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ARTICLE

# Aberration in the structural paradigm of lens protein α crystallin by UV-C irradiation

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**Abstract** The conformation of lens protein α crystallin was investigated using different spectroscopic techniques under normal and UV-C-irradiated condition. The structural elucidation of commercially available lens protein α crystallin under the effects of UV-C irradiation has never been reported earlier. To study the effects of irradiation on the lens protein, we used UV–visible spectroscopy, CD spectroscopy, and steady-state and time-resolved fluorescence measurements along with FTIR study, under increasing doses of UV-C irradiation. Using the secondary and tertiary structural changes as parameters for detecting conformational perturbation, we investigated the structural paradigm shift in the lens protein α crystallin. Increasing doses of UV-C radiation resulted in decreasing β sheet content of α crystallin from 30 to 10%. The fluorescence profile confirmed the formation of ROS species in the protein upon extensive exposure to UV-C irradiation. These results inferred UV-C irradiation may induce alteration of secondary structure of the lens protein leading to impaired biological functioning.

**Keywords** α Crystallin · FTIR · Fluorescence spectroscopy · Secondary structure · UV-C irradiation

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## Introduction

Sunlight is rich in Ultraviolet (UV) radiation which comprises of three wave bands, with varying degrees of effectiveness and causing biological damage: UV-A (320–420 nm), UV-B (280–320 nm) and UV-C (200–280 nm) [1]. UV-C irradiation is effectively blocked from reaching Earth's surface by the ozone layer, but accidental exposure occurs from manufactured sources such as germicidal lamps. Acute exposure to UV irradiation from 245 to 290 nm is absorbed maximally by DNA and can induce cyclobutane dimers between adjacent thymine or cytosine residues, and pyrimidine photoproducts among adjacent pyrimidine residues. Cyclobutane dimers are supposed to be the major contributor to mutation in mammals [2]. UV light also exerts an impact on proteins and induce damage on cells. Moreover, UV light exposure is considered to be one of the environmental factors involved in lens cataractogenesis during ageing. UV-C irradiation is biologically the maximum damaging range of solar radiation. Several hypotheses accounting for the interaction(s) have put forth like generation of free radicals or reactive oxygen species, or modification of protein structures [3]. The detailed interacting mechanisms, however, remain largely unknown. The conformational and functional consequences of UV-C irradiation have already been demonstrated for a variety of proteins [4], but not for α crystallin.

Crystallins are the structural proteins in the eye lens that constitute more than 90% of the total dry mass of the lens. In general, there are three classes of crystallin: α, β and γ and each of them make up one-third of the total mass, α crystallin being the predominant protein of the vertebrate eye lens. It is found as a highly heterogeneous aggregate

with molecular sizes ranging from 300 kDa to over 1000 kDa with an average molecular size of about 800 kDa [5]. Due to the polydisperse nature, very little is known about its quaternary structures. Being a member of the small heat shock protein family (sHSP), its structure consists of the conserved "α crystallin" domain flanked by less conserved N- and C-terminal regions [6], is composed of two subunits: αA and αB [7]. The two isoforms share 57% sequence homology. Native α crystallin oligomer is made up of less than 15 subunits to more than 50 subunits and the ratio of αA to αB in these multimeric complexes is approximately 3:1 [8]. α crystallin has β-sheet as the predominant secondary structure and 15% α-helix, 40% β-sheet and the remainder, random coil and turns [9]. Although αA crystallin is a lens specific protein, αB crystallin is quite ubiquitous and has been found in many tissues such as heart, brain, spinal cord, kidney, and skeletal muscle. αB crystallin has been found to be over-expressed under various stress conditions and increased levels have been found in various neurological diseases such as Alzheimer's and Creutzfeldt-Jacob. αB crystallin has also been found to be interacting with various cytoskeletal proteins and thus function in the remodelling and reorganisation of filament networks [10].

α crystallin has been known to maintain the necessary refractive index of the lens and in order to achieve this function, its concentration is very high, reaching a value of 450 mg/ml at the centre of the lens [11]. In addition to maintaining the refractive index of the eye lens, α crystallin has a chaperone-like activity [12] contributes the continuous growth of eye lens throughout life and no protein turnover in the terminally differentiated lens fibre cells [13]. In the lifetime of a person, lens proteins are subjected to a variety of injuries, stresses and post translational modifications causing them to unfold and denature [14]. Unfolded or denatured proteins tend to aggregate by exposing their hydrophobic regions and leading to formation of insoluble aggregates, thus resulting in light scattering and opacification of lenses [15]. Owing to the high concentration of α crystallin in the lens, they function by binding to unfolded or denatured proteins and controlling non-specific aggregation [16]. β and γ crystallins as well as some housekeeping enzymes such as glyceraldehydes-3-phosphate dehydrogenase, enolase, and leucine amino peptidase has been found to serve as substrates of α crystallin [16].

Uncontrolled aggregation of proteins and several mutations in α crystallin have been known to cause cataract in human population. Cataract, the major cause of blindness worldwide, is caused by protein aggregation within the protected lens environment. With age, covalent protein damage accumulates through several pathways involved in UV radiation, oxidation, deamidation, and truncations. Cataract causes a part of the lens to become opaque, or cloudy. UV irradiation which causes oxidative stress is a considerable risk factor for cataractogenesis [17].

UV-C radiation-induced alteration of lens protein structure has been observed by estimating the irradiation effect on the secondary and tertiary structures of the protein. Besides using fluorescence spectroscopy, UV absorption spectroscopy, time-resolved fluorescence measurements, Fourier transform infrared (FTIR) and circular dichroism (CD) methods have also been applied. This study is significant from the perspective of the crucial role of lens protein to protect human population from the risk of catrogenesis.

## Materials and methods

### Sample preparation

α Crystallin was purchased from Sigma, USA. α crystallin stock solution (1 μM) was prepared in 0.05 M sodium phosphate buffer. Four samples each of 0.25 μM were prepared at pH 7.0.

### UV-C irradiation

A total of 0.25 μM α crystallin samples were exposed to UV-C irradiation in a laminar air flow cabinet (Neo Equipments), for a time period of 30, 60, and 90 min consecutively. A UV-unexposed sample was treated as control throughout the study.

### UV–visible spectroscopy

Absorbance of samples irradiated in UV light for different time exposure and control were measured in UV–visible spectrophotometer (Varian Cary 50 Bio UV–visible Spectrophotometer) using 1.0-cm quartz cells, in the range of 240–340 nm wavelength.

### CD spectroscopy

CD spectra (Jasco J815, Japan) of UV-irradiated and control samples were recorded. To determine the secondary structure, far UV CD measurements were performed (190–250 nm) at 25 °C using cylindrical quartz cuvette of 2 mm path length.

### Steady-state fluorescence spectroscopy

Fluorescence measurements of both native and UV-irradiated samples were carried out on Varian Cary UV Eclipse fluorescence spectrophotometer using 1.0 cm path length

cuvette at 25 °C. The excitation and emission slit widths were set at 5.0 nm. The sample was excited at 270 nm and the emission spectrum was recorded at 300–400 nm.

### Time-resolved fluorescence spectroscopy (TCSPC)

Fluorescence lifetime measurements (TCSPC) were performed using the HORIBA time-correlated single-photon counting. UV-irradiated α crystallin samples were excited using a Nano LED pulsed laser [18]. Excitation was set at 270 nm and emission at 340 nm wavelength. The instrument response function (IRF) was obtained and calibrated using Ludox (Sigma, USA) suspension.

### FTIR spectroscopy

FTIR spectra were recorded using a Perkin Elmer Spectrum 100 spectrometer equipped with a germanium attenuated total reflection (ATR) plate at 297 K using the ATR method at a resolution of 4 $cm^{-1}$, in aqueous solutions. For each spectrum, five scans were recorded. Fourier self-deconvolution (FSD) was used to estimate the number, position, and width of the component bands across 1600 to 1700 $cm^{-1}$ for Amide I region [19]. Further insights into secondary structural contents were determined from the component bands using second order derivative method. Data analysis was performed using Origin Lab (Northampton, USA) and EFTIR (Madison, USA). We considered the Amide I region (1600–1700 $cm^{-1}$) for its predominance in the protein's secondary structures [20].

## Results

### UV–visible spectroscopy

UV visible spectroscopy is used to detect changes in extinction coefficients due to molecular rearrangements and electronic transitions [21]. Herein, we have investigated the structural perturbations of the lens protein α crystallin due to extensive exposure to UV-C irradiation. Firstly, the increase in the extinction value indicated by absorbance helped us to validate the conformational change in the lens protein, upon exposing UV rays [22]. Secondly, UV–Vis absorption profile indicated the formation of aggregates in the protein, characterized by increasing absorbance maxima at 280 nm [23]. Time-scale exposures of the lens protein to UV-C irradiations were increased gradually (Fig. 1), in the range of 30–90 min duration, increasing absorbance value being indicative of conformational alteration.

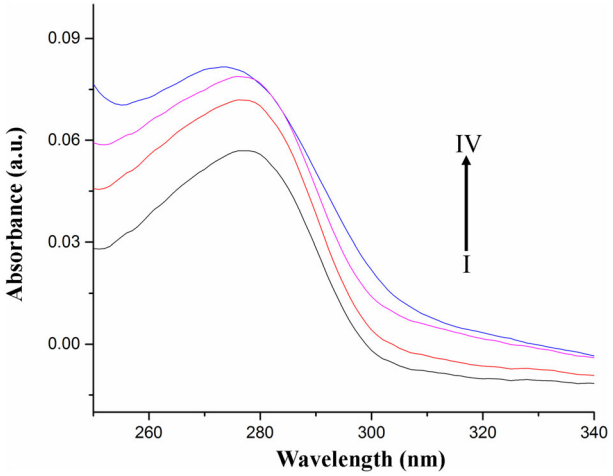


**Fig. 1** UV–visible absorption profile of native and UV-C irradiated α crystallin sample with increasing UV-C exposure from (I) 0 min, (II) 30 min (III) 60 min to (IV) 90 min

### Conformational change by CD spectroscopy

Circular Dichroism studies help us to determine the secondary structure of a protein, on a quantitative and qualitative basis [24]. CD spectroscopy of native and UV-C irradiated α crystallin were performed to study the alteration in secondary structure of the lens protein. UV-C irradiation induced a substantial modification in the β sheet content of the protein (Fig. 2), was observed with a sharp decrease in the peak region at 218 nm compared to native one. β sheet composition changed from 28 to 26% from native state to UV-C irradiated α crystallin. The results have been expressed as MRE (Mean Residual Ellipticity) in deg $cm^2$ $dmol^{-1}$, which is given by $\text{MRE (mdeg)} = \theta_{obs}/(10\, n \cdot c \cdot l)$.

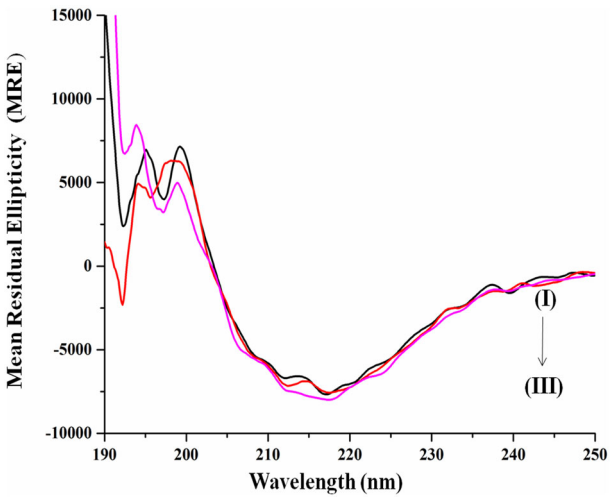


**Fig. 2** CD spectroscopy profile of native and UV-C irradiated α crystallin (0.25 μM) with increasing exposure of UV-C irradiation from (I) 0 min, (II) 30 min and (III) 90 min

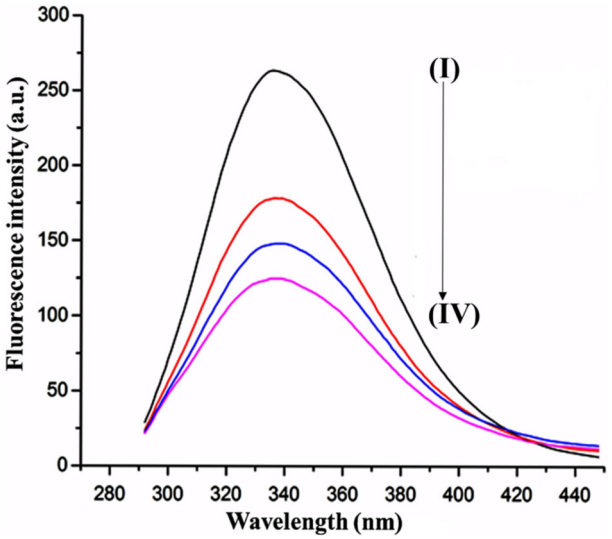


**Fig. 3** Steady-state fluorescence spectrum of native and UV-C irradiated α crystallin (0.25 μM) with increasing exposure of UV-C irradiation from (I) 0 min, (II) 30 min, (III) 60 min and (IV) 90 min. Fluorescence intensity of Tryptophan residues decreased with increasing exposure of UV-C irradiation

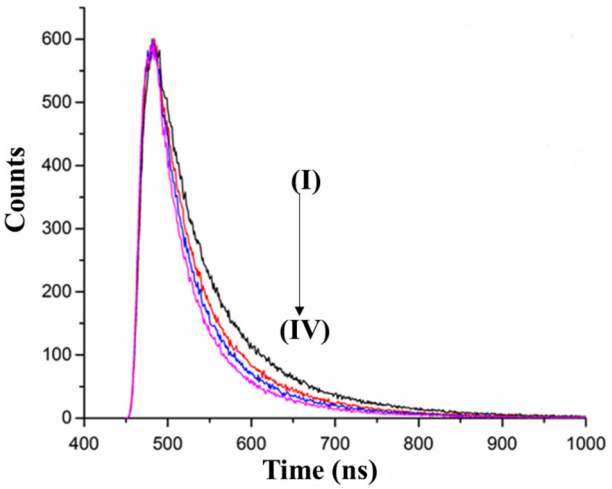


**Fig. 4** Lifetime decay profile of native and UV-C irradiated α crystallin with increasing exposure of UV-C irradiation from (I) 0 min, (II) 30 min, (III) 60 min and (IV) 90 min. A significant decrease in the lifetime of Tryptophan residues from was noted native to 90th minute of UV-C irradiation

Here $\theta_{obs}$ is the observed ellipticity in milli degrees, $c$ is the concentration of protein in Mol $L^{-1}$, $l$ is the length of the light path in centimetres and $n$ is the number of peptide bonds [25].

## Steady-state fluorescence spectroscopy

The structural modification of tertiary system of a protein maybe determined by fluorescence spectroscopic measurements [26]. α crystallin contains 2 tryptophan (Trp) residues in its two subunits, Trp 9 in αA subunit, Trp 9 and Trp 60 in αB subunit [27]. The tryptophan residues absorb UV radiation which imparts damaging effects on the lens protein by photo oxidation of Tryptophan to N-formylkynurenine (NFK), known to act as a photosensitizer [28], which is capable of generating reactive oxygen species (ROS) and free radicals, specifically quenching tryptophan fluorescence [29]. The reactive oxygen species accumulation may cause cross-linking of the protein leading to aggregation. Figure 3 shows gradual decrease in fluorescence intensity of Tryptophan residue with increasing doses of UV-C irradiation. These results were supported by a blue-shift of the emission wavelength of fluorescence spectra by 5 nm. These results evidenced distinct changes in the tertiary structure of the lens protein α crsytallin, due to UV-C irradiation induced photochemical damage [30].

## Time-resolved fluorescence spectroscopy (TCSPC)

Time-resolved fluorescence measurements were carried out to investigate the relaxation time for Tryptophan residue in UV-C irradiated α crystallin (Fig. 4). The lens protein α crystallin possesses 3 tryptophan residues [31], which upon extensive UV-C exposure, had a faster decay compared to native protein samples (Table 1), which decreased from 7.9 to 6.0 ns. These changes, in correlation with the steady-state fluorescence measurements confirmed the change in tertiary structural conformation of the lens protein, upon UV-C irradiation.

## Fourier transform infrared (FTIR) measurements

Proteins are biological macromolecules, which contain certain structural components such as α helix, β sheet. [32].

**Table 1** Secondary structural component analysis of native and UV-C irradiated α crystallin with increasing exposure of UV-C irradiation from (I) 0 min, (II) 30 min to (III) 90 min

| Sample | Secondary structural component analysis by circular dichroism spectroscopy | |
|---|---|---|
| Protein | α-Helix (%) | β-sheet (%) |
| Native | 17.7 | 28.58 |
| 30 min | 18.71 | 26.38 |
| 60 min | 11.53 | 26.83 |
| 90 min | 4.11 | 28.13 |

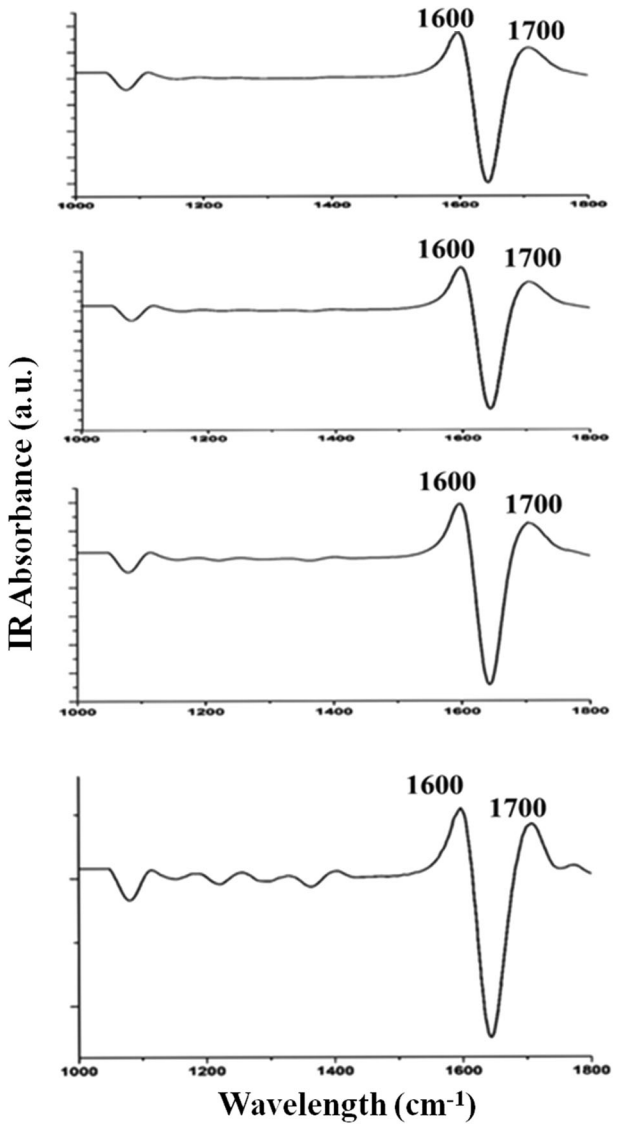


**Fig. 5** FTIR profile of native and irradiated α crystallin with increasing exposure of UV-C irradiation at (I) 0 min, (II) 30 min, (III) 60 min and (IV) 90 min

FTIR measurement is a powerful tool which quantitatively estimated the alteration of the secondary structure of the lens protein α crystallin upon UV-C exposure. Data analysis was performed using Origin Lab (Northampton, USA) and EFTIR (Madison, USA). We considered the Amide I region (1600–1700 $cm^{-1}$) for its predominance in the protein's secondary structures [31].

**Table 3** Alteration in α helix and β sheet content of native and UV-C irradiated α crystallin from 0 min to 90 min, obtained from FTIR spectroscopy in the Amide I region (1600–1700 $cm^{-1}$)

| Sample | Amide I region (1600–1700 $cm^{-1}$) | | |
|---|---|---|---|
| Protein | α-Helix (%) | FWHM | β-sheet (%) |
| Native | 14.51 | 193.52 | 36.75 |
| 30 min | 17.27 | 95.06 | 32.60 |
| 60 min | 15.52 | 90.72 | 31.11 |
| 90 min | 4.71 | 83.8 | 10.10 |

The FTIR curves were deconvoluted and further narrow peaks were obtained by 2nd order derivative method (Fig. 5). The integrated area values have been correspondingly reported in percentages [33]. At low exposure (30 min) to UV-C rays, the lens protein was dominant in β sheet structural content, however, gradually when UV-C exposure time increased to 90 min, the protein almost lost its native state and the β sheet content decreased to 10% from 36% in the native state (Table 2). Full Width Half Maxima (FWHM) is a parameter which is used to detect the broadness and narrowness of the IR spectra peaks. FWHM of pure water is around 294 $cm^{-1}$.

When native α cyrstallin was dissolved in sodium phosphate buffer, the FWHM value markedly narrowed to 83.8, from 193.5 $cm^{-1}$ indicating the peak shifting in amide bonds which further indicates the denaturing and disrupting effects on the amide bond vibrations [33] in presence of increasing UV-C irradiations (Table 3).

## Discussion

This study indicates the structural modification of α crystallin could be altered by the exposure of UV-C irradiation. UV-C-irradiation to native α crystallin resulted in disruption of ordered structure of the protein molecules, which may be implicated as structural perturbations like cross-linking as well as aggregation of the polypeptide chains. The effect on Tryptophan residues in α crystallin by UV-C irradiation may be one of the events that trigger the

**Table 2** Alteration of excited state life time of Tryptophan in native and UV-C irradiated α crystallin with exposure in the range of 0–90 min

| Sample | $\tau_1$ (ns) | $\tau_2$ (ns) | $\tau_3$ (ns) | $\tau_4$ (ns) | $\tau_{average}$ (ns) | $\chi^2$ (Chi-square) |
|---|---|---|---|---|---|---|
| Native | 0.783 | 2.75 | 21.8 | 6.37 | 7.92 | 1.027 |
| 30 min | 0.671 | 2.34 | 18.6 | 6.11 | 6.92 | 1.028 |
| 60 min | 0.678 | 2.32 | 17.9 | 6.22 | 6.77 | 0.996 |
| 90 min | 0.0968 | 0.692 | 2.25 | 21 | 6.01 | 1.119 |

increased oxidation of acid residues in the protein. The structural change due to UV-C irradiation was detected primarily by UV–visible spectroscopy, whereas the changes in secondary structure of α crystallin were confirmed by circular dichroism spectroscopy study indicating marked changes in β-sheet structure with increasing exposure UV-C irradiation primarily at 90 min. The fluorescence spectra analysis revealed that UV-C-irradiation caused changes in the tryptophan microenvironment observed by a change in intensity level of α crystallin. Fluorescence lifetime of tryptophan residue of α crystallin (determined by TCSPC technique) was observed to be significantly decreased from 7.92 to 6.01 ns in presence of UV-C irradiation. The FTIR study further confirmed the change in secondary structure as was evident from the altered content of α-helix and β-sheet structure when exposed to 90 min of UV irradiation exposure compared to the native structure. To summarize, this work divulges the structural alteration in lens protein by the exposure to UV-C irradiation which may have significant impact on its biological function.

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