Small Angle X-ray Scattering from Concentrated Ternary Mixtures of Eye Lens Crystallins

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Introduction

Cataract disease, the leading cause of blindness worldwide, is the end result of increased scattering of light within the human ocular lens[1]. The scattered light emerges from very concentrated (300 to 500 mg/ml) aqueous mixtures of eye lens proteins, primarily those called the crystallins, found within the cytoplasm of the lens fiber cells. To help in developing a quantitative understanding of the light scattering and other properties of these mixtures, we are investigating their short-range liquid structure, using small-angle X-ray scattering.

We have chosen to begin by studying a model ternary mixture consisting of buffer, bovine alpha (α) and bovine gamma-B (γ_B)-crystallin. Both of these proteins are well-studied members of the eye lens crystallin family. α crystallin is a large multisubunit protein of molecular weight 800kD. While the crystal structure of α crystallin is not yet available, its light and X-ray scattering properties are consistent with those of a solution of hard spheres, each sphere having a fairly open structure[2]. γ_B crystallin, on the other hand, is a relatively small, globular protein, 21kD in molecular weight. This protein has strong interprotein attractions and exhibits binary liquid-liquid phase separation in aqueous solution[3]. A high-resolution crystal structure of γ_B crystallin is available.

Using light scattering we find that at a high protein concentration, 300mg/ml, approaching those in the ocular lens, aqueous mixtures of α and γ_B-crystallin at body temperature have the interesting property that they scatter less light than would a linear combination of the component protein solutions[4]. At the same time, however, the phase separation temperature exceeds that of a linear combination of the consituent solutions, and is concave down as the relative proportion of α and γ_B is varied. Thermodynamic analysis of these data indicates that mixtures of α and γ_B-crystallins exhibit enhanced thermal fluctuations of relative protein composition at constant overall concentration. Such fluctuations scatter little light but are nevertheless associated with greater thermodynamic instability.

Methods and Materials

Proteins were isolated from calf lenses using size-exclusion and ion-exchange chromatography, concentrated by ultrafiltration, and analyzed in solutions containing 0.1 M sodium phosphate buffer, pH 7.1, with 20mM dithiothreitol. For the X-ray experiments, samples were mounted in an evacuated, temperature-controlled sample chamber. Measurements were carried out at the SAXS station at beamline 8-ID at the Advanced Photon Source (APS). The X-ray scattering intensity, I(q), as a func-

tion of wavevector, q, was measured in the range $0.1 < q < 3nm^{-1}$. I(q) was obtained for (i) the pure proteins in the concentration range $10 < c < 340$ mg/ml (α) and $10 < c < 380$ mg/ml (γ_B), for (ii) relative protein compositions ranging from all γ_B- to all α-crystallin, at fixed total protein concentrations up to 300 mg/ml, and (iii) as a function of temperature from 37 C down to temperatures near T_c, the critical temperature for phase separation.

Results

The measured I(q) for α-crystallin is consistent with the previous findings of A. Tardieu, M. Delaye and coworkers[2]. These solutions show structure factors primarily consistent with packing of spherical particles. In particular, at high protein concentrations the measured I(q) exhibits a pronounced peak at qR near 3.5 for radius R near 8 nm, consistent with the range $7nm < R_H < 9nm$ we find for the hydrodynamic radius R_H of dilute solutions of the present α-crystallin, using quasielastic light scattering.

Unlike α-crystallin, in the accessible temperature range γ_B-crystallin does not exhibit a relative maximum in I(q). It does, however, show a dramatic increase of scattered intensity at low-q as the temperature is lowered towards the critical temperature. These findings are similar to those previously found by Tardieu and coworkers for mixtures of bovine gamma crystallins[5]. In contrast, I(q) for α crystallin depends only weakly upon temperature. These features of the observed I(q) for γ_B are qualitatively consistent with its upper consolute temperature and nonspherical shape.

Scattering from high concentration α-γ_B mixtures shows features intermediate between those of α and γ_B. We are now in the process of analyzing the mixture data and the single-protein data quantitatively. The present investigations will aid the quantitative study of α-α, γ_B-γ_B and α-γ_B interactions at the very high protein concentrations such as those found in the eye lens cytoplasm. Understanding these interprotein interactions is essential for the rational development of inhibitors of cataractogenesis[6].

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