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In vitro interactions of histones and α-crystallin

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ABSTRACT

The aggregation of crystallins in lenses is associated with cataract formation. We previously reported that mutant crystallins are associated with an increased abundance of histones in knock-in and knockout mouse models. However, very little is known about the specific interactions between lens crystallins and histones. Here, we performed *in vitro* analyses to determine whether α-crystallin interacts with histones directly. Isothermal titration calorimetry revealed a strong histone–α-crystallin binding with a K_d of 4×10^{-7} M, and the thermodynamic parameters suggested that the interaction was both entropy and enthalpy driven. Size-exclusion chromatography further showed that histone–α-crystallin complexes are water soluble but become water insoluble as the concentration of histones is increased. Right-angle light scattering measurements of the water-soluble fractions of histone–α-crystallin mixtures showed a decrease in the oligomeric molecular weight of α-crystallin, indicating that histones alter the oligomerization of α-crystallin. Taken together, these findings reveal for the first time that histones interact with and affect the solubility and aggregation of α-crystallin, indicating that the interaction between α-crystallin and histones in the lens is functionally important.

1. Introduction

α-Crystallin is a major protein of mammalian lenses and is essential for lens transparency. Crystallin aggregation in cataracts in humans is associated with aging, environmental UV stress, and genetic mutations [1–3]. Point mutations in crystallin genes that have been associated with cataracts have been introduced in mouse models to investigate the mechanism of cataractogenesis *in vivo* [4,5].

We previously demonstrated that lens epithelial cells expressing the R116C mutation in αA-crystallin have increased abundances of histones H2B and H4 [6]. Similarly, proteomics analyses on the lenses of *Cryaa*-R49C knock-in mice and young *Cryaa/Cryab* double knockout mice show increases in H2B and H4 as well as H2A [7,8]. Other recent studies also suggest that α-crystallin has a functional relationship with histones, though very little is known about the specific role of histones in the lens [9,10]. In yeast cells, the upregulation of histones was shown to improve cell survival [11]. Although histones can be found in the cell cytoplasm and extracellular space and are involved in inflammation, cancer, and other pathologies [12], their primary function is in the nucleus, where they package DNA into nucleosomes—the basic building blocks of chromatin—and are involved in transcriptional regulation [13–15]. An increase in histones in lenses from the αA-R49C mutant mice may be indicative of an increase in nucleosome density, and a functional increase in the histone/DNA ratio may lead to increased amounts of heterochromatin. The increase in histone transcripts in *Cryaa*-R49C mice suggests that the increase in gene expression of histones may be an early event in cataractogenesis, though the notion that α-crystallin functions as a modulator of the expression of histones has not yet been investigated [16]. Nevertheless, these findings strongly suggest a functional relationship between histones and α-crystallin.

We also discovered that the mutant αA-R49C protein is distributed mainly in the nuclei of transfected lens epithelial cells, where it may bind and sequester histones [17]. Indeed, our proteomic analyses revealed that α-crystallin and histones are colocalized in cataractous lenses [7,8]. As a negatively charged protein, α-crystallin may directly bind to histones, but this possibility has not been tested *in vitro*. It also remains unclear whether α-crystallin exists as a stable complex with histones or serves as a transcriptional inhibitor.

Many chaperones such as Nasp, Npm2 and Asf1 are involved in the sequestration, import, and deposition of histones onto chromatin [18–23]. Histone chaperones are known to bind histones and shield them from non-specific interactions [24]. Additionally, mouse erythroblasts transfer histones and non-histone proteins from the nucleus to the cytoplasm, and a similar process may occur in denucleating lens fiber cells *in vivo* [25]. Investigation of an *in vitro* interaction between histones and α-crystallin may thus provide insight into their functional

Abbreviations: GPC, gel permeation chromatography; HS, high salt; ITC, isothermal titration calorimetry; MALDI-TOF MS, matrix-assisted laser-desorption/ionization-time of flight mass spectrometry; PBS, phosphate-buffered saline; RALS, right-angle light scattering

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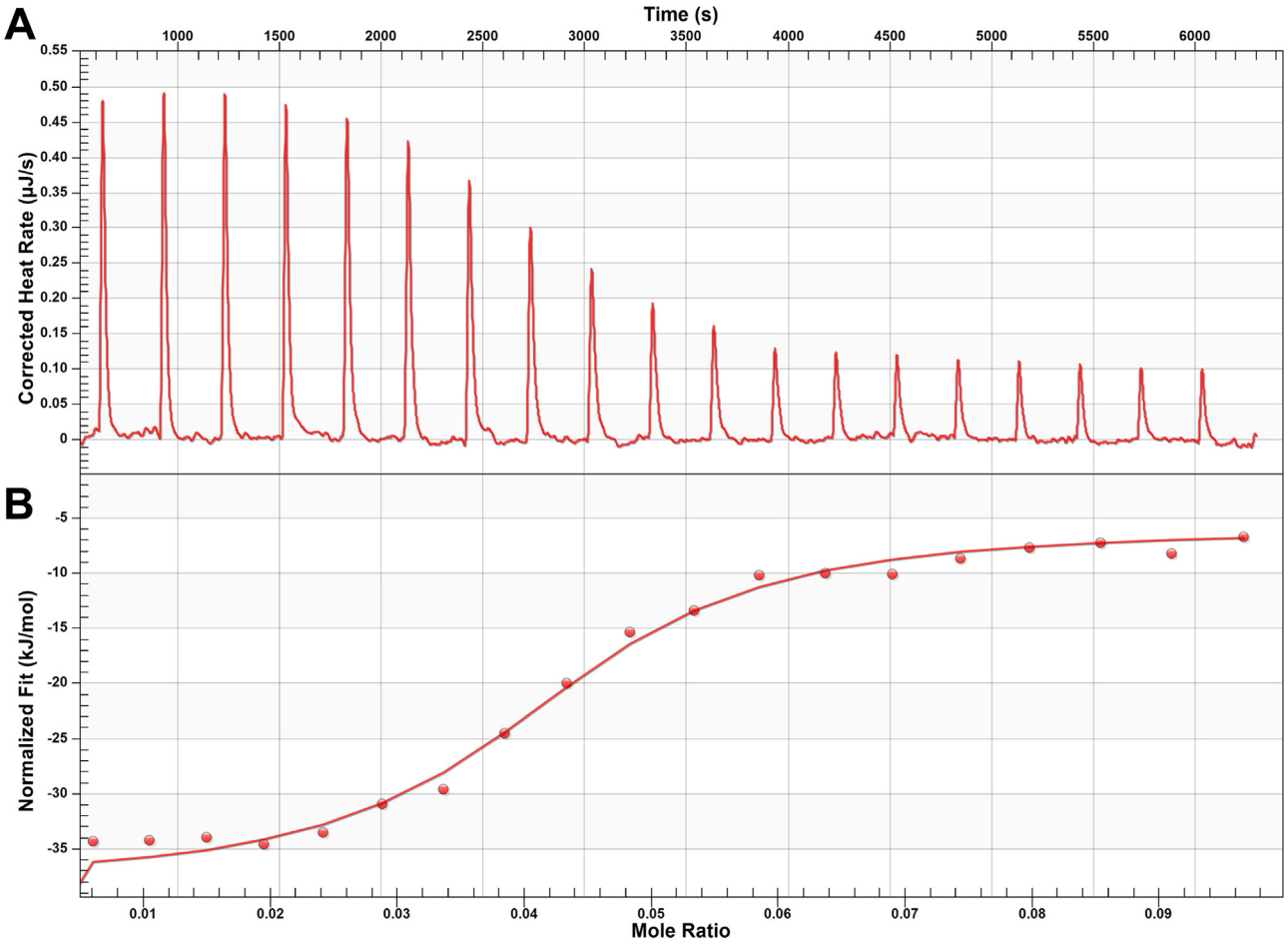

Fig. 1. Isothermal titration calorimetric (ITC) analysis of the interaction between histones and α-crystallin. (A) Changes in current were recorded after sequential injections of 150 µM α-crystallin into a solution of histones (514 µM). (B) Integrated and normalized areas under each peak were plotted against the mole ratio (α-crystallin/histone); the red line shows the fit of the data using the NanoAnalyze program.

Table 1
Thermodynamic parameters of α-, βH, βL-, and γ-crystallin binding to histones.

Sample[a]	K_d	n	ΔH (kJ/mol)	ΔS (J/mol K)	ΔG (kJ/mol)	− TΔS (kJ/mol)	K_a (M^{-1})
α-Crystallin	4.00E−7	0.042	− 26.32	34.20	− 36.52	− 10.20	2.50E6
α-Crystallin HS	2.93E−8	0.040	− 7.60	118.7	− 43.00	− 35.40	3.42E7
βL-Crystallin	8.64E−8	0.036	− 44.80	− 15.05	− 40.32	4.49	1.15E7
βL-Crystallin HS	7.43E−7	0.021	− 37.07	− 6.99	− 34.98	2.08	1.35e6
γ-Crystallin	1.27E−7	0.035	− 55.92	− 55.54	− 39.36	16.56	7.85E6
γ-Crystallin HS	1.02E7	0.010	−65.91	−87.22	−39.90	26.00	9.79E6

[a] Sample concentrations were 150 µM (3 mg/ml) α- or γ-crystallin or 120 µM (3 mg/ml) βL-crystallin with 514 µM (7.2 mg/ml) histones (predissolved for 6 h). HS refers to 0.5 M NaCl-containing phosphate-buffered saline (PBS).

relationship *in vivo*. Therefore, we performed isothermal titration calorimetry (ITC), as well as size-exclusion chromatography and gel electrophoresis, to investigate histone–α-crystallin interactions *in vitro*. The results from this study provide information from which we can design new experiments to better understand the role of histone–α-crystallin interactions in eye tissues and pathologies.

2. Materials and methods

2.1. ITC

Protein–protein interactions between histones and α-crystallin were examined on a nano-ITC instrument (TA Instruments). Histones (bovine) were obtained from Sigma-Aldrich (catalog number H9250) and crystallin proteins (α-, βH-, βL- and γ-crystallin) were purified from porcine lenses [26]; protein solutions were prepared in phosphate-buffered saline (PBS). Preliminary experiments indicated that the time for which the histones were dissolved in PBS was relevant; however, allowing the histones to dissolve in solution for 3 h reduced this effect. MALDI-TOF MS analysis of histones obtained from Sigma showed that this preparation contains the core histones H2a, H2b, H3 and H4 histones (Supplementary Fig. S1).

The ITC instrument was validated using a nano-ITC validation kit according to the manufacturer's instructions. The reference cell was washed three times and then filled with 300 µl deionized and degassed water. The calorimeter was equilibrated to a baseline drift of less than 100 nW over 10 min. Next, the sample cell was washed three times and filled with the histone solution. Twenty sequential injections of 2.50 µl α-crystallin were then made at room temperature (25 °C) with a stirring speed of 350 rpm; the processes for titrating βH-, βL-, and γ-crystallins