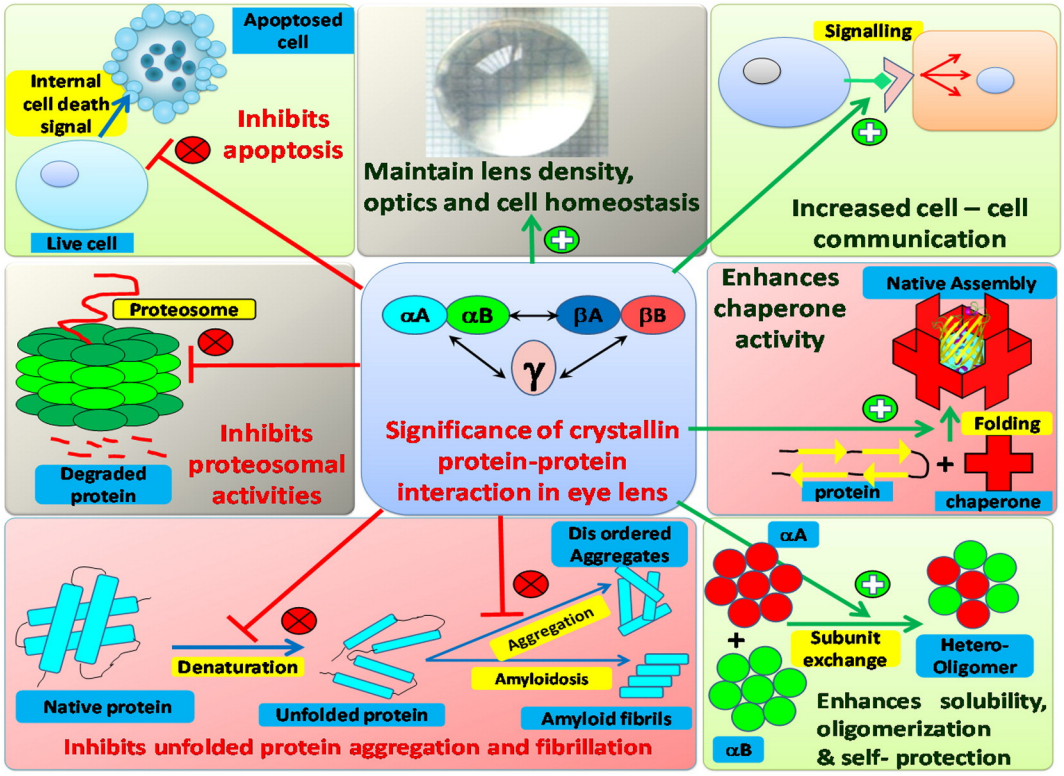

Fig. 1. Schematic illustration depicting the significance of protein–protein interaction in the lens micro-environment.

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