Small-angle X-ray Scattering from Mixtures of Eye Lens Crystallins

G. M. Thurston,[1] L. B. Lurio,[2] V. Lobaskin,[3] P. Schurtenberger,[3] S. G. J. Mochrie[4]
[1]Center for Ophthalmic Research, Brigham and Women's Hospital and
Harvard Medical School, Boston, MA, U.S.A.
[2]Department of Physics, Northern Illinois University, DeKalb, IL, U.S.A.
[3]Department of Physics, Universite Fribourg, Fribourg, Switzerland
[4]Department of Physics, Yale University, New Haven, CT, U.S.A.

Introduction

Cataracts, the leading cause of blindness worldwide, are the end result of gradually increasing light scatter from the human ocular lens [1]. Much of the light scattering occurs within the lens cells. These cells contain proteins called crystallins at concentrations up to 500 mg/mL. High-concentration solutions of the crystallins can have relatively high and uniform refractive indices. At younger ages, they are normally clear enough for vision. However, both the aggregation of crystallins and liquid-liquid phase separation within the cytoplasm can result in a more nonuniform refractive index, which can increase light scatter in cataracts [1]. We seek to understand the driving forces for aggregation and phase separation in solutions of lens crystallins.

Toward this end, we are studying high-concentration model mixtures of two key lens proteins, bovine α-crystallin and γβ-crystallin. Both α and γβ are well-studied proteins that represent two of the three principal classes of structural proteins of the mammalian lens, the α-, β-, and γ-crystallins. To understand the properties of α/γβ mixtures, we are using small-angle x-ray scattering (SAXS), light scattering, phase-boundary determinations, thermodynamic analysis, and Monte Carlo simulation. α-crystallin is a multisubunit protein of ~800,000 g/mol that exhibits repulsive interactions in solution. γβ-crystallin is a globular protein of 21,000 g/mol that exhibits attractive interactions.

Solutions of γβ alone show liquid-liquid phase separation below temperatures near ~0°C, while solutions of α alone show no phase separation under the present buffer conditions. In contrast, we find that α/γβ mixtures can show reversible phase separation well above body temperature. This dramatic rise in phase separation temperature is likely driven by the size disparity between α and γβ, which results in enhanced local fluctuations in protein species composition.

Methods and Materials

Calf α and γβ crystallins were isolated by chromatography and concentrated by ultrafiltration in 0.1 M phosphate buffer containing 20 mM dithiothreitol to retard oxidation. X-ray scattering cross sections Σ(q) vs. wave vector q were measured for $0.01 < q < 0.7$ Å^{-1}, by using 8-keV photons, for concentrations ranging from 2 to 400 mg/mL, for compositions from all α to all γβ, and as functions of temperature.

Results

As reported previously, Σ(q) for α agrees with previous findings of Tardieu and Delaye [2] and coworkers, and under the present buffer conditions, it is approximately consistent with largely temperature-independent packing of spherical particles. Σ(q) for γβ is consistent with previous findings too [3], and the measured form factor for γβ is also consistent with the published crystal structure in the range $0.01 < q < 0.3$ Å^{-1}. By using SAXS, we have measured the mixed second virial coefficient of α and γβ and have found that it is close to the value expected from hard-core α/γβ repulsions.

For high-concentration α/γβ mixtures, we are finding that Monte Carlo simulations that use hard spheres to represent α and that use smaller hard spheres with an attractive Yukawa potential to represent γβ and hard-core interaction between α and γβ generate partial structure factors that, in combination with the appropriate measured form factors, approximate the measured Σ(q). However, the simulation predictions for Σ(q) also exhibit somewhat more γβ-γβ structure than is measured. This could result from the fact that the shape of γβ is not spherical but closer to that of a dumbbell (i.e., two mutually tangent, equal-radius spheres). Monte Carlo simulations that include more-realistic nonspherically symmetric interactions of γβ-crystallin are under construction.

Analytic structure factor models are also under investigation. The Baxter sticky-sphere model is capable of representing the γβ-crystallin Σ(q) close to the experimental uncertainty, but with use of a sphere radius 10% smaller than a sphere equal in volume to γβ. This discrepancy may also result in part from the nonspherical γβ shape. Σ(q) measured for high-concentration α/γβ mixtures can be well-represented, in an ad-hoc fashion, by combining the Baxter sticky-sphere Percus-Yevick model for S(γβ-γβ) with the Percus-Yevick hard-sphere mixture results for the other partial structure factors. Accordingly, a more consistent analytic structure factor model, using

the Baxter sticky-sphere mixture predictions for each of the partial structure factors, will be compared with the data.

We have also made a thermodynamic analysis of the phase boundaries, tie lines, and q ~ 0 light-scattering intensity for solutions whose γβ-crystallin concentrations are near that of the γβ-crystallin/buffer liquid-liquid critical point but that also have added, dilute concentrations of α-crystallin. This analysis, together with an entropy model for sphere mixtures, suggests that clustering of γβ-crystallin molecules, associated with an increasing correlation range near the critical point, is crucial for a quantitative understanding of the measured phase boundaries. Accordingly, we have commenced SAXS measurements of the correlation range vs. temperature for dilute α/concentrated γβ mixtures.

Discussion

We are finding that the principal features of the phase boundaries, light scattering, and x-ray scattering of α/γβ mixtures can be understood in terms of (a) size disparity between α and γβ, leading to separation into phases that differ in protein species composition; (b) attractive interactions between γβ-crystallins, leading both to γβ clustering and to separation into phases that differ in overall protein concentration; and (c) repulsive, hard-core interactions between α-crystallins.

A quantitative understanding of the molecular origins of the properties of realistically concentrated α/γβ mixtures can serve as one basis for studying mixtures that are successively more representative of the eye lens cytoplasm. Such mixtures include β-crystallins, cytoskeletal and membrane elements, and altered proteins associated with aging and cataracts.

Acknowledgments

This work was supported by the National Institutes of Health (EY11840). Use of the APS was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Beamline 8-ID at the APS is supported by DOE (DE-FG02-96ER45593) and the National Science Foundation (DMR 9312543).

References

[1] G. B. Benedek, J. Pande, G. M. Thurston, and J. I. Clark, Prog. Retin. Eye Res. **18**, 391 (1999).

[2] A. Tardieu and M. Delaye, Ann. Rev. Biophys. Bio. **17**, 47 (1988)

[3] P. Petitt, M. E. Edwards, and D. Forciniti, Eur. J. Biochem. **243**, 415-421 (1997).