Small-angle X-ray Scattering Characterization of Lens Crystallin Proteins

A.Y. Mirarefi,[1] A.L. DeVries,[2] A.J. Kiss,[2] S. Boutet,[3] S. Ramakishnan,[4] C.-H.C. Cheng,[2]
I.K. Robinson,[3] C.F. Zukoski[1,4]
[1]Center for Biophysics and Computational Biology, [2]Department of Animal Biology,
[3]Department of Physics, and [4]Department of Chemical and Biomolecular Engineering,
University of Illinois at Urbana-Champaign (UIUC), Urbana, IL, U.S.A.

Introduction

Cataract formation refers to the opacification of the eye lens and is a serious medical condition. More than 50% of humans over the age of 65 develop cataracts, which can result in total blindness. The mechanisms for cataract formation are poorly understood [1]. Changes in the aggregation state of the proteins in the eye lens are responsible for cataract formation. One form of cataract formation occurs when the eye proteins reversibly aggregate upon cooling and the lens undergoes a reversible opacification [2]. This effect has been studied in depth in mammals. While mammalian eyes exhibit cold cataracts beginning at temperatures below 19°C, the lenses of the Antarctic fish, which lives at –2°C, are completely clear [3]. In this study, we characterize differences in eye lens proteins to understand the variations in protein aggregation.

Fish lenses are dense in protein and thus generate a refractive index large enough to focus light [4]. The mechanism for focusing lies in changing the eye shape. In bovine lenses, however, protein densities are not as high, and focusing results from changes in the lens shape. The dense protein state of the fish lens results in large osmotic pressures, unless the proteins are attractive. The evolutionary drive to focus light in an aqueous environment has resulted in a balance of protein concentration and strength of attraction, where the osmotic pressure of the cell matches the external osmotic pressure, and lens transparency is achieved.

The bovine lens has been treated as a system for modelling the physical and biochemical properties of crystallin proteins, which are categorized by size as: α, β, and γ proteins. These proteins interact in complex manners such that the α and β proteins exist as aggregates and the γ proteins are monomeric. The γ proteins have been implicated in cold cataract formation. Even though there is substantially more γ protein in the fish lens, there is no evidence of cold cataract formation.

Two issues of particular interest in a comparative study are the protein interactions that result in γ protein aggregation and how this state of aggregation is influenced by temperature. Here we explore changes that occur in an intact fish lens microstructure as the temperature is varied, and we compare observations on the lenses of fish that live in different climates.

Methods and Materials

I(q) was measured for the whole intact lens of the adult *Dissostichus mawsoni* and *Trematomus bernacchii* and of the temperate big-eyed tuna *Thunnus obesus*. The whole lenses were mounted in a custom-made holder, which was then glued on a peltier cooler by using thermal paste. This setup allowed the temperature to be varied between –20°C and 40°C. Once the samples were mounted on the cold stage, they were placed in the monochromatic x-ray beam at UNI-CAT beamline station 34-ID-C at the APS, and the small-angle x-ray scattering (SAXS) patterns were measured by using a charge-coupled device (CCD) detector located 1090 mm from the sample. The collected images were integrated by using the FIT2D program [5].

Results

Figure 1 shows the integrated SAXS intensity measured on slices of the cortex of the Antartic toothfish

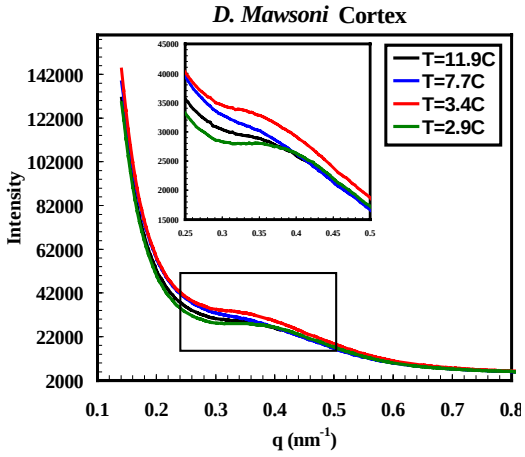

FIG. 1. SAXS intensity measurements of D. mawsoni slices of the cortex. The position of the peak in intensity is seen to vary with temperature, indicating changes in packing.

[*D. mawsoni*] at different temperatures. All scattering curves show a large upturn at low q and a clear peak or shoulder at intermediate q in the intensity for all temperatures. The upturn at low q is associated with long-range structures, while the position of the peak can be related to a distance between neighboring alphas, which are assumed to be the predominant scatterers in the cortex. The position of this peak as well as its height can vary with temperature, suggesting changes in attractions or a temperature-dependent shrinkage of closely packed structures. The alpha crystallin in the lens is a complex of monomers in a variety of sizes. The structure of the complex is debatable, but it appears to be amorphous [6].

Discussion

The difference in the curves shown in Fig. 2 is believed to come from different states of aggregation of the crystallins. SAXS measurements of dilute separated fractions (form factor) of the α, β, and γ proteins did not show any peaks. The origin of the peaks observed is then the contribution of the structure factor to the total intensity. We can extract the neighbor distance between complexes for each of the species measured. For the *T. obesus*, we find the characteristic neighbor distance is 12 ± 2 nm and 13 ± 2 nm for both Antarctic fishes. These values are smaller than the maximum diameter of 18 ± 2 nm of the largest scattering elements for the warm-blooded cow lenses [7]. The Antarctic fishes are unique in that they are highly dense and no cataract is observed at −12°C (manuscript in preparation).

The microstructure of the lens is created from the attractions between the particles. These attractions move particles closer together than they would be on average, thus creating voids or defects in dense gels. The upturn at low q is associated with the clusters of densely packed α proteins.

The alpha aggregate size in the dissected lenses of the Antarctic and temperate fishes measured with SAXS is smaller than the size that is measured with dynamic light scattering. The microstructure does not appear to change much at these volume fractions at this length scale. The temperature effects are apparently weakly dependent on the species. This is interesting, given the climate that these fish live in.

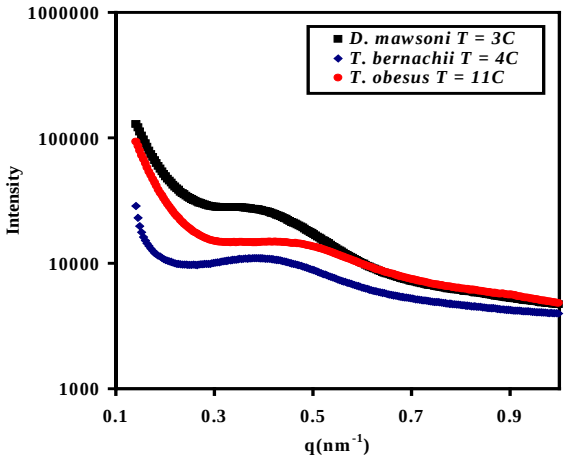

FIG. 2. The integrated SAXS intensity measured on slices of cortex for three different species of fish. The temperatures at which the data were taken were close to the physiological temperatures at which the individual fish live.

Acknowledgments

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. UNI-CAT is supported by the UIUC Materials Research Laboratory (DOE; State of Illinois Board of High Education, Higher Education Cooperation Act; and National Science Foundation;), Oak Ridge National Laboratory (DOE under contract with UT-Battelle, LLC); National Institute of Standards and Technology (U.S. Department of Commerce); and UOP LLC.

References

[1] J.I. Clark, *Principle and Practice of Opthamology* (Saunders College Publishing, Philadelphia, PA, 1994).
[2] J.A. Thomson, P. Schurtenberger, G.M. Thurston, and G.B. Benedek, Proc. Natl. Acad. Sci. U.S.A. **84**, 7079 (1987).
[3] M. Delaye, J.I. Clark, and G.B. Benedek, Biochem. Biophys. Res. Commun. **100**(2), 908-914 (1981).
[4] J. Horwitz, Semin. Cell. Dev. Biol. **11**(1), 53-60 (2000).
[5] A. Hammersley, *Computer Code FIT2D, Version 10.27, Reference Manual* (European Synchrotron Radiation Facility, Grenoble, France, 1998).
[6] J. Vanhoudt, S. Abgar, T. Aerts and J. Clauwaert, Biochemistry **39**, 4483-4492 (2000).
[7] M. Delaye and A. Tardieu, Nature **302**, 415 (1983).