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Effects of cataract-causing mutations W59C and W151C on βB2-crystallin structure, stability and folding

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ABSTRACT

β/γ-Crystallins, the predominant structural proteins in vertebrate lens with lifelong stability to maintain lens transparency, share a high similarity in their primary sequences and tertiary structures. Four conserved Trp residues have been shown to be important to γ-crystallin structure, stability and protection against UV irradiation, whereas their roles in β-crystallins remain elusive. Herein we found that two congenital cataract-causing mutations, W59C and W151C, dramatically decreased βB2-crystallin solubility and stability against thermal and guanidine hydrochloride-induced denaturation. The two mutated proteins were prone to form aggregates when irradiated by UV light in the tubes or exogenously expressed in the cells. Although W59 and W151 are structurally identical in β/γ-crystallin domains, substituting them by Cys led to dissimilar influences on βB2-crystallin stability. Our results suggested that the conserved Trp residues might play a more crucial role in the correct folding and structural integrity of β-crystallin domains than in γ-crystallins.

1. Introduction

Cataract, the opacification of vertebrate lens, is the leading cause of human blindness worldwide. Cataractogenesis is a multifactorial process and the influencing factors including but not limited to aging, UV irradiation, metabolic disorders and inherited mutations [1,2]. Cataracts induced by diverse factors generally have the same features at the protein level, that is, the aggregation and deposition of various crystallins, the predominant cytoplasmic proteins in the lens [3–5]. In vertebrates, crystallins can be catalogued into three families: α-, β- and γ-crystallins. Various crystallins have similar molecule weights of the polypeptides (20–30 kDa), but differ significantly in their oligomeric sizes [4]. α-Crystallin, which forms large oligomers ranging from 10-mers to over 40-mers [6,7], belongs to the small heat shock protein family and acts as a chaperone to protect the other proteins in the lens [8]. β/γ-Crystallins, the major structural proteins in the lens, share a similar fold in their tertiary structures composing of four Greek-key motifs divided into two domains. The major difference between β- and γ-crystallins is the ability to form oligomers. β-Crystallins can form homomers or heteromers ranging from dimer to octamer, while γ-crystallins are exclusively monomeric [4]. The lifelong stability of various crystallins is believed to play a crucial role in the maintenance of lens optical properties and consequently crystallin mutations account for about half of congenital cataracts caused by genetic disorders [9].

Sequence alignment indicates that there are four highly conserved Trp residues, two at each of the two domains, in various β/γ-crystallins. These four Trp residues in γ-crystallins have been shown be important to not only structural stability but also efficient quenching of the fluorescence induced by the absorbed UV light [10–15]. In support of these findings, substitution of any one of these four conserved Trp residues has been associated with congenital cataract formation [16–19]. The W43R mutation in γD-crystallin, which was associated with autosomal dominant congenital cataract in a Chinese family [16], altered the native structure and decreased the stability against heat and UV irradiation of γD-crystallin [16,20,21]. However, no biochemical and biophysical studies are available for the roles of the conserved Trp residues in β-crystallins.

Two mutations in βB2-crystallin, W59C and W151C, has been linked to autosomal dominant congenital cataracts in Indian and Chinese families [17–19]. W151C has been shown to easily form aggregates in human lens epithelial cells [18]. W59C is associated with total cataract in an India family [17], while W151C is linked to central nuclear cataracts in an Indian family [19] and progressive

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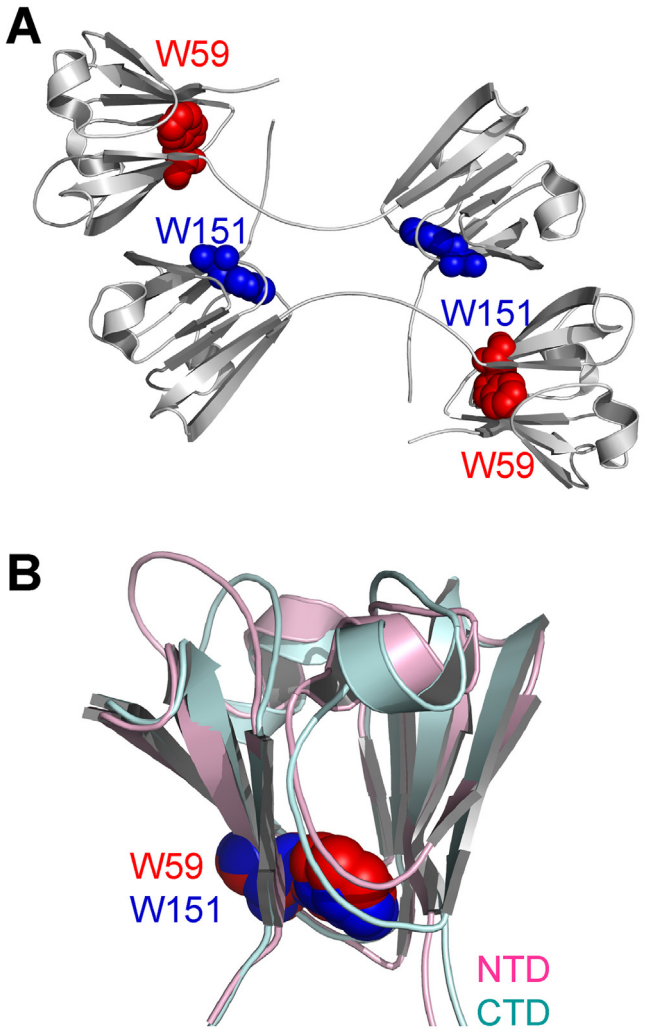

Fig. 1. W59 and W151 are structurally identical residues in β/γ-crystallin domains formed by four Greek-key motifs. (A) Crystal structure of βB2-crystallin (PDB ID: 1YTQ). W59 and W151 are highlighted by the space-filling model. (B) Alignment of the N- and C-terminal domains of βB2-crystallin. The position and side-chain orientation of W59 and W51 are almost superimposed, implying that they are structurally identical. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

membranous cataracts in a Chinese family [18]. Structurally, these two Trp residues are located at the β-strand of Greek-key motif 2 and 4, respectively (Fig. 1A). When the crystal structures of the N-terminal domain (NTD) and C-terminal domain (CTD) are aligned, W59 and W151 are almost superimposed (Fig. 1B), implying that these two Trp residues are structurally identical in the domain structures of βB2-crystallin. However, it is unclear yet whether these two mutations affect βB2-crystallin structure and stability or not. In this research, this problem was addressed by comparing the structural features and biophysical properties of the W59C and W151C mutants of βB2-crystallin. Our results showed that these two mutations remarkably decreased βB2-crystallin solubility and stability, implying that the conserved Trp residues are essential for β-crystallin structure and stability.

2. Materials and methods

2.1. Chemicals

Kanamycin was from AMRESCO. Sodium dodecyl sulfate (SDS), imidazole and 1-anilinonaphtalene-8-sulfonate (ANS) were purchased from Sigma-Aldrich. Dithiothreitol (DTT) and isopropyl-1-thio-β-D-galactopyranoside (IPTG) were obtained from Promega. All other reagents were local products of analytical grade.

2.2. Plasmids and site-directed mutagenesis

The wild type (WT) *CRYBB2* gene was cloned from the human lens total cDNA as described previously [22]. The mutants were constructed by site-directed mutagenesis according to the standard procedures using the following forward (F) and reverse (R) primers: W59C-F: CAGGCTGGACCCTGCGTGGGCTATGAACA; W59C-R: TGTTCATAGCCCACGCAGGGTCCAGCC; W151C-F: CAGAGTGGCACGTGTGTTGGCTACCAGTA; W151C-R: TACTGGTAGCCAACACACGTGCCACTCTG. The plasmids used for overexpression in *E. coli* were prepared by inserting the genes into pET28a (Novagen). The plasmids for eukaryotic cell transfection were constructed using the vectors of pEGFP-C3 and pcDNA3.1-Flag as described previously [23]. All plasmids were verified by sequencing.

2.3. Protein expression, purification and characterization

The WT and mutated proteins were overexpressed in *E. coli* Rosetta (DE3). Details of protein expression and purification were the same as those described elsewhere [22]. In brief, the overexpression of the recombinant proteins was induced by 0.1 mM IPTG. The *E. coli* cells were cultured for 20 h at 16 °C after induction. The His-tagged recombinant proteins were purified by Ni-NTA affinity chromatography followed by size-exclusion chromatography (SEC) using a Hiload 16/60 Superdex 200 prep-grade column equipped on an ÄKTA purifier in buffer A (20 mM sodium phosphate, 0.15 M NaCl, 1 mM DTT, 1 mM EDTA, pH 7.2). The purity of the proteins was above 95% as evaluated by SDS-PAGE and SEC analysis. The SDS-PAGE analysis was performed using a 12.5% acrylamide gel under reducing conditions. SEC analysis was carried out using a Superdex 200HR 10/300GL column at 4 °C. The SEC profiles were collected with a flow rate of 0.4 ml/min. All samples were prepared in buffer A with a final concentration of 0.2 mg/ml.

2.4. Solubility measurements

The maximum solubility of the WT and mutated βB2-crystallins were measured by concentrating the protein solutions in the Vivaspin 500 micro-concentrators centrifuged at 15,000*g* at 20 °C until the concentration of soluble fractions maintained constant. The final values were the average of three independent repetitions.

2.5. Spectroscopic experiments

Details regarding the spectroscopic measurements were the same as those described previously [22]. In brief, the UV absorption spectra were obtained on an Ultraspec 4300 pro UV/Visible spectrophotometer (Amersham Pharmacia Biotech). The far-UV circular dichroism (CD) spectra were measured on a Jasco J-715 spectrophotometer (Jasco Corp.) using a 1 mm pathlength cell, a resolution of 0.2 nm and a wavelength range of 190–250 nm. The fluorescence spectra were recorded on F-2500 fluorescence spectrophotometer (Hitachi Ltd.) using a 1-cm-pathlength cuvette. The intrinsic Trp fluorescence was excited at 295 nm and the extrinsic ANS fluorescence was excited at 380 nm. The samples for ANS fluorescence were prepared by equilibrating the protein solutions with 75-fold ANS molecules for 30 min in the dark. All spectroscopic experiments were performed at 25 °C.