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Role of α-Crystallin protein-protein interactions in disorders of the system and its therapeutic approaches: a new study

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Abstract: α- Crystallin, a major eye lens protein with chaperone activity is vital in cataract development. As a member of the small heat shock protein superfamily, α-Crystallin is able to recognise and bind denatured or unfolded proteins, thereby preventing their aggregation. An important constituent of eye drops and artificial tears is polyethylene glycol-400(PEG-400) which can interact with α- Crystallin and lead to alterations in its tertiary structure, namely the global transition to a non-native form. Another protein, which is characterised by its presence in the insoluble fraction of vertebrate nuclear fiber cells, is Galectin-Related Interfiber Protein (GRIFIN) in the Ocular lens which is a novel binding partner of α-Crystallin. Formation of complexes involving such proteins may influence cell elongation and suture formation during lens development. The binding of PEG-400 or GRIFIN with α-Crystallin may serve as a rationale for the discovery of various therapeutic molecules used for the treatment of eye-related diseases involving crystallin proteins.

Keywords: α-Crystallin, Protein Interactions, PEG-400, GRIFIN, Complex Formation, Therapeutic Molecules, Eye Diseases

1. Introduction

α- Crystallin is a significant protein in the eye lens which is necessary for lens transparency. It is also known as a molecular chaperone because it binds to partially unfolded polypeptides, preserving their integrity in a refolding competent state, making it an essential component of stress responses. As a result, the cell is shielded from the consequences of irreversible protein aggregation [1-3]. *In vitro* native interactions between α- Crystallin and a variety of proteins were suggested by recent research. Many complicated systemic diseases, such as diabetes or other retinal conditions like age-related macular degeneration, uveitis, trauma, and ischemia [4, 5], are thought to involve such interactions in significant ways. In protein aggregation diseases, a change in the protein's native conformation may alter its functionality, resulting in pathological consequences. Because the formation of protein aggregates is the root cause of numerous diseases, a thorough comprehension of the forces or interactions that influence protein aggregation is necessary for developing therapeutic strategies to treat these diseases [6, 7]. Low-level toxicity polyethylene-glycol (PEG 400) is a key component of eye tears and is used in ophthalmic solutions to alleviate burning, irritation, or eye dryness or discomfort. Although a number of thermodynamic parameters suggested that van der Waals forces and hydrogen bonding drive interactions between -crystallin and PEG 400, no such alterations in secondary structure have been elucidated [8, 9]. GRIFIN (galectin-related interfiber protein), a novel binding partner, is one of the lens structural proteins that has been shown to interact with - crystallin. Due to sequence divergence at two positions (N48K and R72V), GRIFIN lacks lactose binding activity—a requirement for -galactoside binding—and is related to the galectin superfamily of proteins [10, 11]. GRIFIN only makes up about 0.5 percent of the water-soluble lens protein, and little is known about this protein's potential physiological function in the lens. However, the high concentration of this protein in the tissue suggests that it may play a role in the refractive index that the lens needs to focus light on the retina. Therefore, the effective packing of protein in the concentrated lens cytoplasm may be due to the interactions that GRIFIN has with α- Crystallin.

During cellular stress, crystallin binds to and inhibits denatured cytoskeletal proteins. This activity could be attributed to its ability to bind to copper and stop the formation of reactive oxygen species. As a result, it is possible that α- Crystallin's interactions with PEG 400 or GRIFIN play a significant role in a number of diseases that could potentially benefit from therapeutic applications [12, 13]. The intravenous injection of -crystallin, for instance, may shield the

retinal ganglion cells from apoptosis and ROS production simultaneously.

2. Interactions of α- Crystallin with PEG- 400

Multi-spectroscopic methods, molecular modeling, and molecular docking were used to investigate α- Crystallin's interaction with PEG-400, a crucial component of the eye drops. Studies using UV-Vis spectroscopy revealed a value of 0.9 105 M-1, indicating that PEG-400 and α- Crystallin bind and interact well. Fluorescence quenching studies revealed the existence of a dynamic model of quenching as a result of an increase in the Stern-Volmer constant as a function of temperature [8, 14,15]. Using the machine learning-based PrankWeb application, a binding pocket with three and one chain in the active hetero-tetrameric form of - crystallin was discovered that may play a significant role in interacting with PEG-400. It's possible that residues like Pro-160, His-18, Ser-85, and Ser-53 play a significant role in this interaction. The fact that PEG-400 plays a crucial role in reducing the symptoms of ocular surface disease (OSD) is what makes it so important. This is because its interaction with α- Crystallin plays a significant role in cataract formation, research into this interaction has a clinical application (Table 1) [1,16].

CD spectroscopy studies showed that when interacting with PEG-400, - crystallin's secondary structure changed significantly. In addition, PEG-400-incubated crystallin showed a significant increase in ANS fluorescence, suggesting that the crystallin's structure was changed to a molten globule-like state (MG) [17-19]. As a result, exposing these interactions between PEG-400 and crystallin is a cutting-edge research project that has the potential to serve as a foundation for the creation of therapeutic molecules that could help combat α- Crystallin -directed ocular disease (Fig 1).

3. Interactions of α- Crystallin with GRIFIN (galectin-related interfiber protein) in the ocular lens

Denatured or unfolded proteins are the source of α- Crystallin ability to interact with a wide range of substrate proteins. Since α- Crystallin is known to play a role in cataractogenesis and makes up most of the lens-soluble proteins, it is important to know how crystallins interact with other proteins in their native environment [10]. GRIFIN (galectin-related interfiber protein) was a significant protein that was found to be uniquely associated in a complex with the lenses of transgenic mice. In the presence of ATP, GRIFIN was found to interact with - crystallin; consequently, it was evident that physiological ATP concentrations might probably enhance GRIFIN and - crystallin interactions.

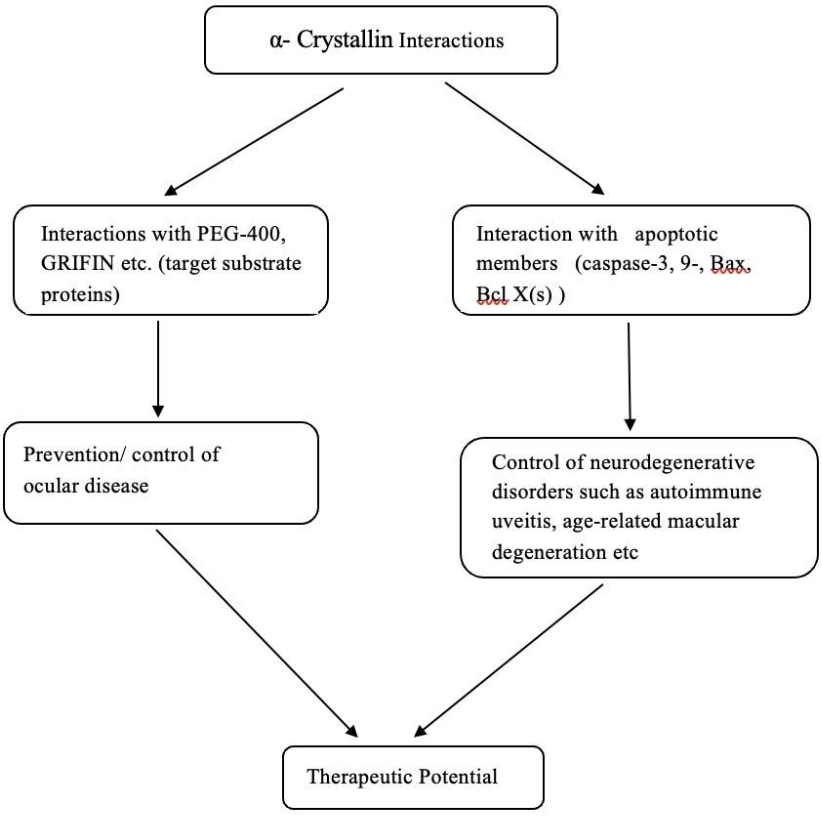

Figure 1. α- Crystallin interactions with other proteins or apoptotic members help to regulate many ocular/systemic diseases under control and thus its therapeutic potential may be explored.

Table 1. α- Crystallin interacts with proteins and play a regulatory role in the vertebrate system.

Target protein substrate interacting with α- Crystallin	Role of Such Interactions	Disorder regulated	Reference
PEG-400	Pro-160, His-18, Ser-85 and Ser-53 residues of α-Crystallin interact with substrate to prevent ocular disease	Ocular diseases such as cataract or retinal trauma	[1, 16, 17]
GRIFIN	Interactions mediate lens fibre cell elongation during lens development	cataractogenesis	[20, 21]
Apoptotic proteins (Caspase-3, Bcl X(s), Bax)	Anti-apoptotic activity; inhibit the activation of apoptotic members and in turn, the onset of neurodegenerative disorders.	Autoimmune uveitis, age-related macular degeneration	[26, 29, 30]

Because they cause hydrophobic sites to be exposed and, as a result, stabilize the structure of -crystallin, fluctuations in ATP may play a significant regulatory role in regulating the crystallin–GRIFIN interactions [20, 21]. It is believed that the galectins play a crucial role in mediating cellular interactions with elements of the extracellular matrix (Table 1). The proper cell elongation and organization of sutures are maintained by the dynamic interactions that take place between the membrane-associated complexes of fiber cells and the underlying lens capsule. Additionally, the association of GRIFIN-crystallin with the plasma membrane of lens fiber cells may guarantee that they have an impact on cell elongation throughout lens development [22, 24]. The elongation and migration of lens fiber cells during lens development are thought to be influenced by these interactions, which may also be involved in ocular lens disorders.

4. Therapeutic potential of α- Crystallin Interactions

α- Crystallins are known to be apoptosis inhibitors in both mitochondrial and death receptor-mediated pathways; B- crystallin prevents procaspase-3 from maturing into caspase-3 by interacting with it. Both A- and B- interact directly with caspase-3, Bcl-X(S), and Bax at the same time. Crystallins regulate PKC and AKT signaling pathways in lens epithelial cells to prevent UVA-induced apoptosis (Table 1) [25]. Attempts to deliver α- Crystallins tagged with a cell penetration peptide are made possible by these advantages of the former. In lens epithelial cells, this method of delivering α- Crystallin into cells has demonstrated improved resistance to heat and oxidative stress [25, 26].

Within the ACD domain of α- Crystallin, two peptide interaction sites —70FVIFLDVKHFSPEDLTVK88— in αA and —73DRFSVNLDVKHFSPEELKVK92 — in αB were found. These peptide interaction sites are also referred to as ‘mini - crystallin chaperones’ or ‘MACs’ [27, 28]. It may be possible to further investigate the therapeutic potential of the interaction sequences in that are responsible for binding to other proteins. A MAC derived from αB- crystallin was found to inhibit oxidative stress-mediated apoptosis in retinal epithelial cells, whereas the inhibition of amyloid fibrillogenesis and toxicity of the MAC from αA- crystallin could be utilized for Alzheimer's disease treatment [29, 30].

4. Conclusions

α- Crystallin is a molecular chaperone which protect vertebrate eye lens from protein aggregation. It interacts with various other proteins of the eye lens which plays an important role in ocular and other systemic diseases. Any abnormal interactions between the chaperone and its target substrate proteins may result in an increased protein aggregation and disease; for instance the substrate-chaperone interaction between αB- crystallin and its substrates involve various interactive domains which have been characterised extensively. αA- crystallin is known to possess anti-apoptotic activity which may inhibit the activation of caspase-3 and -9 and thereby responsible for regulating any neurodegenerative disease. Such a function may be directly related to its chaperone activity. Two interacting molecules which have been identified to associate with α- Crystallin are PEG-400, an important constituent of eye drops and GRIFIN, which upon interacting with α- Crystallin induces the

exposure of hydrophobic sites and influence lens cell elongation during its development. Such interactions are believed to hold a therapeutic potential as they inhibit amyloid fibrillogenesis as well. An intravenous injection of 'mini-chaperone' peptides of α-Crystallin in the ocular lens is responsible for keeping ocular diseases under control. The therapeutic potential of α-Crystallin is being explored further with the help of proteomics studies as well which may unleash the importance of such crystallin- substrate interactions in upcoming research fields.

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