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Review

Differential role of arginine mutations on the structure and functions of α-crystallin☆

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ABSTRACT

Background: α-Crystallin is a major protein of the eye lens in vertebrates. It is composed of two subunits, αA- and αB-crystallin. α-Crystallin is an oligomeric protein having these two subunits in 3:1 ratio. It belongs to small heat shock protein family and exhibits molecular chaperone function, which plays an important role in maintaining the lens transparency. Apart from chaperone function, both subunits also exhibit anti-apoptotic property. Comparison of their primary sequences reveals that αA- and αB-crystallin posses 13 and 14 arginine residues, respectively. Several of them undergo mutations which eventually lead to various eye diseases such as congenital cataract, juvenile cataract, and retinal degeneration. Interestingly, many arginine residues of these subunits are modified during glycation and even some are truncated during aging. All these facts indicate the importance of arginine residues in α-crystallin.

Scope of review: In this review, we will emphasize the recent *in vitro* and *in vivo* findings related to congenital cataract causing arginine mutations in α-crystallin.

Major conclusions: Congenital cataract causing arginine mutations alters the structure and decreases the chaperone function of α-crystallin. These mutations also affect the lens morphology and phenotypes. Interestingly, non-natural arginine mutations (generated for mimicking the glycation and truncation environment) improve the chaperone function of α-crystallin which may play an important role in maintaining the eye lens transparency during aging.

General significance: The neutralization of positive charge on the guanidino group of arginine residues is not always detrimental to the functionality of α-crystallin.

This article is part of a Special Issue entitled Crystallin Biochemistry in Health and Disease.

1. Introduction

The eye is a remarkable sensory organ which imparts vision to perceive the world around us. Vision impairment takes place when there is a loss in the transparency of the vertebrate eye lens. The human lens is a transparent tissue in the eye with a refractive index of 1.40 [1]. The loss in transparency and the development of opacity of the ocular lens is termed as cataract. It is mainly classified into four categories on the basis of age. First is the congenital or infantile cataract which takes place in the first year of life; followed by juvenile cataract which happens within the first decade of life. The third category is the pre-senile cataract which happens before 45 years and the last is the senile cataract which occurs beyond 45 years [2]. As per World Health Organisation (WHO) report of 2012, cataract is a global health burden. According to this report nearly 39 million people around the world are blind among which ~18 million are affected by cataract. Therefore reasons behind cataract formation need to be understood properly.

Lens development is a vital event in the organogenesis of the eye [3]. The ocular lens is a transparent avascular tissue which is formed by the proliferation and differentiation of the lens epithelial cells. Lens development is a complex process and this development is broadly categorized into four phases [4] (Fig. 1). The initial phase of development leads to the association of head ectoderm with the optic vesicles. Usually, primary and secondary lens fiber cells are generated in phases 2 and 3, respectively. In the final phase, these fiber cells continue to accumulate over the old fiber cells. In this phase, when the fiber cells begin to elongate, a massive increase in the concentration of crystallin proteins takes place [5–7]. These crystallin proteins provide the structural integrity to the lens fiber cells and they continue to do so for the rest of the life span of the organism. Crystallin proteins as well as secondary fiber cells persist for the entire life span without significant turnover in the lens [8].

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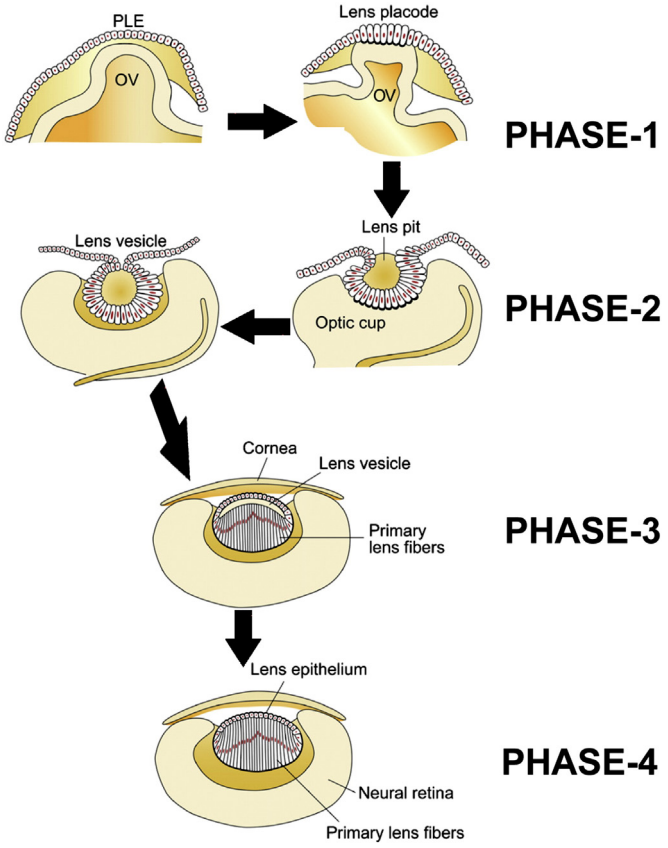

Fig. 1. General phases of lens development. Phase-1. The vertebrate lens initially develops a surface ectoderm which is exposed to multiple inducing factors during the gastrulation stage. Association of the presumptive lens ectoderm (PLE) with the optic vesicle (OV) leads to the formation of lens placode. Phase-2. Invagination of the lens placode forms the lens pit and consequently forming the lens vesicle. Cells in the anterior of the lens vesicle lead to the formation of lens epithelium while those in the posterior give rise to primary lens fiber cells. Phase-3. Differentiation of the lens epithelial cells near the equator gives rise to the secondary fiber cells. Phase-4. The lens then continues the addition of the lens fiber cells over the old fiber cells. It acquires the correct mechanical stiffness required for focussing light. These secondary fiber cells persist for the whole life span without any turnover in the lens cells. (Reproduced with permission from The International Journal of Biochemistry & Cell Biology 40 (2008) 317–323).

The term "Crystallin" denotes to a set of soluble proteins which are mainly responsible for the refractive properties of eye lens. Lens consists of three major crystallin proteins (α-, β- and γ-crystallins) and the concentration of these three crystallin proteins together is 400 mg/ml [9]. Among them, α-crystallin is the major one. It exists as a highly heterogeneous aggregate with an average molecular weight of 650–800 kDa [10]. It has two subunits (αA- and αB-crystallin) and these two subunits generally associate in 3:1 ratio to form a large oligomeric protein (~40 mer) [11]. αA-crystallin comprises of 173 amino acid residues and αB-crystallin consists of 175 amino acid residues. They share ~60% sequence similarity (Fig. 2) and belong to small heat shock protein (sHSP) family. Both of them are major β-sheet protein and possess highly conserved "α-crystallin domain" (~80–100 amino acid residues). This domain is flanked by an N-terminal domain and preceded by a C-terminal tail [10]. Besides lens, these two subunits are also found in other non-lenticular tissues like retina [12]. In retina, they are expressed mostly in the ganglion cell, inner synaptic layers, and in photoreceptors [13–15]. αA- and αB-crystallin knockout studies revealed that they play a critical role in lens development [16–18].

Besides, their contribution to the lens development, both αA- and αB-crystallin exhibit molecular chaperone function. In 1992, it was Horwitz who for the first time demonstrated that α-crystallin exhibits molecular chaperone function [19]. Since then, several studies revealed that α-crystallin can prevent the thermally and chemically induced aggregation of different client proteins including β- and γ-crystallins [20–22]. More importantly, α-crystallin drew more attention when it was found that it can prevent aggregation of client proteins including γ-crystallin against UV-irradiation [23,24]. It can also assist the refolding of different denatured enzymes in an ATP-independent manner [25]. All these findings are more relevant to the *in vivo* chaperone function of α-crystallin, where lens proteins are often exposed to several external insults including UV-irradiation, thermal and chemical stresses which can induce protein aggregation and thereby cause cataract. It is believed that the molecular chaperone function of α-crystallin is indispensible for eye lens transparency and protects retina and retinal pigment epithelium (RPE) from stress induced degeneration [15,26].

Several point mutations in α-crystallin are found in various eye diseases such as cataract, and retinal degeneration [27,28]. For example, G98R in αA-crystallin gives rise to pre-senile cataract in an Indian family [27]. Similarly an autosomal recessive congenital cataract mutation in αA-crystallin is found at the 9th position where the tryptophan residue gets converted into a termination codon (W9X) [28]. Point mutations in αB-crystallin gene are also reported in the literature [29,30]. A point mutation in αB-crystallin (P20S) causes autosomal dominant congenital cataract [29]. Mutation in the αB-crystallin gene is also associated with retinal degeneration [30]. Interestingly, when we carefully looked into the different mutations associated with the four categories of cataract, we observed that the frequency of arginine mutations both in αA- and αB-crystallin is more in congenital cataract. Apart from these mutations, the modification of several arginine residues also takes place in this long lived α-crystallin protein due to glycation which is an important post-translational modification in the eye lens [31]. Even, truncation of some arginine residues in α-crystallin takes place during aging [32]. All these facts suggest that arginine residue may be playing a crucial role in the structure, stability, and molecular chaperone function of α-crystallin. The purpose of this review is to emphasize the recent findings on congenital cataract causing arginine mutations both in αA- and αB-crystallin in various families and how such mutations affect their structure and function and promote their aggregation inside eye lens during cataractogenesis. Another purpose

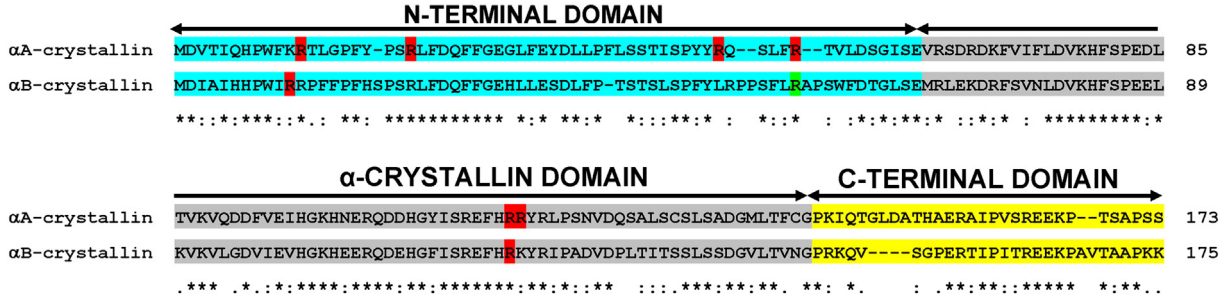

Fig. 2. Sequence alignment of αA- and αB-crystallin. Sequences of αA- and αB-crystallin were aligned using the program ClustalW. The N-terminal domain (NTD) is shown in turquoise, the "α-crystallin domain" (ACD) is shown in light gray and the C-terminal domain (CTD) is shown in yellow. The symbols (*, : and .) represent identical residue, conserved substitution and semi-conserved substitution respectively. The arginine residues which are involved in juvenile and congenital cataract are marked in green and red respectively.

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