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Deciphering the Interactions of Crystallins with Metal Ions/ ATP and its Applications: A Novel Study

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Abstract: Crystallins are the predominant proteins of the eye lens which prevent the heat and oxidative-induced stress-induced aggregation of other proteins. They may be classified into two superfamilies, the α- and βγ- crystallins. The βγ- crystallins are long-lived structural proteins which refract light onto the retina. The microbial crystallins can not only bind to calcium ions, but even able to coordinate other ions such as Mg^{2+}, Sr^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} etc. Such metal ions may influence the stability and aggregation propensity of human γS- crystallin as well. Previous studies had even revealed the binding of αA- and αB- crystallins with Cu^{2+} ions and suppressed the formation of Cu^{2+} mediated oxygen species and thus protected ascorbic acid from oxidation by copper ions. The residues 71-88 present in mini αA- crystallin, a peptide of αA- crystallin were found to be responsible for the prevention of oxidation. The binding of metal ions to crystallins may influence the formation of protein aggregates, and thus cataract or other disorders but there are some ions which may even help to improve the chaperone activity of α crystallins. Adenosine triphosphate (ATP), the energy currency of the cell improves the chaperone activity of α-crystallins by regulating the chaperone-target substrate interactions. This minireview explores various insights of the interactions of crystallins with metal ions and ATP which may help in the search for more therapeutic molecules in near future.

Keywords: Crystallins, Chaperone Activity, Interactions, Metal Ions, Protein Aggregates, Disorders, ATP, Therapeutic Molecules

1. Introduction

The transparency of the lens is highly dependent on various homeostatic processes such as truncation, glycation, oxidation etc. which are some major post-translational modifications as well. Oxidation is strongly associated with aggregation involving disulphide and non-disulphide bonds thus it has extensive impact on sulphur-containing amino acids cysteine and methionine [1, 2]. Multiple disulphides in human gamma crystallins had been reported and its increased propensity towards disulphide bond formation, dimerisation, aggregation in vitro. Some metal ions such as Cu^{2+} may interact with human γD- crystallin and result in its aggregation. The increased concentration of copper with age and cataract lens suggests its potential role in cataract formation [3, 4]. A recent study confirmed the role of Cys111 serine residue in copper mediated precipitation by γD- crystallin [1, 5].

The vertebrate β- and γ- crystallins are responsible for making up the refractive tissues of the eye lens, comprising about 50% of the dry weight. It has been found that primarily microbial βγ- crystallins consist of a characteristic double clamp Ca^{2+} binding motif. Interactions of βγ- crystallins hold important implications for lens homeostasis i.e. zinc or copper ions are responsible for increasing the chaperone activity of lens α- crystallins [6, 7]. In contrast to the vertebrate γ- crystallins which do not possess significant cation interactions in the lens, these particular crystallins are able to interact with a variety of cations such as calcium, strontium, zinc, copper etc or even lead which influence the stability and interactions of the crystallin proteins [8, 9]. Among crystallins, αA- crystallin is one of the abundant protein of eye lens which exhibits chaperone-like function and responsible for the maintenance of lens transparency [10, 11]. Copper is present in eye lens in micromolar concentration and bound to lens protein [12, 13]. It can bind to the protein and suppress the Cu^{2+}-mediated oxidation of reactive oxygen species; it has even been found to modulate the chaperone activity of αA-crystallin.

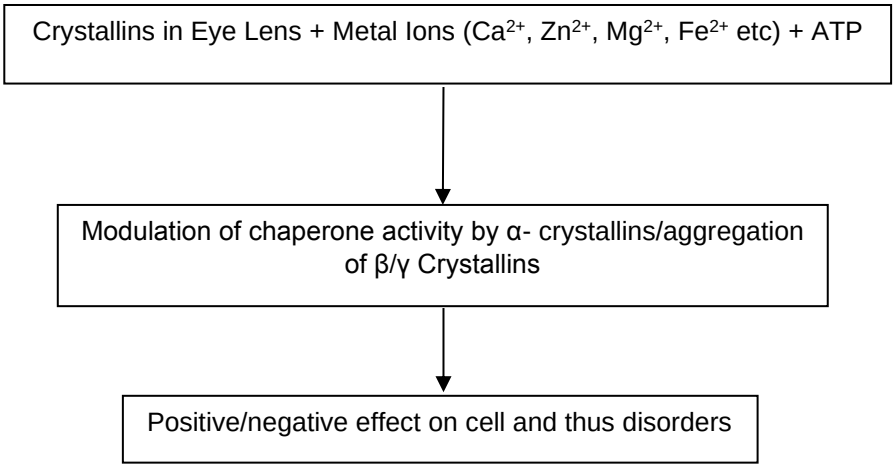

Figure 1. the crystallin interacts with metal ion/ ATP and has a positive/ negative effect on cell which may lead to various disorders of eye or other organs.

The mini αA- crystallin possesses a sequence αA70-80 which is a Cu^{2+} binding site and responsible for the suppression of Cu^{2+}-induced oxidation of ascorbic acid. ATP (adenosine triphosphate), also known as the energy currency of the cell is known for its property but its interaction with α-crystallin was unknown even then. A recent study had revealed its importance in modulation of crystallin interactions with various target substrates and thus useful in enhancing the chaperone activity of α-crystallin [1].

Such ion-crystallin interaction may influence the structure and stability of not only human crystallins but even in case of fish otolith and lens where environmentally common cations such as Sr^{2+}, Fe^{2+} are present, including Mn^{2+}, Co^{2+}, Pb^{2+} etc. (Figure 1) Significant research on the effect of such non-oxidising cations or ATP on the stability of crystallin proteins is yet to be conducted in a greater detail.

2. Copper and metal ions interact with human γD crystallin and βγ- crystallins and promote their stability

The involvement of cysteine residues in disulphide-mediated crosslinking of human γD-crystallins had been determined. Cys111 in recombinant human γD crystallins was found to be critically involved in disulphide bond formation; authors had observed an increased propensity of certain γ-crystallin residues towards disulphide bond formation or aggregation during cataracts [1, 5]. Its mutation to alanine was able to abolish dimerisation completely. When αB- crystallin was added, it was unable to protect Cys111 from dimerisation but instead Cu^{2+}-induced hγD-crystallin aggregation was suppressed up to 50% and 80% respectively by the mutants C109A and C111A respectively [1].

The microbial βγ- crystallins from the tunicate Ciona intestinalis (Ci-βγ) was able to interact with calcium ions and thereby located in a light-sensitive sensory organ which is highly enriched in other metal ions too [2,7]. Ci-βγ possesses two functional Ca^{2+}-binding sites which is also more refractive. Previous studies had reported the weak interactions of lens βγ-crystallins with calcium, but their two stable domain structures suggested that these crystallins had eschewed calcium activity in all vertebrates except for Ci-βγ [14]. Copper and zinc ions increase the chaperone activity of lens α-crystallins whereas their interactions with human γD- crystallin leads to the formation of aggregates, as evident by elevated copper levels [15,16]. A biophysical characterisation for Ci-βγ in the presence of a variety of divalent cations revealed that all tryptophan residues of the organism were buried in the hydrophobic core of the protein [17, 18]. An increased thermal unfolding midpoint (Tm) from 46°C to 94°C revealed the stabilisation of the βγ-crystallin residue in the presence of Ca^{2+} whereas similar stabilities were revealed in the presence of other metal ions Sr^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+} etc as evident from their higher Tm values for each [19].

In the healthy lens, γ-crystallins possessed weak interactions, but their interaction with small cations highlighted their ability to tolerate potentially destabilising interactions to a certain extent. The addition of Ca^{2+}, Sr^{2+}, Mg^{2+} or Mn^{2+} did not alter the thermal unfolding of human γS crystallin but could produce soluble aggregates in excess. The Zn^{2+}-induced aggregation was reversible upon EDTA addition for all the tested proteins, thereby supporting the coordination of zinc ions to HγS crystallin via solvent-accessible cysteines which cause intermolecular bridging. Prior investigations even revealed Cu^{2+}-induced aggregation of HγS crystallin, after addition of 1 equivalent Cu^{2+}; the solvent-accessible residues of C109, C111 were found to be responsible for aggregation, which could be blocked by GSSG [20].

Thus an increased cation concentration was responsible for promoting γ-crystallin directly or indirectly via displacement of copper from α-crystallins. Such a displacement alters the protein-protein interactions and disrupts protein function in prions or other aggregation disorders [21, 22].

3. α-crystallin contains a specific copper-binding site which modulates its chaperone activity

Previous studies revealed that α- crystallin can bind to copper ion and prevent the copper-mediated oxidation of ascorbic acid [23]. Mass spectrometry and isothermal titration calorimetric assays determined the direct measurement of Cu^{2+} ions which interacts with a specific peptide on αA- crystallin i.e. mini-αA crystallin. CD spectroscopy studies revealed an alteration in secondary structure of the crystallin protein while a substitution of a His residue by Ala in mini αA- crystallin abolished the redox suppression activity of the particular peptide [13].

Bis-ANS binds to hydrophobic proteins and peptides which leads to a several fold enhancement of the probe fluorescence. The interaction of mini αA-crystallin with the hydrophobic probe led to a maximum emission complex of around 490nm, but a prior binding of Cu^{2+} to the peptide decreased Bis-ANS binding. The chaperone activity of αA- crystallin was tested in the presence of Cu^{2+} ion using a target substrate citrate synthase (CS) and a significant increase in the aggregation of the denaturing CS was observed. Upon addition of mini αA- crystallin an efficient suppression of CS aggregation was observed, thereby revealing that Cu^{2+}ion rather results in a degradation of any target substrate [13, 24].

When other metal ions such as Zn^{2+}, Fe^{2+} was added to mini αA- crystallin to check whether their presence affects the binding of Cu^{2+} to the peptide the hydrophobic probe Bis-ANS was used for testing it [13,14]. But unlike Cu^{2+}-treated mini αA- crystallin, Zn^{2+}- or Fe^{2+}- treated crystallin did not show any loss in the probe fluorescence, thereby suggesting probably that no metal ions were bound to the peptide or the bound ions may have been displaced by Bis-ANS. Thus the data suggested the presence of a specific copper-binding site (residues 70-88, which is revealed using a deletion mutant of αA- crystallin lacking a portion of the mini-chaperone sequence) which is not affected by the presence of any other metal ion [13].

The presence of two histidines and one arginine is a requirement for one copper-binding site but mini αA- crystallin lacks Arg residue, so it could be possible that the presence of a single His residue along with Lys and the N-ter amino group is responsible for copper coordination in the peptide residue [24, 25]. Thereby the role of Cu^{2+} or other metal ions on the chaperone activity of αA- crystallin have been elucidated, with further research still on the run (Table I).

Table I. Effect of metal ions or other molecules in the cell on the chaperone activity of various crystallins of the eye lens.

Type of Crystallin	Organism	Metal Ion/Molecule which binds to Crystallin Protein	Impact on cell	Reference
γ-crystallin	Human			
a. γD- crystallin b. γS- crystallin		Cu^{2+} Cu^{2+}	Negative Negative, Cu^{2+} causes aggregation	[1,5,15,16] [17,18,19,20]
βγ- crystallin	*Ciona intestinalis* (*Ci-βγ*)	Ca^{2+}, Zn^{2+}, Mg^{2+}, Cu^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}	Positive, improvement of thermal stabilities of βγ-crystallin	[2,7,17,18,19,20]
α-crystallin a. αA b. αB	Human	Cu^{2+}, Zn^{2+}, Fe^{2+}	Cu^{2+} has a negative effect, Zn^{2+} or Fe^{2+} did not show any effect in the presence of Cu2+	[13,24,25]
		ATP (adenosine triphosphate)	Enhances chaperone function of α-crystallin as it improves the chaperone-client substrate complex associations	[26-34]

4. Interaction of ATP with α-crystallin and sHSPs and the future implications of such interactions

The exact impact of metal ions on crystallins has long been of interest, even now. At the same time, the role of adenosine triphosphate (ATP) which is an important fuel of life for humans and Mycobacterium species is known [26]. Interactions of ATP with small heat shock proteins (sHSPs) especially Hsp20 and Hsp27 are known to affect their structure and function.

ATP plays a major role in the synthesis of several macromolecules essential for cell survival. Small heat shock proteins (sHSPs) constitutes a certain class of chaperones under molecular stress, characterised by the conserved 'α-crystallin domain' (ACD) whose molecular mass ranges between 12-43kDa [27, 28]. Mutations and post-translational modifications in sHSP lead to cataract formation in the lens [29, 30, 31]; the disorder is prevented by the chaperone function of α- crystallin which has a major role in the transparency of the eye lens. Since the human lens contains about 3mM ATP, thus the probability of its interaction with α-crystallin is high inside the lens. The C-terminal extension of αB interacts with ATP via the β4-β8 groove of the ACD [32]. Such a binding has altered the chaperone function of the protein because ATP enhances the association between the chaperone and client proteins, thereby improving the chaperone activities of both αA and αB subunits of α-crystallin. It even improves αB mediated refolding of denatured client proteins like lactate dehydrogenase or xylanase II in its molten globule state [33, 34]. ATP is also responsible for increasing the structural stability of α-crystallin, and even maintaining the stability of chaperone-client complexes of crystallin proteins.

Thus, it may be inferred that metal ions such as Ca^{2+} is responsible for the formation of aggregates upon interaction with crystallins such as human γD-crystallin, whereas zinc or copper ions are responsible for increased chaperone activity of α-crystallins but formed aggregates upon interacting with human γD-crystallin. The presence of residues α70-80 in mini αA-crystallin was responsible for an increased chaperone activity of the crystallin protein, in the presence of Cu^{2+} ion which, in turn was responsible for aggregate formation (without the presence of the peptide). An increased concentration of metal ions can thereby be responsible for cataract formation, owing to their ability to form increased aggregates. On the other hand, ATP concentration in the eye lens could enhance the increased chaperone activity of the eye lens protein α-crystallin and improve its structural ability and its association with various target substrates.

An increased concentration of metal ions may thus prove futile and lead to eye disorders such as cataract while ATP is useful for an improved chaperone function of α-crystallin. A detailed understanding of their interaction with crystallin proteins may help to decipher their applications in targeted drug delivery or therapeutic approaches in near future.

5. Conclusion

The role of some metal ions on α- crystallin, a small heat shock protein with chaperone-like activity has been well documented. In some pathologies such as Parkinson's disease or Alzheimer's disease, an accumulation of metal ions has been observed. Various metal ions such as zinc, copper, calcium hold a significant role on the chaperone activity of crystallins at various concentrations. Zinc or copper increase the chaperone activity, at concentrations of about 0.1 and 1mM respectively whereas calcium inhibits its activity completely at a concentration of 1mM. The presence of residues α70-80 in mini αA- crystallin may be responsible for an increased chaperone activity of the peptide, in the presence of Cu^{2+} ions. An increased ATP concentration in eye lens could enhance the increased chaperone activity of crystallins and thus revealing its biological importance in the living cells.

The importance of such metal ion-crystallin interactions is vital to decipher the underlying mechanisms behind cataract or other pathologies. Future studies on such detailed interactions would help to unleash the importance of these factors i.e. how metal ions could augment the phenotype in the genetically predisposed condition.

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Conflict of interest

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