Production and Characterization of Human AlphaA, AlphaB, and GammaC Lens Crystallin

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The leading cause of blindness worldwide is cataract disease, which is the end result of increased scattering of light within the ocular lens. This increased scattering is due to a change in the interactions between different lens crystallins, which is reflected in the liquid-liquid phase diagrams of their concentrated mixtures. Phase diagrams of human lens crystallins have not been studied as extensively as those of the calf lens. We have produced human lens crystallins for this purpose. *E.coli* BLR (DE3) was transformed with plasmids containing the genes for human alphaA, alphaB, and gammaC crystallin. The cells were grown to log phase, then induced to produce crystallin with isopropyl β-D-thiogalactopyranoside (IPTG). Cells were then centrifuged, lysed, and the supernatants were subjected to size exclusion chromatography to separate the crystallin from cellular protein. The crystallins were then run on an SDS PAGE gel to assay their purity. Titrations were also performed on the human crystallins in parallel with calf crystallin to help determine the surface charge on the proteins. Once the individual crystallins have been fully characterized, concentrated mixtures similar to those within the human lens can be produced for studies of the phase diagram and its molecular basis via light, neutron, and X-ray scattering experiments.