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Expression of alphaA crystallin: A human lens protein

Abstract

One of the leading causes of serious visual impairment in young children is congenital cataracts (1). Cataracts and subsequent reductions in vision are the result of opacification of the lens. The human lens is primarily made up of water-soluble proteins called crystallins, which are divided into three groups, alpha, beta, and gamma (2). Mutations in all three of the types have been found to cause the formation of cataracts, although the exact mechanisms remain elusive (3). An autosomal dominant mutation in human alphaA crystallin was discovered to be the cause of one type of congenital cataract in humans (3). In order to study the mechanism by which this mutation results in cataract formation it is necessary to first study the normal form of human alphaA crystallin. This project accomplishes the expression, isolation, and purification of human alphaA crystallin.

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K. J. Lampi

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"Expression of AlphaA Crystallin: a human lens protein."

By

Lori Lee Jaffray

A thesis submitted to the faculty of the
College of Optometry
Pacific University
Forest Grove, Oregon
For the degree of
Doctor of Optometry
December 2000

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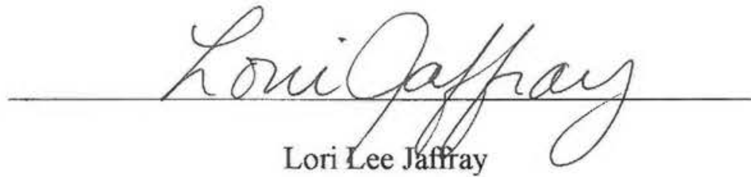
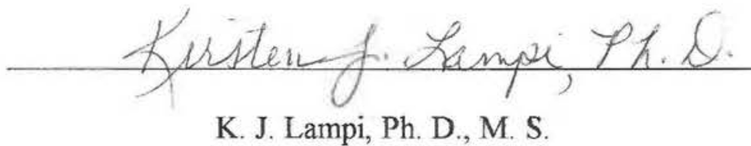


Lori Lee Jaffray

Professor in Charge of Research



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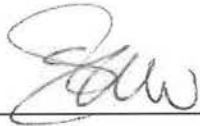

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Biography of Author

Lori Lee Jaffray attended Lindsay Thurber Comprehensive High School in Red Deer, Alberta from 1988 to 1991, where she earned the Rutherford Scholarship for academic achievement. After high school, Lori traveled to Sao Paulo, Brazil for one year to attend school there and to learn Portuguese. Upon returning to Canada, Lori attended Red Deer College from 1992 to 1994, and began her pre-Optometry courses. In order to complete her degree in science, Lori transferred to University of Alberta in Edmonton. Lori completed her Bachelor's degree in Science, with a specialty in Biochemistry from 1994 to 1997. While attending the University of Alberta, Lori was a member of the executive of the Biochemistry Students Association.

In the fall of 1997, Lori began her Optometry degree at Pacific University. While there, Lori has been very active in the Amigos, attending numerous screenings and traveling to Costa Rica in 2000. Lori has also been a member of the BSK honors fraternity since her first year and was co-president of the chapter from 1999-2000. Lori plans to graduate from Optometry in May 2000 and return home to Canada.

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Abstract

One of the leading causes of serious visual impairment in young children is congenital cataracts (1). Cataracts and subsequent reductions in vision are the result of opacification of the lens. The human lens is primarily made up of water-soluble proteins called crystallins, which are divided into three groups, alpha, beta, and gamma (2). Mutations in all three of the types have been found to cause the formation of cataracts, although the exact mechanisms remain elusive (3). An autosomal dominant mutation in human alphaA crystallin was discovered to be the cause of one type of congenital cataract in humans (3). In order to study the mechanism by which this mutation results in cataract formation it is necessary to first study the normal form of human alphaA crystallin. This project accomplishes the expression, isolation, and purification of human alphaA crystallin.

Introduction

The human lens is a unique and complex organ. Its primary function is to eliminate retinal blur. The strict control of lens transparency is required to achieve this goal (4). Proper nutrient metabolism as well as regular folding of the lens crystallins, which make up 90% of the lens proteins, are essential to maintaining transparency (2,5). There are three classes of crystallins in the lens, alpha, beta, and gamma, which interact and pack together in a complex manner so that light can pass through the lens with minimum scattering. Any disruption in the ordered packing of the crystallin proteins would lead to cataracts. The exact three dimensional structures of all the crystallins are not yet known, but based on their secondary structures, they are thought to be made up

mainly of beta sheet and alpha helical subunits, which ultimately influence their folding tendencies into three dimensional structures (2,6). The purpose of this project is to express and purify human alphaA crystallin so that it can be further studied.

Alpha crystallin is the largest of the three crystallins, based on molecular weight (2), and comprises 40-50% of total lens proteins (5,7). There are two main subtypes of alpha crystallin, alphaA and alphaB (2). Although their N-terminal regions differ, alphaA and alphaB share a 57% C-terminal sequence homology. Therefore, despite some similarities, these proteins differ in their relative functions (5,7,8). AlphaB appears to be produced in response to stress in many non-ocular tissues in the body (8,9). AlphaA, on the other hand, appears to play a major structural role in the lens, and is not found to be abundant in any other human tissue (8). Approximately 32 subunits of alphaA and alphaB come together via hydrophobic interactions to form a multimer of approximately 800kDa (6,8,10). In part, this considerable size and complexity has limited the study of alpha crystallin's structure (8,9).

A conserved region of 100 amino acids in both alphaA and alphaB crystallin make them distant relatives of a functionally related group of proteins called heat shock proteins (HSP's) (9,11). However, strong evidence suggests that alphaA is the more stable of the two alpha crystallins (7). HSP's have been studied in a number of different organisms, and although their structures may be vastly different, their universal function appears to be to aide in proper protein folding and breakdown, and to maintain the correct conformations of proteins during times of stress (11).

The proteins in the lens are formed during fetal development and do not continue to replicate throughout life. In this static environment, where there is a lack of protein

turnover, repair and maintenance mechanisms are of paramount importance (5). A protein that assists in the folding of other proteins, is called a molecular chaperone (11). In the lens, alphaA is believed to demonstrate molecular chaperone-like activity (5,7,11). Ensuring the correct folding of the other crystallins and protecting them from incorrect aggregations is essential to maintaining proper lens transparency (11). By recognizing unfolded or partially denatured regions, and sequestering them, alphaA crystallin may be the vital link in understanding cataract formation.

By studying the normal aging process of the lens, which results in cataracts, it has been shown that the crystallins become more insoluble over time (2). High molecular weight aggregates of the crystallins begin to appear and increase in size with age (2). Breakdown in the proper folding of the crystallins and the formation of disulfide bridges between proteins may account for the formation of these aggregates (2). The role of alphaA crystallin appears to be to prevent these processes from occurring and therefore plays a vital role in preventing or slowing cataract formation (8).

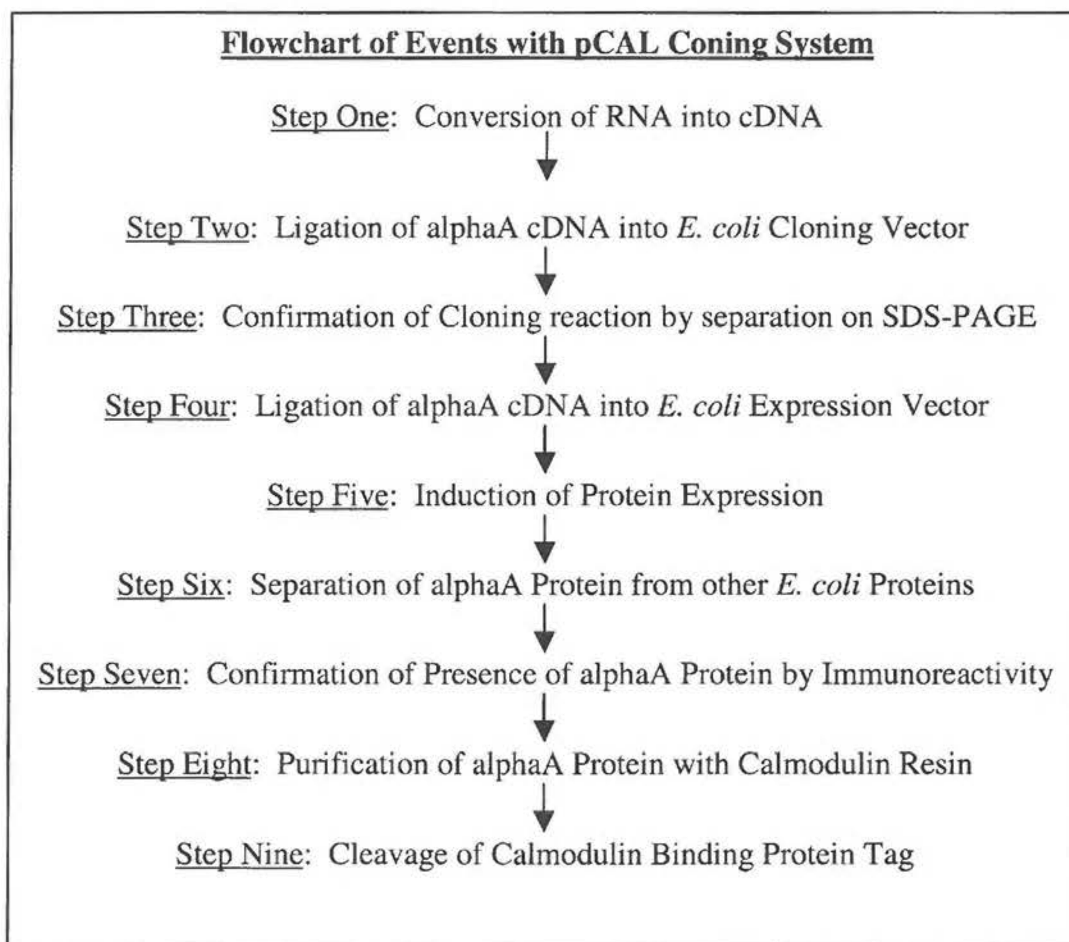
Mutations in the genes of all three types of crystallins have been found to cause cataracts (3,10,12). Of particular interest to this project was the discovery of an autosomal dominant congenital cataract (ADCC) due to a mis-sense mutation in the human alpha crystallin gene (CRYAA), by Litt et al. Litt et al studied a particular family, in which 13 individuals, over 4 generations, were afflicted with congenital cataracts. Genetic testing showed that the same mis-sense mutation on chromosome 21 existed in all afflicted family members while other family members lacked the mutation (3). The single base pair change in the DNA sequence of the alpha crystallin gene results in an amino acid arginine(R116) being converted to a cysteine(C116) residue (3).

Although this small change may appear moderately harmless on the surface, each of these two amino acids have significantly different properties, when considered in terms of their effects on secondary structure of a protein. As was previously mentioned, the crystallins are primarily made up of alpha helices and beta sheets. Although any of the 20 amino acids can be incorporated into alpha helices and beta sheets, certain ones are more stable and sterically suitable for these structures than others (10). Cysteine is a key player in the formation of disulfide bridges, and is therefore capable of disrupting alpha helix and beta sheet formations (10). Disulfide bridges play a role in proper protein folding and any changes in the formation of these bridges can have profound effects on the final structure.

In order to determine if alphaA crystallin's structure is being altered by the mis-sense mutation, one must first study the normal form of alphaA crystallin. Little is known about the exact three-dimensional structure of alphaA crystallin at this time. Many theoretical models have been proposed, mainly spherical or globular in nature, and are largely based on its secondary structure (6,11). By determining the exact folding nature of both normal alphaA and the mutated form of alphaA, it may be possible to determine how the mutation leads to cataract formation. Three-dimensional studies of the two forms of alphaA, including nuclear magnetic resonance (NMR) and electron spin resonance (ESR) may ultimately be employed to determine the differences between the two similar proteins. **This project focuses on the expression and purification of normal human alphaA crystallin.**

Materials and Methods

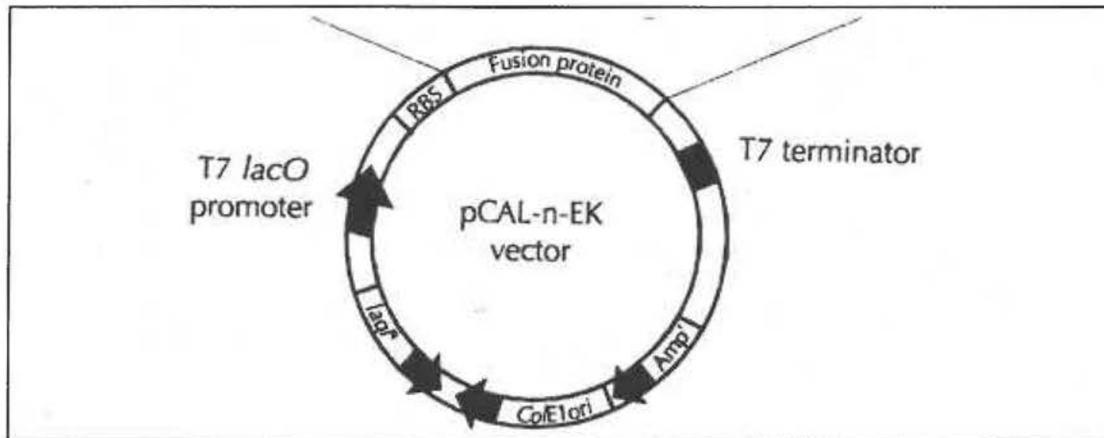
Figure 1



Step One

Total human RNA was previously extracted from donor lenses (13). A cDNA copy of the genetic material was produced via reverse transcriptase-polymerase chain reaction (RT-PCR) and gene specific primers. Once the cDNA of alphaA was obtained, primers were designed specifically to allow ligation into the pCAL-N-EK expression vector shown in Figure 2 (Stratagene Cloning Systems, La Jolla, CA)

Figure 2



The pCAL vector was chosen in part because it contains a Calmodulin binding peptide (CBP) tag. This allows alphaA DNA to be inserted as the "fusion protein" in the diagram. Once alphaA is ligated into the vector, it allows for easy purification with a Calmodulin affinity resin. Also contained in the pCAL vector is a recognition site for a site-specific protease enterokinase (EK), located between the CBP tag and the alphaA insert. This protease (EK) can later be used to cleave the CBP tag, leaving only the alphaA protein. The T7 promotor is highly effective, allowing efficient multiplication of the vector and alphaA insert.

Primers were specifically designed to allow ligation to a specific section of alphaA DNA. Primers must be able to anneal to the alphaA DNA, and also to separate from the alphaA DNA during the different phases of the PCR reaction. Once the gene specific primers were designed, 1µl of alphaA cDNA was amplified by PCR. Standard PCR protocol was performed in a thermal cycle machine, using 2.5 units of Taq Polymerase. The PCR products were then resolved on a 1% agarose gel and stained with Ethidium Bromide. The bands were excised from the gel and weighed. The amplified

DNA was then purified from the agarose gel and an optic density (OD) reading was taken to determine the concentration of DNA. (An OD reading equal to 1 at 260nm equals 50μg/mL microgram of double stranded DNA.) The alphaA DNA was then ligated with 1μL of pCAL vector and incubated at room temperature for four hours.

Step Two

Transformation of the ligation product into the XL1-Blu strain of *E. coli* cells was performed. As the XL1-Blu strain of *E. coli* lacks the T7 promoter region necessary for protein expression, it was specifically used to amplify the alphaA DNA insert, while keeping protein expression to a minimum. The ligation vector product was incubated with the XL1-Blu cells in culture broth (SOC medium) at 37°C for one hour on a shaker. Several aliquots of the transformation reaction mixture were plated onto agar plates containing Ampicillin, to prevent contaminating bacteria growth. The plates were inverted and incubated at 37°C overnight.

Step Three

Several colonies of cells were picked from the agar plates described above and allowed to amplify in culture broth overnight at 37°C. Culture broths of *E. coli* were spun down and cells were lyzed with strong detergents. The plasmids from the lysed cells were isolated. These plasmid preparations were screened for the presence of the alphaA insert using gene specific primers to alphaA in a PCR reaction. The PCR products were visualized on a 1.5% agarose gel.

Step Four

Positive colonies were transformed into BL21(DE3)pLysS cells. These DE3 cells are another strain of *E. coli* cells. The DE3 cells contain the T7 promoter region and are

therefore used for protein expression of the alphaA insert. By varying the growth conditions of the DE3 cells using IPTG, overexpression of the alphaA protein is possible. Once again, the transformation reaction mixtures were plated onto agar, containing Ampicillin, and were incubated overnight at 37°C.

Several colonies were picked and the transformed DE3 cells were cultured separately in Luria culture broth (LB broth) containing Ampicillin, Chloramphenicol, and Glucose for three hours, with continuous shaking at 37°C. In order to monitor the growth of the cells, an OD reading at 600nm was periodically taken.

Step Five

Once an adequate concentration of cells was acquired, half the cells were stored undisturbed at 4°C as a control. To the other half of the cells, Isopropyl-beta-*D*-thiogalactopyranoside (IPTG) was added to induce protein expression. The induced cells were shaken at 37°C for two hours. A small portion of cells were then centrifuged down into pellets and stored in a 30% glycerol stock at -80°C to allow cells to be amplified in the future as needed.

Step Six

To determine levels of protein induction, *E. coli* proteins from the cell lysates were first separated from alphaA protein on a sodium dodecyl sulfate-poly acrylamide gel (SDS-PAGE). Protein concentration of the cell lysate was determined by a modified biuret method according to manufacturers instructions (BCA Protein Assay, PIERCE, Rockford, Il). The protein samples were diluted to approximately the same concentration and were separated on an SDS-PAGE gel. The gel was stained with Coomassie Blue to visualize the bands of the expressed proteins.

Step Seven

To confirm that the desired protein had been expressed, an immunoblot was performed using a specific anti-alpha crystallin antibody. Proteins separated on the SDS-PAGE gel were transferred to blotting paper, polyvinylidene difluoride (PVDF), by electrophoresis using NOVEX mini-apparatus according to manufacturers protocol (NOVEX Experimental Technology, San Diego, CA). Following incubation with the primary anti-alpha antibody, positive immunoreactivity was detected by incubating with secondary goat-antirabbit IgG antibody, conjugated to alkaline phosphatase. An immunoreactive band was visualized by color development upon cleavage of alkaline phosphatase substrate.

Step Eight

After confirmation of alphaA expression, the expressed protein was purified. The alphaA protein was cleaved from the Calmodulin binding protein of the pCAL vector. After induction and pelleting of the cells, they were lysed with a mixture of Lysosyme, Pepstatin A, dithiothreitol (DTT), and phenylmethanesulfonyl fluoride (PMSF). The cells were centrifuged to separate the soluble proteins, the supernatant, from the cell remnant pellet. The supernatants were incubated with a Calmodulin binding resin column for three hours. This resin binds to the tag on the expressed protein. The resin was spun down to separate the resin from the binding buffer containing unwanted *E. coli* proteins. A portion of the supernatant buffer was removed from the resin in preparation for visualization on an SDS-PAGE gel. The resin was washed several times with binding buffer and portions from these washes were also prepared for a gel. A buffer containing EGTA was used to elute alphaA. Samples of these elutions along with the washes,

supernatant buffer, and a sample of unbound alphaA were all visualized on a SDS-PAGE gel. After purification of the protein, an attempt was made to cleave off the CBP tag.

Step Nine

The elutions from the column were first equilibrated with an Enterokinase (EK) cleavage buffer and were incubated with EK protease overnight at 37°C. The results of the cleavage reaction were subsequently separated on an SDS-PAGE gel.

After several unsuccessful attempts at cleaving the CBP, it was decided to try a second expression kit that did not include a binding tag. It was believed that the alphaA crystalline remained bound to the resin and therefore an alternate method was required to purify the protein. Subsequently the pCR-T7/CT TOPO-TA expression kit was chosen (Invitrogen Corporation, Carlsbad, CA). Although this kit would make purification of the expressed protein more difficult, due to the lack of a binding tag, it was felt that it was a necessary step in order to get purified native alphaA protein.

Alternate Cloning Method

Methods to express protein using the TOPO kit parallel those procedures described above with the pCAL kit, with the notable exception of the tag. Briefly, the TOPO kit method is described below.

Ligation into TOPO Cloning Vector

The alphaA DNA was ligated into the T7/CT TOPO vector. The ligated vector was then transformed into Top 10F' cells. These cells were amplified and the alphaA ligated insert was purified using a minispin kit according to manufacturer's protocol (Qiagen Inc., Valencia, CA).

Transformation into Expression Cells and Protein Induction

Plasmids containing alphaA were transformed into DE3 expression cells and incubated at 37°C to culture several liters of cells. Protein expression was induced with IPTG and allowed to incubate. The DE3 cells were lysed and the supernatants containing the soluble proteins were separated onto an SDS-PAGE gel.

Immunoreactivity and Sequence Confirmation

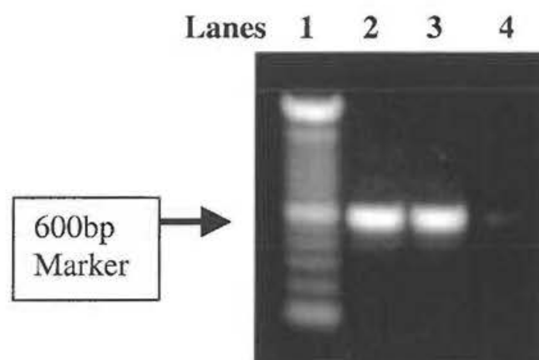
Immunoblotting was performed to detect alphaA. The alphaA protein was purified using anion-exchange and gel filtration chromatography. DNA sequencing further confirmed the presence of alphaA insert and agreed with the published sequence (GenBank Accession No. U05569).

Results

Transcription and PCR Reactions (pCAL kit)

AlphaA crystallin was cloned and expressed. The following figures outline the results at each step within the process. The first step in cloning alphaA was the PCR transcription of the gene as shown in Figure 3. Using the gene specific primers, alphaA cDNA was transcribed from donor RNA and amplified via PCR. The PCR reactions were visualized on agarose gels (lane 2, 3, and 4) with known molecular weight calibrating basepair (bp) standards (lane 1).

Figure 3

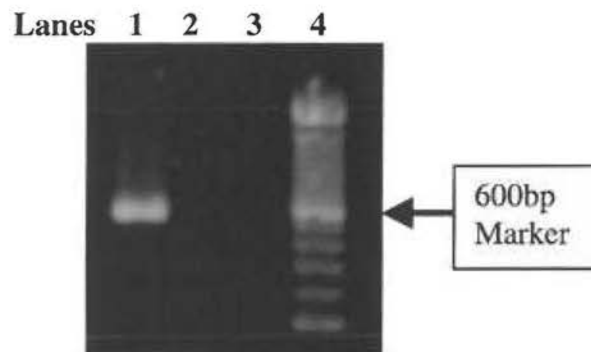


The lack of bands in lane 4 in Figure 3, indicates a colony in which cloning was not successful. Heavy bands of approximately 600bp when compared to standards, are evident in lanes 2 and 3 in Figure 3, and correspond to the size of the alphaA cDNA plus the CBP tag. This indicates the presence of alphaA cDNA and confirms that the transcription and PCR reactions were successful.

Ligation and Transformation

After amplification, the alphaA cDNA was ligated into the pCAL vector and cultured in a strain of *E. coli* cells. Following isolation of the plasmids from selected colonies, a PCR screen was performed (Figure 4) to confirm ligation and aliquots were separated on an agarose gel (lanes 1,2,3).

Figure 4



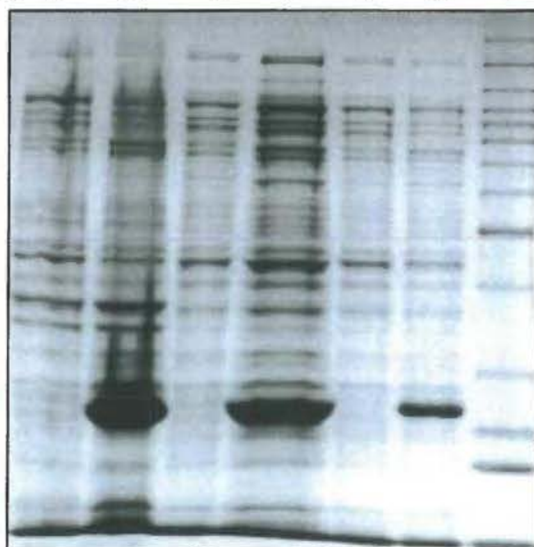
When compared to molecular weight standards in lane 4, Figure 4 shows a heavily stained band in lane 1 corresponding to 600bp. This confirms the presence of alphaA insert with the CBP tag in the plasmid from one of the picked colonies of XL1-Blu cells. Lanes 2 and 3 are from colonies in which no ligation occurred. Thus, the band in lane 1 demonstrates that ligation and transformation of the cloning cells was successful.

Induction and Expression

After completion of cloning, the alphaA protein was expressed. The protein product of alphaA crystallin was expressed via transformation and induction of DE3 expression cells with IPTG. Lysed portions of DE3 cells were run through a Calmodulin resin column in order to separate the alphaA protein from all the other cell proteins. Each pair of lanes, (1-2, 3-4, 5-6) in Figure 5, corresponds to the non-induced control and induced portions of DE3 cell colonies respectively. The elutions of these different cell lines were separated on an SDS-PAGE gel to visualize the expressed alphaA product (Figure 5). The heavily stained bands in lanes 2, 4, and 6 of Figure 5 approximately correspond to 25 kDa, when compared with the molecular weight markers in lanes 1 and 7. This is in agreement with the expected molecular weight of the alphaA protein with the CBP tag attached. Figure 5 clearly shows that protein was induced in at least 3 different colonies of DE3 cells. The presence of alphaA protein was definitively confirmed by detection with an anti-alpha antibody, as shown in Figure 6. The immunoblot showed an intensely stained band at 27kDa, just above the 25kDa marker (Figure 6).

Figure 5

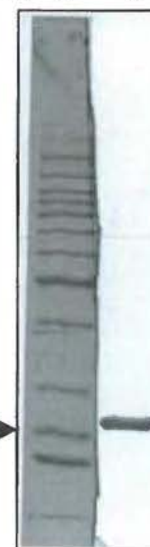
Lanes 1 2 3 4 5 6 7



25kDa Marker

Figure 6

Lanes 1 2

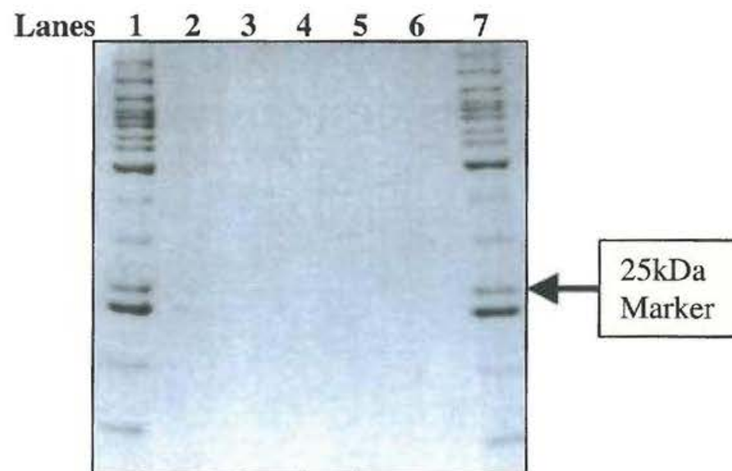


This is where alphaA crystallin is expected to resolve by SDS-PAGE. No other proteins were detected in the immunoblot (lane 2), indicating specific immunoreactivity of the induced alphaA protein.

Purification and Removal of Tag

In order to separate the alphaA protein from the CBP, a cleavage reaction with Enterokinase was performed. The products of these reactions were resolved on a gel in Figure 7 (lanes 2 through 6) to confirm cleavage.

Figure 7

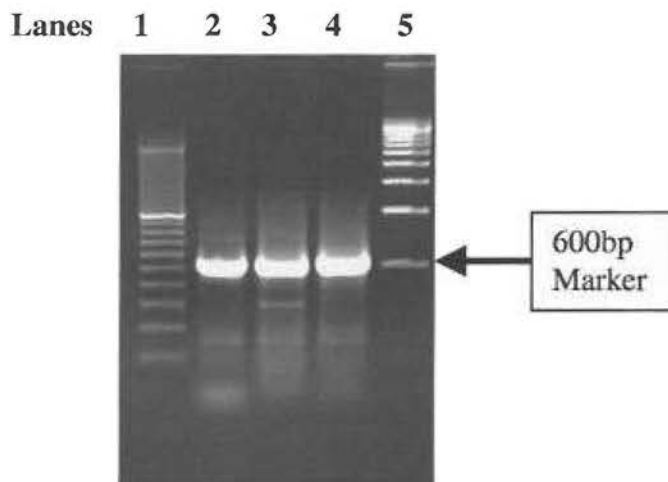


The lack of bands in lanes 2 through 6 in Figure 7 indicates an absence of the alphaA protein. The tagged alphaA remained bound to the column which resulted in the absence of protein in the elutions. Therefore, when the column was regenerated, the bound protein was discarded. Further attempts to cleave alphaA from the binding protein were made by varying the reaction conditions with similar negative results. This was surprising since the tag had been successfully cleaved off of a beta crystallin in a similarly designed study (14).

Cloning with TOPO Kit

In order to express protein without a tag, a second system was employed. With the TOPO kit, alphaA cDNA was amplified using gene specific primers. The correct size of the cDNA was checked by separating the products on an agarose gel (figure 8). The alphaA was then ligated into Top10F' cells which were cultured at 37°C.

Figure 8



Bands in lanes 2, 3, and 4 of Figure 8 are consistent with the approximate length (600bp) for alphaA cDNA, when compared to the standard markers. After transformation and expression of alphaA protein in the TOPO system, the proteins were purified by anion exchange and gel filtration chromatography. The elutions from these columns were separated on an SDS-PAGE gel (Figure 9). The heavy bands in lanes 3 (anion exchange) and 4 (gel filtration) correspond to the correct size of the alphaA crystallin protein. The other bands in lanes 3 and 4 are proteins, which were eluted from the chromatography column. However, they do not correspond to the correct molecular weight for alphaA crystallin. Lanes 1 and 2 of Figure 9 correspond to molecular weight markers and *E. coli* cell lysate respectively.

Figure 9

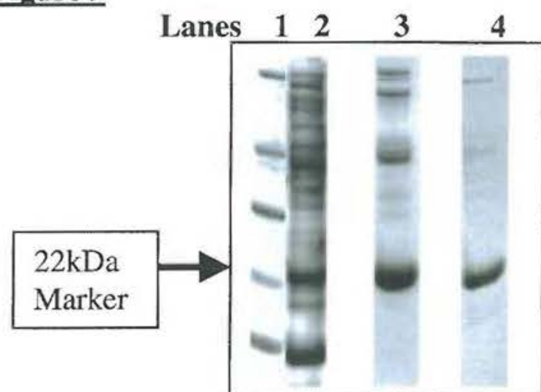
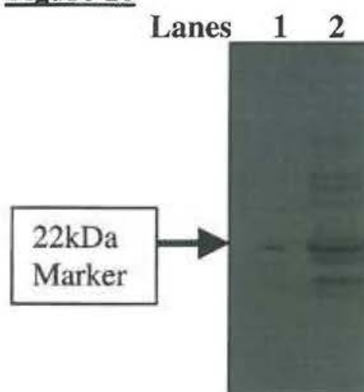


Figure 10



The results of an Immunoblot, performed to confirm the presence of alphaA via gel filtration chromatography, are shown in Figure 10. The heavily stained band in lane 1 of Figure 10 shows the correct molecular weight of alphaA (without the CBP tag) and confirms the presence of alphaA protein. In order to ultimately complete this project and confirm the presence of alphaA protein, a sample of alphaA DNA was sent to the Core labs at OHSU for sequencing. The experimental sequence used for this project agreed with the published sequence for alphaA crystallin (GenBank Accession No. U05569). Thus, alphaA crystallin was successfully purified with the TOPO system.

Discussion

AlphaA crystallin was successfully cloned and expressed by two separate systems based on the following three lines of evidence. Initially, a positive PCR screening was obtained for both kits. Secondly, resolution of expressed protein on SDS-PAGE was achieved with both systems. Finally, immunoreactivity with a specific alphaA antibody was visualized with the two separate systems.

The pCAL and TOPO systems require somewhat different protocols to achieve the same results. While the pCAL kit employs a built in purification step with its CBP, the TOPO kit requires a much less predictable method of purification. The TOPO kit

requires the use of chromatography to isolate alphaA from the ligated vector in which it is amplified.

The use of the pCAL kit, although it did not yield the desired outcome, did stimulate some interesting questions. Most importantly, why the enterokinase cleavage step failed? As the same kit was used in a simultaneous project with another crystallin with excellent results, it demonstrated that the pCAL kit does in fact work (14). The reason it failed with alphaA crystallin must lie in the nature of alphaA crystallin itself.

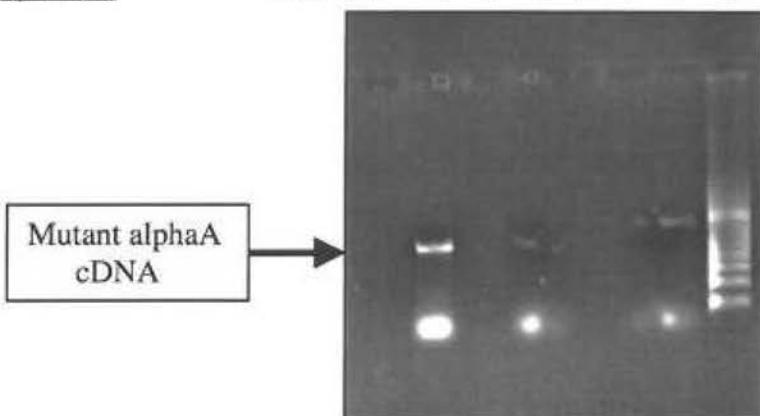
From more recent theories, it is suggested that the N-terminus of alphaA crystallin is buried and would not have been available for cleavage by the EK enzyme (6). The N-terminus may be buried within a single monomer of alphaA or it may be buried within the larger multimer of alphaA. Furthermore, the C-terminus and not the N-terminus has been shown to be available for modification during the aging process (15). Therefore, the logical reason for the lack of cleavage by the enterokinase with the pCAL system is that the N-terminal tag was buried and unavailable to the enzyme.

The TOPO kit however, allowed the expression and purification of alphaA crystallin to be successfully completed. The lack of a binding tag eliminated the need for availability of the N-terminus of alphaA and, as such, the problem encountered with the pCAL kit disappeared. While additional purification steps were required, the alphaA protein obtained was approximately 90% pure (14).

With alphaA crystallin available in soluble form, it may now be possible to study its three-dimensional structure via NMR and ESR techniques. The mutated form of alphaA crystallin, discovered by Litt et al, has also been created via site-directed mutagenesis (14). An initial agarose gel of the PCR cloning step of the mutated form of

alphaA is shown in Figure 11 (14). In addition, primers have been designed for the mutated form of alphaA-crystallin to enable future projects (14).

Figure 11 **Lanes 1 2 3 4 5 6 7 8**



The further expression and purification of mutated alphaA will allow the two types of alphaA to be comparatively studied. With these new achievements, it will be possible to compare the two forms of human alphaA crystallin in their native forms. The remaining goals of studying the alteration of the three-dimensional configuration and determining and its role in the formation of congenital cataracts may prove invaluable to the future treatment and prevention of needless vision loss.

Conclusions

The expression and purification of alphaA crystallin is an important step in studying its role in cataract formation. Determining the biochemical nature of alphaA crystallin may elucidate the mechanisms by which cataracts develops.

The goal of expressing alphaA crystallin has allowed many interesting facts about alphaA crystallin to be uncovered. The invaluable information learned about the N-terminal region of alphaA crystallin will allow future studies to be better designed.

AlphaA crystallin needs to be studied in greater detail to uncover the role it plays in the formation of cataract in both the young and old. The ultimate goal of prevention of the devastating point mutation in alphaA crystallin may also be achieved. Only with continued research of all the types of crystallins will the mechanisms of cataract formation be elicited and possible solutions be hypothesized.

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Appendix A

Glossary

Materials Used

cDNA – Complementary DNA synthesized by RNA-directed DNA polymerase using RNA as a template.

CBP – Calmodulin Binding Protein Tag

DTT - Dithiothreitol

EGTA – Ethyleneglycol-bis-(beta-amino-ethyl ether)-N,N,N',N'-tetraacetic acid

EK – Enterokinase enzyme

Ethidium Bromide – A form of dye that prevents transcription and DNA replication by binding to specific regions of the DNA molecule.

IPTG – Isopropyl-beta-*D*-thiogalactopyranoside

LB – Luria broth - Combination of NaCl, Tryptone, yeast extract, deionized water at a pH of 7.0

PVDF – Polyvinylidene Difluoride – filter membrane

PMSF – Phenylmethanesulfonyl Fluoride

RNA – A linear, usually single-stranded polymer of ribonucleotides. RNA encodes the information needed to synthesize proteins.

SDS-PAGE - Sodium Dodecyl Sulfate – Poly Acrylamide Gel Electrophoresis

SOC – Culture media containing of NaCl, Tryptone, yeast extract, $MgCl_2$, $MgSO_4$, and filter sterilized 2M glucose solution

Protein Chemistry Definitions

Alpha helix – A secondary structure that occurs in many proteins; a right-handed helix with 3.6 amino acid residues per turn stabilized by hydrogen bonds.

Beta sheet – A secondary structure, occurring in many proteins, resulting from the regular folding of polypeptide chains.

C-terminus – The carboxyl end of a polypeptide chain of a protein molecule.

N-terminus – The amino end of a polypeptide chain of a protein molecule.

Ligation – The joining of two DNA strands by the formation of a phosphodiester bond between their terminal nucleotides.

Mis-sense mutation – A mutation in which a codon (3 nucleotides in sequence) undergoes a nucleotide change such that it codes for a different amino acid, often resulting in the production of a nonfunctional protein.

Monomer – A single polypeptide chain, that is able to combine with other like or unlike molecules to form larger polypeptide complexes.

Multimer – A protein molecule composed of more than one polypeptide chain.

Plasmid – A small, closed entity of double-stranded DNA forming an extrachromosomal self-replicating genetic element in many bacteria and some eukaryotes, often carrying genetic sequences that give the host cell a survival advantage such as resistance to antibiotics.

Point mutation – A mutation consisting of an alteration in a single nucleotide of a nucleic acid strand.

Primer – A short single-stranded DNA fragment that is required to initiate polymerization of new DNA nucleotides.

Promoter – A nucleotide sequence on a DNA strand that will initiate transcription.

Protease – Any enzyme that catalyzes the hydrolysis of a protein into smaller substance parts.

Secondary Structure – A protein structure; folding, twisting, coiled, often springlike chain resulting when hydrogen bonds form between the adjacent parts of a molecule, as in an alpha helix or beta sheet.

Vector (Cloning) – A plasmid used in recombinant DNA experiments as an acceptor of foreign DNA.

Vector (Expression) – A cloning vehicle designed to allow and promote the expression of an inserted gene.

Techniques

Anion Exchange Chromatography – A process in which anions in solution exchange with anions in an insoluble matrix or resin.

Chromatography – A technique for separating components from a mixture by placing the mixture in a mobile phase that is passed over a stationary phase.

ESR – Electron Spin Resonance

Gel Filtration Chromatography – A separation technique based on molecular size in which the mobile phase is a liquid and the stationary phase consists of three-dimensional networks of cross-linked polymer chains, such as beads of porous polymeric material

NMR – Nuclear Magnetic Resonance

PCR – Polymerase Chain Reaction – A process for amplifying a DNA molecule by up to 10^6 to 10^9 fold; extremely important in biotechnology and in research.

DNA Annealing – the renaturation of complementary single-stranded DNA molecules into double-stranded DNA following their earlier denaturation; involves the formation of hydrogen bonds between pairs of bases.