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Review

Corneal crystallins and the development of cellular transparency

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Abstract

Past studies have established that the cornea like the lens abundantly expresses a few water-soluble enzyme/proteins in a taxon specific fashion. Based on these similarities it has been proposed that the lens and the cornea form a structural unit, the 'refracton', that has co-evolved through gene sharing to maximize light transmission and refraction to the retina. Thus far, the analogy between corneal crystallins and lens crystallins has been limited to similarities in the abundant expression, with few reports concerning their structural function. This review covers recent studies that establish a clear relationship between expression of corneal crystallins and light scattering from corneal stromal cells, i.e. keratocytes, that support a structural role for corneal crystallins in the development of transparency similar to that of lens crystallins that would be consistent with the 'refracton' hypothesis.

Keywords: Corneal crystallins; Corneal transparency; Keratocytes; Light scattering

Contents

1. Introduction

Holt and Kinoshita [1] were the first to report that water-soluble extracts from the bovine cornea contained a single prominent protein fraction that was initially recognized as bovine corneal protein 54 (BCP54) [2,3]. Later studies have identified BCP54 as aldehyde dehydrogenase 3A1 [4,5] that comprises from 20 to 40% of the total water-soluble protein content of the bovine corneal epithelium. More recent studies have shown that unlike non-transparent tissues and organs other than lens, the corneas from a wide range of species abundantly express a few water-soluble enzyme/proteins (Table 1) [3,6–14], many of which are identical to the abundantly expressed taxon-specific lens crystallins, including aldehyde dehydrogenase 1A1 (ALDH1A1)/η-crystallin, α-enolase/τ-crystallin, glutathione-*S*-transferase/Ω-crystallin, lactic dehydrogenase/ε-crystallin, glyceraldehyde-3-phosphate dehydrogenase (G3PDH)/π-crystallin, and arginino-succinate lyase/δ-crystallin [6,8]. Interestingly, several of these corneal enzyme/proteins are abundantly expressed in the lens of the same species, notably arginino-succinate lyase/δ-crystallin in the chicken and

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Table 1
Known corneal crystallins and their lens counterparts

Corneal crystallin	Species	References	Lens crystallin	Species
ALDH1A1	Rabbit, human, pig	[8]	η-Crystallin	Elephant shrew
α-Enolase	Human, mouse, chicken, crocodile, toad/frog	[6,9]	τ-Crystallin	Lamprey, crocodile
Glutathione-*S*-transferase	Squid, mouse	[6]	Ω-Crystallin	Cephalopods
Lactic dehydrogenase	Rabbit, human, chicken, pig	[7]	ε-Crystallin	Duck, crocodiles
G3PDH	Rabbit, human, chicken	[7]	π-Crystallin	Geckos
Arginino-succinate lyase	Chicken	[6]	δ-Crystallin	Birds, reptiles
α, β, γ-Crystallin	Indian toad and frog	[10]	α, β, γ-Crystallin	Mammals
Triose phosphate isomerase	Crocodile	[9]		
TKT	Mammals	[8,11]		
BCP54/ALDH3A1	Most mammals	[3,6]		
Isocitrate dehydrogenase	Bovine	[12]		
Gelsolin	Fish	[13,14]		
Actin	Fish, mouse	[7,13,14]		
Peptidl-prolyl *cistrans* isomerase	Chicken	[6]		
Pyruvate kinase	Chicken	[7]		
Annexin II	Chicken	[7]		
Protein disulfide isomerase	Chicken	[7]		

glutathione-*S*-transferase/Ω-crystallin in the squid. Furthermore, the anuran (toad and frog) corneal epithelium, which can transdifferentiate to regenerate the lens, abundantly expresses ubiquitous, vertebrate lens α, β and γ-crystallin in addition to the taxon-specific crystallin α-enolase/τ-crystallin [10]. Overall, the similarity in expression of these proteins in the cornea and lens, both in abundance and taxon-specificity, has lead many investigators to refer to these proteins as corneal crystallins [15,16].

While the critical function of corneal crystallins has yet to be established, the fact that the lens and cornea are unique in being transparent has prompted the hypothesis that they form a structural unit, the 'refracton', that has evolved through a process of gene sharing to meet the demands required for the development and maintenance of transparency and refraction of light necessary for vision [17,18]. Indeed, many of the corneal and lens crystallins show important functional properties such as chaperones and metabolic enzymes that could play a role in protecting cells from light-induced stress. In mammals the most common corneal crystallin identified has been ALDH3A1, which has been shown to be protective against UV and oxidative stress-induced apoptosis [19–21]. ALDH3A1 and ALDH1A1 also metabolize hexanal and 4-hydroxynonenal and malondialdehyde, the major products of lipid peroxidation, while generating NADPH, a UV absorber [22–24]. ALDH3A1 may also directly absorb UV light and protects against UV-induced inactivation of other intracellular enzymes, either through UV-absorption or chaperone-like activity [22]. Overall, water-soluble proteins from the cornea while accounting for 20% of the total protein may account for over 50% of the total UVB-light absorption, and have been called "absorbins" by some investigators [25].

In addition to the metabolic and UV-absorptive roles, crystallins are thought to play a structural function by directly influencing light scattering within the cornea. In the lens, high concentration of crystallin proteins have been shown to provide short-range order within the cytoplasm of the lens fibers reducing light scattering from macromolecules as occur in more dilute solutions [26–29]. It has therefore been proposed that corneal crystallin proteins serve a similar function in corneal epithelial cells and stromal keratocytes (fibroblasts) by accumulating to a high proportion of the soluble protein and limiting light scattering [8,30].

While there is strong theoretical basis supporting crystallin proteins, transparency and the 'refracton' theory, experimental evidence confirming these relationships are only now being reported. These studies suggest that there is an association between expression of corneal crystallins and cellular transparency, particular involving the stromal keratocyte. This paper reviews the evidence linking corneal crystallin protein expression and light scattering from the cornea and establishes that adult corneas that are transparent show high expression levels for corneal crystallins in stromal cells, i.e. keratocytes. More importantly, decreased corneal crystallin protein expression during early postnatal development or after injury is associated with loss of corneal transparency and marked light scattering from stromal keratocytes. Finally, decreased expression of corneal crystallins in cultured stromal keratocytes is associated with increased in vitro light scattering. Taken together these findings strongly support a role for corneal crystallins in the development and maintenance of transparency and the 'refracton' theory for the evolution of transparency and refraction within the eye.

2. Cellular transparency in the normal cornea

The normal cornea is composed of 3 distinct tissue layers (Fig. 1), the anterior corneal epithelium, the corneal stroma and the posterior corneal endothelium. While the thickness of the corneal epithelium is fairly constant, ranging from 45 to 50 μm in the human, mouse and rabbit [31–33], the corneal stromal thickness varies considerably depending on the species and can be quite thin in mice (70–90 μm depending on strain), thicker in rabbits and humans (325–500 μm, respectively) and very thick in pigs and cows (1 mm) [32–35]. The corneal stroma for the most part contains regularly arranged collagen fibers of uniform thickness and spacing that are organized into thicker