Biochemical and Biophysical Research Communications xxx (2015) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Role of Aquaporin 0 in lens biomechanics

S. Sindhu Kumari [a], Neha Gupta [a], Alan Shiels [c], Paul G. FitzGerald [d], Anil G. Menon [e], Richard T. Mathias [a, b], Kulandaiappan Varadaraj [a, b, *]

[a] Physiology and Biophysics, Stony Brook University, NY, USA
[b] SUNY Eye Institute, NY, USA
[c] Washington University School of Medicine, St. Louis, MO, USA
[d] Cell Biology and Human Anatomy, School of Medicine, University of California, Davis, CA, USA
[e] University of Cincinnati College of Medicine, Cincinnati, OH, USA

ARTICLE INFO

Article history:
Received 19 April 2015
Available online xxx

Keywords:
AQP0
AQP5
CP49
Filensin
Aquaporin
Lens
Fiber cell adhesion
Lens biomechanics
Accommodation
Presbyopia

ABSTRACT

Maintenance of proper biomechanics of the eye lens is important for its structural integrity and for the process of accommodation to focus near and far objects. Several studies have shown specialized cytoskeletal systems such as the beaded filament **(BF)** and spectrin-actin networks contribute to mammalian lens biomechanics; mutations or deletion in these proteins alters lens biomechanics. Aquaporin 0 (AQP0), which constitutes ~45% of the total membrane proteins of lens fiber cells, has been shown to function as a water channel and a structural cell-to-cell adhesion **(CTCA)** protein. Our recent *ex vivo* study on AQP0 knockout (**AQP0 KO**) mouse lenses showed the CTCA function of AQP0 could be crucial for establishing the refractive index gradient. However, biomechanical studies on the role of AQP0 are lacking. The present investigation used wild type (WT), AQP5 KO (**AQP5**$^{-/-}$), AQP0 KO (heterozygous KO: **AQP0**$^{+/-}$; homozygous KO: **AQP0**$^{-/-}$; all in C57BL/6J) and WT-FVB/N mouse lenses to learn more about the role of fiber cell AQPs in lens biomechanics. Electron microscopic images exhibited decreases in lens fiber cell compaction and increases in extracellular space due to deletion of even one copy of AQP0. Biomechanical assay revealed that loss of one or both copies of AQP0 caused a significant reduction in compressive load-bearing capacity of the lenses compared to WT lenses. Conversely, loss of AQP5 did not alter the lens load-bearing ability. Compressive load-bearing at the suture area of AQP0$^{+/-}$ lenses showed easy separation while WT lens remained intact. These data from KO mouse lenses in conjunction with previous studies on lens-specific BF proteins (CP49 and filensin) suggest that AQP0 and BF proteins could act co-operatively in establishing normal lens biomechanics. We hypothesize that AQP0, with its prolific expression at the fiber cell membrane, could provide anchorage for cytoskeletal structures like BFs and together they help to confer fiber cell shape, architecture and integrity. To our knowledge, this is the first report identifying the involvement of an aquaporin in lens biomechanics. Since accommodation is required in human lenses for proper focusing, alteration in the adhesion and/or water channel functions of AQP0 could contribute to presbyopia.

1. Introduction

The mammalian ocular lens consists of two types of cells, epithelial and fiber cells. Epithelial cells express Aquaporin 1 (AQP1) and AQP5, high permeability water channels, while fiber cells express AQP0, a much less efficient water channel, and AQP5.

AQP0 is the most abundant protein in the fiber cell membrane. Mutation and knockout of AQP0 causes lens cataract. It plays various roles in lens biology. First, AQP0 functions as a water channel [1,2]. AQP water channels, gap junction channels and solute transporters play significant roles in creating a microcirculation within the avascular lens. The circulation provides nourishment to central fiber cells and disposes of their metabolic wastes, thus helping to maintain transparency and homeostasis [3–5]. C-terminal phosphorylation affects calmodulin binding and regulation of AQP0 [6,7]. AQP0 also functions as a structural fiber cell-to-fiber cell adhesion (**CTCA**) protein [8–12]. A genetically-

* Corresponding author. Physiology and Biophysics, Stony Brook University, NY, USA.
E-mail address: kulandaiappan.varadaraj@stonybrook.edu (K. Varadaraj).

http://dx.doi.org/10.1016/j.bbrc.2015.04.138
0006-291X/

engineered transgenic mouse model expressing AQP1 in fiber cells of AQP0 KO mice showed only partial recovery from cataract. In this model, AQP1 more than compensated for the reduced water permeability due to KO of AQP0, implying an additional unique function for AQP0 [11,12]. Ultrastructural studies revealed loss of integrity of the characteristic fiber cell hexagonal arrangement in AQP0 null or mutant lenses. This is consistent with a role for AQP0 in maintaining the cellular architecture of the fiber cells [12–15]. A role for AQP0 in establishing and maintaining the lens refractive index gradient has also been recently suggested [16].

Does AQP0 have a role in maintaining lens biomechanics? The lens has to maintain its biomechanical properties for sharp focusing on the retina. Presbyopia generally develops with age as the ability of the lens to accommodate is compromised [17]. Two cytoskeletal structures, as the BFs and the actin-spectrin network, participate in maintaining lens biomechanics [18,19]. Lens BFs have been shown to interact and colocalize with AQP0 [20], suggesting there could be a biomechanical role for AQP0.

The present investigation was undertaken to determine the role of AQP0 in establishing and/or maintaining the biomechanical properties of the lens. For these studies we compared lenses obtained from WT, AQP5 KO, and AQP0 KO mice. Our data strongly suggest that AQP0 may impart appropriate stiffness and elasticity in different parts of the lens, probably through its water channel and CTCA functions.

2. Materials and methods

2.1. Animals

Wild type (WT), AQP5 KO ($AQP5^{-/-}$), AQP0 KO heterozygous ($AQP0^{+/-}$) and homozygous KO ($AQP0^{-/-}$) mouse in C57BL/6J background, and WT-FVB/N mouse were used. All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by Stony Brook University Animal Care and Use Committee.

2.2. Genotyping

Genotyping by PCR using primers described by Alizadeh et al. [21] and competitive PCR using primers described by Simirskii et al. [22] were performed to confirm the absence or presence of CP49 deletion mutation in the mouse strains.

2.3. Analysis of lens AQP0, CP49 and filensin protein expression

To analyze the expression of AQP0 in WT-C57BL/6J, $AQP5^{-/-}$, $AQP0^{+/-}$ and WT-FVB/N mouse, whole lens proteins were extracted using NuPAGE LDS sample buffer (Invitrogen). Samples were resolved in 4–12% gradient NuPAGE gel (Invitrogen). Western blotting was done as described [3,5,21] using AQP0 antibody. Antibody binding was detected using alkaline phosphatase kit (Vector Laboratories, CA).

Lens outer cortex **(OC)** membrane preparations (4M urea-washed) of WT-C57BL/6J, $AQP5^{-/-}$, $AQP0^{+/-}$ and WT-FVB/N mouse were done as described previously [16]. Membrane pellets were extracted using LDS sample buffer. Equal amounts were used for Western blotting using CP49 or filensin antibody as described above.

2.4. Light microscopic and ultrastructural analyses of lenses

Light microscopic analysis was performed as described [16]. Scanning electron microscopic studies of WT and $AQP0^{+/-}$ lenses were performed as in Kumari et al. [12] (see Supplement 1).

2.5. Lens morphometric analysis

Lens was placed on a milled circular dimple (~50 μm diameter) of a testing chamber filled with physiological saline. Sagittal images were captured and axial (**ax**) and equatorial (**eq**) diameters were calculated using a micrometer and ImageJ software (http://imagej.nih.gov/ij/). Lens volume (V) and aspect ratio (AR) were calculated: $V = (4/3)\pi r_{eq}^2 r_{ax}$; $AR = r_{eq}/r_{ax}$; r_{ax} and r_{eq} are corresponding axial and equatorial radii (see Supplement 1).

2.6. Compression stress test to evaluate lens biomechanics

Compression stress testing was conducted to study biomechanical stiffness of 1-month or 2.5-month-old WT and experimental lenses as described previously [19,23], with slight modifications (Details given in Supplement 1). Each lens was subjected to gentle unrestricted compressive stress using weighed glass coverslips (18 × 9 mm: 72 mg or 18 × 18 mm: 144 mg). Sagittal images of lens shape alterations were digitized. ImageJ software (NIH) was used to measure lens ax and eq diameters which were converted to compression-stress (T): T =((d-d0)/d0), where d is the ax or eq diameter at a given load, and d0 is the corresponding ax or eq diameter at zero load. 'T' was plotted against load (mg).

2.7. Effect of load on lens suture integrity

Anterior side of one-month-old WT-C57BL/6J or $AQP0^{+/-}$ lens showing 'Y' suture was oriented to face the objective of an inverted confocal microscope and imaged with or without coverslip load under low and high magnifications; images were analyzed using Photoshop 9.

2.8. Statistical analysis

SigmaPlot 10 was used for Student's t-tests. P values ≤0.05 was considered significant.

3. Results and discussion

Except where otherwise indicated, lenses from mice of C57BL/6J genetic background were used in these studies. The exception is some control studies of WT lenses in FVB/N background. Initially

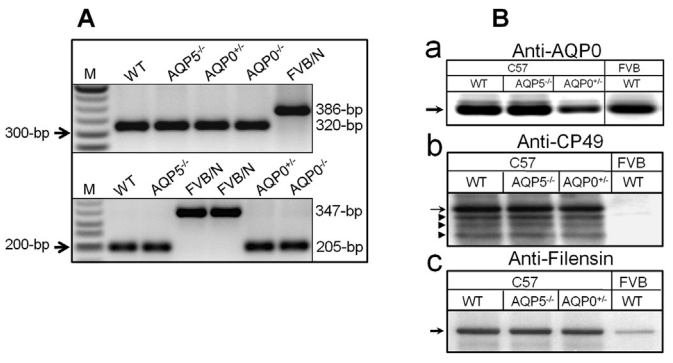

Fig. 1. (A) Genotyping. Top panel: PCR using primers as in Alizadeh et al. [21]; 320 bp, indicates presence of intact CP49 allele; 386 bp, indicates presence of mutant CP49 allele. **M**-Marker (50-bp Ladder). **Bottom panel**: Competitive PCR [22]. 205-bp indicates presence of intact CP49 allele; 347-bp indicates presence of mutant CP49 allele. **(B)** Western blotting of lens total membrane proteins using **(a)** AQP0 antibody (arrow, AQP0: 28 kDa) and cortex total membrane proteins using **(b)** CP49 antibody (arrow, CP49: ~49 kDa) or **(c)** filensin antibody (arrow, filensin: ~95 kDa). Arrowheads: lower immunoreactive bands to anti-CP49.

Please cite this article in press as: S. Sindhu Kumari, et al., Role of Aquaporin 0 in lens biomechanics, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.04.138