Characterization of Myocilin–Myocilin Interactions

Michael P. Fautsch and Douglas H. Johnson

**Purpose.** To determine whether myocilin (MYOC; also referred to as TIGR) is present as a complex in human aqueous humor, whether part of the complex formation may be due to MYOC–MYOC interactions and to characterize the sites of interaction.

**Methods.** Human aqueous humor was analyzed by using a gel filtration column for the identification of MYOC complexes. MYOC–MYOC interactions were studied by yeast two-hybrid system. Expression of full-length and truncated MYOC proteins in AH109 yeast was analyzed for growth and color on minimal medium. Site-directed mutagenesis was used to selectively mutate eight leucine residues within the leucine zipper motif. In vitro transcription and translation was used to verify yeast two-hybrid analysis.

**Results.** MYOC was found to be present in human aqueous humor as a complex ranging from 120 to 180 kDa. Expression of full-length MYOC in yeast as well as in vitro binding studies revealed that MYOC can interact with itself. MYOC–MYOC interactions occurred mainly within amino acids 117-166, a region containing a leucine zipper domain. Glycine substitution for selective leucine residues confirmed that MYOC–MYOC interactions occurred mainly within the leucine zipper domain.

**Conclusions.** MYOC is present in human aqueous humor, not as a monomer but as a complex. Part of this complex may form due to MYOC–MYOC interactions that take place mainly within the leucine zipper domain. (Invest Ophthalmol Vis Sci. 2001;42:2324–2331)

**Materials and Methods**

**Collection of Aqueous Humor**

Human aqueous humor was collected from six patients undergoing cataract surgery. This study was approved by the Mayo Clinic Institutional Review Board and conformed to the Declaration of Helsinki. No evidence of glaucoma or other ocular disease was present. At the start of the surgery, a paracentesis was made in the peripheral cornea and a 30-gauge cannula was inserted through the paracentesis tract. The tip of the cannula was placed in the mid anterior chamber, and 50 to 150 μl of aqueous was aspirated. Aqueous samples were lyophilized and stored at -20°C until use.

**Gel Filtration of Aqueous Humor**

Protein concentrations of the six individual aqueous humor samples were measured using a Bradford assay (Bio-Rad, Hercules, CA). Protein concentrations ranged from 0.16 to 0.35 mg/ml. Individual aqueous humor samples were combined and concentrated to 200 μl by using a filter (Microcon 10; Millipore, Bedford, MA). This produced a total of 111 μg of aqueous humor protein, which was then added to a gel filtration column (Superdex-200; Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated at pH 7.4 in PBS. A 150-μl aliquot from each of the 25 fractions (500 μl) was analyzed for MYOC by using an anti-MYOC antibody and with anti-rabbit Ig conjugated to horseradish peroxidase.

Although the function of MYOC is unknown, it contains a hydrophobic N-terminal signal peptide sequence, indicating that it is secreted. An extracellular function of MYOC is supported by its presence in the aqueous humor of normal, JOAG, and POAG samples.

MYOC expression in vitro can be increased by several different factors, including stress, stretch, steroid treatment, and TGF-β. Dexamethasone treatment of the anterior segment of cultured human eyes has shown that MYOC expression increases in a time-dependent manner similar to the time course for the development of steroid glaucoma. Excess secretion of normal, nonmutant MYOC has been proposed as a mechanism of steroid-induced glaucoma. In support of this hypothesis, we have found that infusion of recombinant MYOC increases IOP in cultured eyes, whereas heat-denatured MYOC or various other control proteins in similar or higher concentrations do not.

Structural studies with recombinant MYOC suggest that MYOC may function as a dimer or tetramer. Gel filtration of bovine and monkey aqueous humor indicates that MYOC is present in complexes, possibly as large oligomeric structures.

We examined human aqueous humor to determine whether MYOC is present as a monomer or is found in a complex. We examined the ability of MYOC to interact with itself, and we describe the region responsible for MYOC–MYOC interactions.
characterization of MYOC–MYOC interactions

Table 1. Oligonucleotides Used in the Study

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Oligo Name</th>
<th>Sequence</th>
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<tr>
<td>MYOC</td>
<td>MYOC-5'</td>
<td>5'-ATGGGAATTCTAGGAGAGCTAGGTCAGAGAAGC-3'</td>
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<tr>
<td>MYOC-3'</td>
<td>5'-ATGGGATCCCTATCATGGAAGAGCTTCATGTC-3'</td>
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</tr>
<tr>
<td>C terminus</td>
<td>C-term-5'</td>
<td>5'-ATGGGAATTCTAGGAGAGCTAGGTCAGAGAAGC-3'</td>
</tr>
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<td>Stop-3'</td>
<td>5'-ATGGGATCCCTATCATGGAAGAGCTTCATGTC-3'</td>
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<td>N-term-3'</td>
<td>5'-ATGGGATCCCTATCATGGAAGAGCTTCATGTC-3'</td>
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<td>No zipper</td>
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<td></td>
<td>Delete-3'</td>
<td>5'-CAGAGGAGGGGCTTGGACAGAAGGACC-3'</td>
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</table>

All fragments were cloned into pGADT7-AD. MYOC and C-terminal fragments were also cloned into pGBK7-BD. Base pair substitutions are underlined.

* MYOC-3' was used as 3' primer.
† MYOC-5' was used as 5' primer.

Placement Construction of Full-Length MYOC and Truncated Fragments

The MYOC cDNA template used in this study was described in our previous work. The oligonucleotides used to amplify each MYOC deletion construct are described in Table 1. All MYOC deletion constructs were obtained by PCR and cloned into a plasmid (pCR2.1; Invitrogen, Carlsbad, CA). The appropriate sized insert was isolated from pCR2.1 with restriction enzymes EcoRI and BamHI. Fragments corresponding to MYOC-binding domain (BD; base pairs [bp] 133-1551, aa 33-504) and C terminus (bp 541-1551, aa 169-504) were ligated into another plasmid (pGBKT7-BD; Clontech, Palo Alto, CA) immediately downstream of the Gal4 DNA binding domain (BD) and an epitope tag from the transcription factor c-myc. Fragments corresponding to MYOC-activation domain (AD; bp 133-1551, aa 33-504), C terminus (bp 541-1551, aa 169-504), Gln368Stop (bp 133-1551, aa 33-504), and N terminus (bp 133-1551, aa 33-504), "half zipper" (bp 133-1551, aa 33-504), and "no zipper" (bp 133-1551, aa 33-504), were ligated into pGBK7-BD (Clontech) immediately downstream of the Gal4 AD and hemagglutinin (HA) epitope tag. The MYOC DNA sequence and amino acid numbers are based on GenBank accession number U85257. The sequence of all deletion constructs was verified using a DNA sequencer (Perkin Elmer-Applied Biosystems, Foster City, CA).

Site-Directed Mutagenesis of MYOC

For site-directed mutagenesis of MYOC, oligonucleotides containing base pair substitutions that change leucine amino acids to glycine were generated (Table 1). These oligonucleotides were used to amplify 5' and 3' fragments that contain the appropriate base pair substitutions. Corresponding 5' and 3' fragments were fused, using oligonucleotides MYOC-5' and MYOC-3', by the technique of gene splicing by overlap extension (SOEing) and ligated into pCR2.1. The appropriate sized inserts were isolated from pCR2.1, by using restriction enzymes EcoRI and BamHI, and were cloned into pGADT7-AD. Mutated constructs are identical with MYOC-AD, except for the following leucine-to-glycine amino acid changes: L12 (aa 117, 124); L78 (aa 159, 166); L1234 (aa 117, 124, 131, 138); L5678 (aa 145, 152, 159, 166); L18 (aa 117, 124, 131, 138, 145, 152, 159, 166). The construct "delete zipper" contains the amino and carboxyl ends of MYOC but does not have aa 117-168. MYOC was used as the DNA template for L12, L78, and delete zipper amplification. L12 was used as the DNA template for L1234 and L78 as the DNA template for L5678. For amplification of L18 fragments, L1234 was used as the DNA template for the 5' fragment and L5678 as the DNA template for the 3' fragment. The sequence of all mutated constructs was verified with the DNA sequencer (Perkin Elmer-Applied Biosystems).

Yeast Two-Hybrid System

The two-hybrid system is a transcriptional assay engineered to examine protein–protein interactions. The two-hybrid system uses the finding that transcription factors often contain both a DNA-binding domain and an activation domain. These domains can remain functional, even if contained on separate proteins, provided that the proteins interact with each other. The yeast two-hybrid system exploits this phenomenon by fusing a separate BD and AD to a pair of potentially interacting proteins. Coexpression of interacting proteins in yeast results in transcription of reporter genes that code for nutritional markers (adenine, histidine) and galactosidase. If a protein fused to the BD and a protein fused to the AD interact, yeast containing these proteins will grow on minimal medium and produce a blue color if an appropriate substrate is present. Noninteracting proteins result in no growth and no color.

The two-hybrid system was purchased from Clontech. pGBK7-BD and pGADT7-AD plasmids containing the inserted MYOC DNA sequence were transformed into AH109 yeast using a yeast-transformation system (Yeastmaker; Clontech) and allowed to grow at 30°C for 2 to 4 days on plates containing selective medium without tryptophan and leucine (-LT). Because pGBK7-BD contains the gene for tryptophan and pGADT7-AD contains the gene for leucine, transformed
AH109 yeast plated on −LT medium grows only if the yeast contains both plasmids. For growth studies, individual colonies were isolated from −LT plates, diluted into medium, and optical densities at 600 nm (OD600) were obtained. Equal amounts of yeast from individual colonies were spotted onto agar plates containing selective medium without tryptophan, leucine, histidine, adenine (−AH1LT) or into 3 ml −ALHT medium. Plates were incubated at 30°C until colonies appeared (2–5 days). Liquid cultures were grown at 30°C with shaking for 48 hours. If expressed proteins interact, yeast will grow on −ALHT medium. Cultures were assayed for growth by measurement at OD600. Growth of yeast containing MYOC deletion or mutation constructs was calculated as a percentage of MYOC-AD on a minimum of 12 individual yeast colonies obtained from four independent experiments. Plates were scanned with a digital scanner (Eastman Kodak, Rochester, NY).

In addition to growth on −ALHT medium, yeast containing interacting proteins also induces expression of βgalactosidase. To determine βgalactosidase activity, yeast from 1.5 ml liquid culture was pelleted by centrifugation. Yeast was washed three times in modified RIPA buffer. Pellets were resuspended in 100 μl Z buffer and lysed by four freeze-thaw cycles (2 minutes in liquid nitrogen followed by 2 minutes at 37°C). Z buffer (70 μl) containing 0.3% β-mercaptoethanol was added, along with 160 μl of 6 mg/ml o-nitrophenyl β-galactopyranoside (ONPG). Samples were incubated at 30°C until they appeared yellow and were assayed at OD420. β-Galactosidase activity was calculated as a percentage of MYOC-AD on a minimum of nine yeast colonies obtained from three independent experiments.

In Vitro Binding Studies

Confirmation of yeast two-hybrid assay results was obtained using an in vitro binding analysis. Constructs used in the yeast two-hybrid study were transcribed and translated in vitro with a coupled transcription-translation system (TNT Quick; Promega, Madison, WI). Briefly, 40 μl of the master mix, containing T7 RNA polymerase, nucleotides, salts, RNase inhibitor, and rabbit reticulocyte lysate, was combined with 30 μl of the master mix, containing T7 RNA polymerase, nucleotides, salts, and tryptophan (−ONPG). Samples were incubated at 30°C until they appeared blue. Liquid cultures were grown at 30°C with shaking for 48 hours. If expressed proteins interact, yeast will grow on −ONPG. Samples were incubated overnight at 4°C with a polyclonal antibody (Clontech) was used as the primary antibody and anti-mouse Ig (Zymed, South San Francisco, CA) as the secondary antibody. The antibody-antigen complex was detected using ECL Western blot signal-detection reagent (Amersham).

To determine whether MYOC is found as a monomer (53-55 kDa) or migrates as a complex in human aqueous humor, a gel filtration column was used. Three distinct protein peaks were identified by absorbance of the individual fractions collected from the column (Figs. 1B, 1C; fractions 15-16, 18-19, 22-23). Protein standards determined that fraction 15 was approximately 167 kDa, fraction 19 was 71 kDa, and fraction 23 was 23 kDa. SDS-PAGE gels demonstrated that fractions 15-17 contained a 55-kDa protein and a small amount of a 70-kDa and 23-kDa protein. Fractions 18-21 contained large amounts of a 68-kDa protein, presumably albumin, and smaller amounts of a doublet at approximately 58 and 80 kDa (Fig. 1B).

To determine which fractions collected from the gel filtration column contained MYOC, Western blot analysis of fractions 1-25 was performed. Only fractions 15-17 were positive for MYOC protein (Fig. 1C). This suggests that MYOC is not found in aqueous humor as a monomer (55 kDa) but is present as a complex. This complex ranges in size between 120 and 180 kDa with most of the complex approximately 150 kDa. Nonfractionated aqueous humor verified the antibody specificity for MYOC (Figs. 1A, 1C).

Yeast Two-Hybrid System

Because MYOC is found as a complex in aqueous humor, we examined the possibility that part of the complex formation was due to MYOC–MYOC interactions. Full-length MYOC constructs (minus the 32-aa signal peptide sequence) were fused to the AD or BD of Gal4 transcription factor (Figs. 2A, 2B). These constructs were transformed into AH109 yeast and grown on selective medium without the amino acids leucine and tryptophan (−LT, Fig. 2C). Growth on −LT medium indicated that the yeast contained both the MYOC-BD and the MYOC-AD plasmids. Four individual yeast colonies were isolated from the −LT plates and tested for the ability to grow on selective medium without adenine, histidine, leucine, and tryptophan (−ALHT). Coexpression of MYOC-BD and MYOC-AD in yeast resulted in growth on −ALHT plates, which indicates that MYOC-MYOC interactions occurred (Fig. 2C). Two controls were run: MYOC-BD expressed in the presence of vector alone (Fig. 2C) and MYOC-AD expressed with a nonrelated control protein, lamin C-BD (data not shown). The absence of growth with these controls suggests that MYOC interacts with itself in a specific manner.

To determine whether MYOC–MYOC interactions occur in the N- or C-terminal regions of the molecule, the Gln368Stop mutation and a C terminus construct were amplified and fused to AD (Fig. 2B). Coexpression of MYOC-BD and Gln368Stop in yeast resulted in growth on −ALHT medium indicating an interaction in the N-terminal region. However, yeast coexpressing MYOC-BD/C terminus did not grow well on −ALHT medium, indicating the C-terminal region did not interact with full-length MYOC. This was confirmed in liquid culture where growth studies showed MYOC-BD/C terminus grew (12.4% ± 2.5%; mean ± SEM; n = 15) at only a fraction of MYOC-BD/MYOC-AD (100% ± 3.4%; n = 15). These results indicated that an MYOC–MYOC interaction occurred in the N-terminal region of the MYOC molecule.

To define the MYOC N-terminal interaction region, fragments corresponding to aa 33-168 (N terminus), aa 33-144 (half zipper), and aa 33-116 (no zipper) were cloned downstream of AD. These MYOC fragments were able to interact with MYOC-BD, confirming an N-terminal interaction (Fig. 2C). Although the coexpression of MYOC-BD/no zipper grew on solid agar, in liquid culture the growth rate was slow (38.5% ± 4.7%; n = 16), suggesting that the region between aa 117-167, which contains a 32-aa N-terminal tag was run for size comparison and antibody specificity (Fig. 1B; lane 2).

RESULTS

Gel Filtration of Aqueous Humor

The initial evaluation of human aqueous humor on SDS-PAGE revealed two MYOC-reacting bands of approximately 53 and 55 kDa (Fig. 1A; lane 1). Bacteria-produced recombinant MYOC (which contains a 32-aa N-terminal tag) was run for size comparison and antibody specificity (Fig. 1B; lane 2).
contains the leucine zipper domain, is important for MYOC–MYOC interactions.

**Site-Directed Mutagenesis**

To verify MYOC–MYOC interactions within the leucine zipper, site-directed mutagenesis was used to mutate MYOC cDNA so that the corresponding protein products contained glycine in place of the eight leucine residues involved in the MYOC leucine zipper (L18; Fig. 3B). Glycine was used for substitution because its preference for forming α-helix structures was low (0.43) when compared with the high preference of leucines for α-helix structures (1.34).\(^{32,33}\) Coexpression of L18 with MYOC-BD in yeast reduced growth to 24% (\(n = 15\); Fig. 3C). This correlated to β-galactosidase activity, which was reduced to 17% (\(n = 10\)) when compared with MYOC-BD/MYOC-AD (100% ± 2%; \(n = 11\)). This verified that the leucine zipper was involved in MYOC–MYOC interactions (Fig. 3B).

To determine which part of the leucine zipper had the most effect on MYOC interactions, specific leucine residues in the leucine zipper motif were modified. Glycine residues substituted for leucines at aa 117 and 124 (L12) resulted in growth reduction of almost 70% (31% ± 3%; \(n = 16\)) when coexpressed with MYOC-BD. Additional substitutions of leucine 131 and 138 (L1234) did not further reduce growth (37% ± 4%; \(n = 14\)), indicating that the interaction was similar to MYOC-BD/L12. Substitution of leucine 159 and 166 (L178) resulted in an increase in growth (131% ± 8.1%; \(n = 12\)), indicating that the MYOC-BD/L78 interaction was strong. Further mutation of aa 145 and 152 (L5678) resulted in very little change in growth when compared with MYOC-AD/MYOC-BD (89% ± 7%; \(n = 13\)). Taken together, these results indicate that the N-terminal region of the leucine zipper is critical for MYOC–MYOC interactions.

**In Vitro Binding Studies**

To examine MYOC–MYOC interactions in vitro, MYOC constructs were transcribed-translated and analyzed for the ability to complex with full-length MYOC after immunoprecipitation with anti-HA antibody, which recognizes an epitope found on all AD-containing constructs). Initially, several controls were performed to show the specificity of the anti-HA antibody. Only MYOC-AD was immunoprecipitated with anti-HA antibody after translation (Fig. 4A; lane 1). MYOC-BD was not immunoprecipitated, indicating the antibody recognized only an AD-containing protein and did not recognize the BD-containing protein (Fig. 4A; lane 2).

To determine whether full-length MYOC was capable of interacting with itself in vitro, MYOC-AD was cotranslated with MYOC-BD and immunoprecipitated. Figure 4A (lane 4) shows the presence of MYOC-AD (compare lane 4 with lane 1). Because MYOC-AD and MYOC-BD are the same size, the same membrane was immunoblotted with a c-myc antibody generated against an N-terminal tag specific for BD-containing products (Fig. 4B). Only BD-containing proteins that complex with AD proteins can be identified. Lane 4 shows the presence of MYOC-BD, indicating that an interaction between MYOC-AD and MYOC-BD occurred. MYOC-AD (without MYOC-BD) was not detected, indicating c-myc antibody specificity for BD-containing proteins (Fig. 4B; lane 1). MYOC-BD (without MYOC-AD) was also not detected, because it was not immunoprecipitated with anti-HA antibody (Fig. 4B; lane 2).

To verify that the C-terminal region is not involved in MYOC–MYOC interactions, the C terminus was cotranslated...
with MYOC-BD. Although C terminus was immunoprecipitated with anti-HA (Figs. 4A, 4B; lane 6), no MYOC-BD was present. This indicates that no interaction between C terminus and MYOC-BD occurred (Figs. 4A, 4B; lane 6). This result was confirmed when C terminus was inserted into pGBK7-BD and cotranslated with MYOC-AD. No C terminus band was immunoprecipitated with MYOC-AD (Figs. 4A, 4B; lane 11).

To confirm the involvement of the leucine zipper motif region, several N-terminal MYOC deletion constructs were cotranslated in vitro with MYOC-BD. MYOC-BD was able to complex with Gln368Stop and half zipper–translated products (Fig. 4; lanes 7, 8). Immunoprecipitation of L18 (which contains eight leucine-to-glycine substitutions within the leucine zipper), cotranslated with MYOC-BD, resulted in a banding pattern similar to immunoprecipitation of L18 alone (compare Figs 4A; lanes 3, 5). This indicates that very little interaction with MYOC-BD occurred. This was supported by Western blot analysis, which indicated that a low level of MYOC-BD was able to interact with L18.

**DISCUSSION**

Leucine zipper motifs are a series of heptad repeats characterized by a leucine found at every seventh amino acid. X-ray diffraction and NMR spectroscopy have demonstrated that leucine zippers dimerize by forming parallel coiled–coil α helices that wrap around one another through electrostatic (hydrophobic) interaction of the leucine side chains. Initially identified in transcription factors, leucine zippers have been found in a wide variety of proteins and, through their ability to homo- or heterodimerize, play a pivotal role in regulating the proteins’ function.

Our analysis of MYOC–MYOC interactions in vivo and in vitro shows that MYOC is capable of forming interactions with itself mostly through the leucine zipper motif found between aa 117 and 166. Substitution with glycine for the eight leucines involved in forming the zipper reduced the ability to form MYOC–MYOC interactions as evidenced by a 74% reduction in growth rate. These substitutions presumably disrupt the intermolecular electrostatic interaction between MYOC amino acids and may interfere with the presumed α-helical nature of the N terminus. Disruption of the N-terminal region of the leucine zipper appears to be more important for MYOC–MYOC interactions, in that substitution of the first two leucines in the leucine zipper (L12) dramatically affected MYOC interactions when compared with mutation of any of the other six leucines making up the leucine zipper.

The effect of any of these substitutions does not appear to change the predicted secondary structure of the C-terminal region that contains the olfactomedin-homology domain (data not shown). Olfactomedin-like proteins have been hypothesized to form oligomeric complexes, suggesting that interactions within this region of MYOC may also be responsible for MYOC–MYOC interactions. Results of our study indicate that the olfactomedin homology found within MYOC plays a minimal role in interactions within MYOC. However, the importance of this region is well documented; almost all known MYOC mutations have been identified within the C-terminal region. Identification of additional MYOC-binding partners is important in determining the function of the C terminus.

Although interactions within the leucine zipper account for most binding between MYOC molecules, additional residues within the first 116 amino acids may also be involved in
MYOC–MYOC interactions. The no zipper construct (aa 33-116) was still able to bind to full-length MYOC (Fig. 2). Fusing aa 33-116 to C-terminal aa 167-504 (delete zipper) produced a construct that was also able to interact with MYOC, which supports the presence of an additional MYOC–MYOC binding region outside the leucine zipper (Fig. 3). Several explanations could account for this. One possibility is that the truncated protein products expressed from the no zipper and delete zipper constructs expose amino acids that normally are hidden in MYOC because of structural constraints. This therefore could result in nonphysiological binding. An alternative possibility is that aa 33-116 are important for MYOC interactions. This region contains the amino portion of the predicted α-helical region of MYOC. Whether this truncated MYOC fragment can still maintain its α-helix structure and interact with full-length MYOC is unknown. Of interest is the occurrence at every seventh amino acid of three leucines (aa 84, 91, and 98) in this N-terminal region. Although this “mini” leucine zipper is not in-frame with the leucine zipper starting at aa 117, it is possible that this region is also partly responsible for MYOC–MYOC interactions.

In addition to its α-helix structure, the N-terminal region contains two cysteine residues that could be important in disulfide bond formation (aa 47 and 61). We have found that MYOC also forms complexes when gel electrophoresed under nonreducing conditions, suggesting that intermolecular and/or intramolecular disulfide bond formation could also be responsible for MYOC complex formation. This confirms an earlier
observation by Nguyen et al. Further analysis of the cysteine residues and disulfide bond formation will help delineate their importance.

Although our data suggest the importance of the N terminus in MYOC–MYOC interactions, the amino acid sequence immediately downstream of the leucine zipper motif may also be involved. Coexpression of MYOC-BD with Gln368Stop resulted in the highest growth rates when compared with N terminus, half zipper, or no zipper (Fig. 2C). This indicates that aa 168-368 may be involved in MYOC–MYOC interactions. However, several studies with C terminus (which contains aa 168-368) may be involved in MYOC–MYOC interactions. Furthermore, as little as two amino acid substitutions within the leucine zipper (L12) reduced MYOC–MYOC interaction significantly. Because this portion of the study involves truncated and mutated proteins, we cannot rule out the possibility that these modifications have distal effects and have altered the downstream structure, interfering with potential interactions outside the leucine zipper.

The finding that MYOC is in large complexes in human aqueous humor and that MYOC–MYOC interactions occur mainly through the leucine zipper region supports the possibility that MYOC is present in human aqueous humor as a dimer. Previous studies found recombinant MYOC in complexes similar to a dimer or tetramer structure when cross-linked with glutaraldehyde. Studies in bovine and monkey aqueous humor suggest MYOC is present as a dimer and possibly as tetramer or oligomeric structures. It is possible additional MYOC molecules interact upstream of the leucine zipper, resulting in a tetramer formation. Furthermore, an MYOC dimer may form with other proteins, possibly the 70- and 23-kDa proteins found in the same gel filtration fractions (Fig. 1B). Further analysis of the MYOC complex using immunoprecipitation studies will help identify the true nature of the MYOC complex in human aqueous humor.

Although the function of MYOC is unknown, the finding of MYOC in complexes has prompted the idea that MYOC and mutated forms of MYOC may interfere with aqueous outflow through the trabecular meshwork by plugging outflow channels. However, there is also evidence for a potential intracellular role. It is of interest that aqueous humor samples collected from patients with JOAG who harbor the Gln368Stop mutation still contain wild-type MYOC but do not contain the mutated form. Similarly, expression of mutant MYOC in trabecular meshwork cells indicates that the mutant forms are translated but not secreted. This suggests that mutant MYOC may not plug outflow pathways, but rather could alter the secretion of normal MYOC or other secreted proteins whose function may be to maintain the integrity of the trabecular meshwork and its extracellular matrix.

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**References**


