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Cataract-linked mutation R188H promotes βB2-crystallin aggregation and fibrillization during acid denaturation

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ABSTRACT

Cataract is characterized by the formation of light-scattering protein aggregates in the lens. β/γ-Crystallins are the predominant structural proteins in the cytosol of lens fiber cells, and more than fifty β/γ-crystallin mutations have been linked to autosomal dominant congenital cataract. However, the structural role of these mutations in the formation of the core structures of amorphous aggregates or amyloid-like fibrils has not been elucidated yet. In this research, we studied the effects of the V187M and R188H mutations on the aggregation and fibrillization of βB2-crystallin during acid denaturation. The behavior of V187M was the same as the WT protein, suggesting that the residue at position 187 contributed little to the aggregation/fibrillization process. R188H promoted the formation of amorphous aggregates at pH above 3 and accelerated fibrillization at pH 3. The distinct behaviors of the mutants suggested that the residue at position 188 might play a regulatory role in βB2-crystallin aggregation/fibrillization but not reside in the core of the aggregates/fibrils.

1. Introduction

Protein misfolding and aggregation are prevalent phenomena observed in numerous diseases, which are generally called protein folding diseases or conformational diseases [1]. The occurrence of aggregation under certain conditions can be regarded as an intrinsic property of proteins due to the marginable stability of proteins [2]. In the cells, incompletely folded proteins, misfolded proteins and aggregates are abolished by the protein quality-control system [3]. Once the aggregated proteins escape the cellular quality-control mechanism, cellular functions may be impaired by the lack of functional proteins, the toxic effects of the aggregates or the accumulation of large aggregates. Cataract, which is characterized by the opacification of the lens, is the most obvious and well-known disease caused by the appearance of large protein aggregates [4].

Lens is a highly differentiated organ with the function of transmitting and focusing light on the retina, and has been used as an excellent model to investigate fundamental biological processes for several decades [5]. In mature vertebrate lens fiber cells, the organelles are fully degraded to reduce the scattering of visible light [6]. Due to the lack of turnover machineries, the proteins in the lens are required to maintain a lifelong stability against various intracellular and environmental stresses. Consistently, crystallins, the most abundant soluble proteins in vertebrate lens, are unique in their high stability and extreme solubility up to ~300 mg/ml [7,8]. There are three classes of crystallins in vertebrate lens: α-, β- and γ-crystallins. α-Crystallins are small heat shock proteins with chaperone-like function to inhibit protein aggregation [9]. β/γ-Crystallins are structural proteins with similar tertiary structures composing four Greek-key motifs divided into two domains [7]. The major difference between β- and γ-crystallins is their oligomeric states: β-crystallins exist as homo- or hetero-oligomers, while γ-crystallins are exclusively monomers. The importance of crystallins in lens structure and function has been revealed by the extremely high concentration of crystallins in lens fiber cells and a number of cataract-linked mutations [7,10,11].

Although cataract can be induced by numerous factors, the precipitation of β/γ-crystallins is a general feature for both congenital and aging cataract. Thus, understanding crystallin aggregation is one of the key points to elucidate the molecular mechanism of cataract and to develop non-surgical methods to prevent or delay cataract [12]. In cataract lens, crystallins may deposit in amorphous

Abbreviations: ANS, 1-anilinonaphtalene-8-sulfonate; BSA, bovine serum albumin; CD, circular dichroism; DTT, dithiothreitol; EM, electron microscope; E_{max}, maximum emission wavelength of intrinsic Trp fluorescence; IPTG, isopropyl-1-thio-β-D-galactopyranoside; SEC, size-exclusion chromatography; SDS, sodium dodecyl sulfate; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; ThT, thioflavin T; WT, wild type.

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aggregates or amyloid-like fibrils. Despite of the morphological difference, the formation of both forms of aggregates is generally initiated by partially or fully unfolding of crystallins, which reorganize to pack via intermolecular β-sheet structures and grow into large aggregates thereafter [12,13]. The extensive studies in the monomeric protein γD-crystallin have led to several aggregation models depending on the conditions at which the aggregates are formed. It seems that the C-terminal domain of γD-crystallin is responsible to the formation of the core of amyloid-like fibrils, while both N- and C-terminal domain contribute to thermal aggregation [14–19]. However up to now, little is known about the details regarding the aggregation mechanism of oligomeric β-crystallins. Moreover, it is also unclear whether the cataract-linked mutations just destabilize crystallins or could play a role in the progression of aggregation. In this research, we addressed this problem by using two cataract-linked mutations (V187M and R188H) located at the last β-strand of Greek-key motif 4 in βB2-crystallin [20–22]. Acid-induced denaturation and aggregation was selected as a model system to distinguish the effects of mutations on protein stability and aggregation. Our results suggested that R188H but not V187M significantly promoted βB2-crystallin aggregation and fibrillization during acid-induced denaturation. Based on the results herein and the previous results [17,22], we proposed that His188 in the R188H mutant may promote aggregation by stabilizing intermolecular interactions among regions flanking the core of the aggregates.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), dithiothreitol (DTT), EDTA, isopropyl-1-thio-β-D-galactopyranoside (IPTG), sodium dodecyl sulfate (SDS), 1-anilinonaphtalene-8-sulfonate (ANS) and thioflavin T (ThT) were Sigma products. All the other chemicals were local products of analytical grade.

2.2. Protein expression and purification

The expression and purification of the wild type (WT) and mutated human βB2-crystallins were the same as those described previously [22,23]. In brief, recombinant proteins were overexpressed in *Escherichia coli* Rosetta (DE3) cells. After induction by 0.1 mM IPTG, the cells were grown in the Luria–Bertani medium for 4 h at 37 °C for the WT protein and V187M or 15 h at 20 °C for R188H. The recombinant proteins were purified from the soluble fractions of cell lysis by a Ni–NTA affinity column, followed by a Hiload 16/60 Superdex 200 prep-grade column. The purity of the final products was checked by SDS–PAGE and size-exclusion chromatography (SEC) using a Superdex 200HR 10/30 column equipped on an ÄKTA purifier. The protein concentration was determined by the Bradford method using BSA as a standard [24]. The purified proteins were dissolved in buffer A containing 20 mM sodium phosphate, 150 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT. The pH of the protein solutions was adjusted by adding HCl or NaOH.

2.3. Spectroscopy

Details regarding the spectroscopic measurements were the same as those described previously [22,25]. In brief, the protein concentration was 0.2 mg/ml for all spectroscopic experiments. The high-concentration protein solutions used for fibrillization were diluted in buffer A before spectroscopic measurements. The far-UV circular dichroism (CD) spectra were measured on a Jasco J-715 spectropolarimeter. The fluorescence spectra were recorded on a Hitachi F-2500 fluorescence spectrophotometer. The excitation wavelengths of the intrinsic Trp fluorescence, ANS fluorescence and ThT fluorescence were 295 nm, 380 nm and 440 nm. Parameter *A*, a sensitive parameter to reflect the position and shape of the Trp fluorescence spectra [26], was calculated by dividing the fluorescence intensity at 320 nm by that at 365 nm (I_{320}/I_{365}). The resonance Rayleigh light scattering was measured with an excitation wavelength at 295 nm [27]. The turbidity of the protein solutions was monitored by the absorbance at 400 nm (A_{400}) using an Ultraspec 4300 pro UV/Visible spectrophotometer.

2.4. Size-exclusion chromatography

Size-exclusion chromatography (SEC) analysis was conducted using a Superdex 200HR 10/30 column equipped on an ÄKTA FPLC as described previously [22,23]. In brief, the column was pre-equilibrated with buffer A at a given pH, and then 100 μl protein solutions were injected into the column and run at a flow rate of 0.4 ml/min at 4 °C. The protein concentration used for SEC analysis was 1 mg/ml. The column was calibrated using standard molecular weight markers as described elsewhere [22].

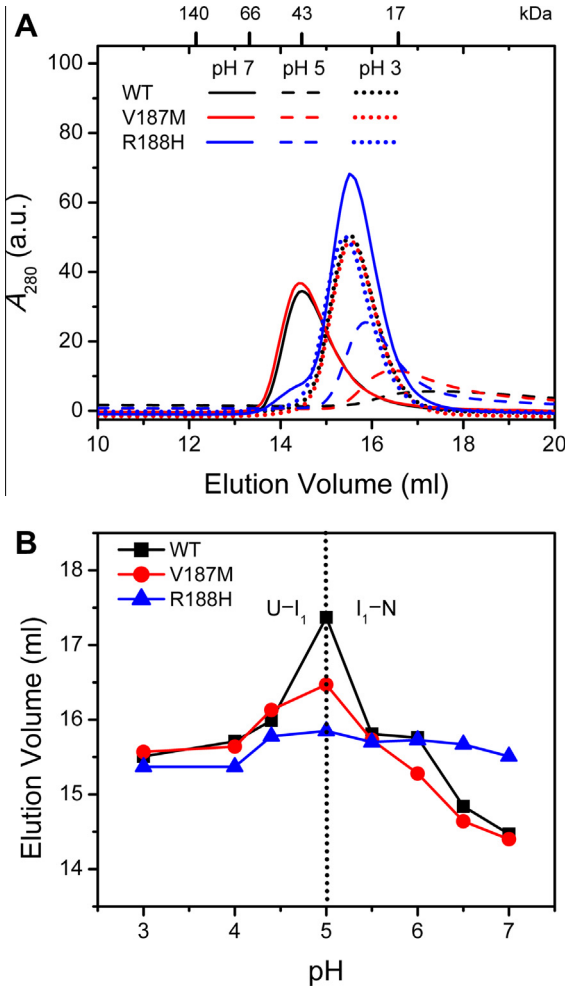

Fig. 1. Changes in the oligomeric states of the WT and mutated βB2-crystallins during acid denaturation. (A) Representative SEC profiles of the proteins. The SEC analysis was performed using a protein concentration of 1 mg/ml and carried out at 4 °C in buffer A. The positions of the standard proteins used for calibration are labeled on the top of the panel. (B) pH-dependence of the elution volumes of the main peaks in the SEC profiles. Both the WT protein and V187M eluted as a single peak under various pH conditions. The elution profiles of R188H contained a main peak from monomers and a minor peak from dimers under neutral conditions, and only the elution volume of the main peak was measured and presented.