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Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of α-crystallin chaperone activity☆

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Abstract

α-Crystallin, a molecular chaperone of the eye lens, plays an important role in maintaining the transparency of the lens by preventing the aggregation/inactivation of several proteins and enzymes in addition to its structural role. α-Crystallin is a long-lived protein and is susceptible to several posttranslational modifications during aging, more so in certain clinical conditions such as diabetes. Nonenzymatic glycation of lens proteins and decline in the chaperone-like function of α-crystallin have been reported in diabetic conditions. Therefore, inhibitors of nonenzymatic protein glycation appear to be a potential target to preserve the chaperone activity of α-crystallin and to combat cataract under hyperglycemic conditions. In this study, we investigated the antiglycating potential of cumin *in vitro* and its ability to modulate the chaperone-like activity of α-crystallin vis-à-vis the progression of diabetic cataract *in vivo*. Aqueous extract of cumin was tested for its antiglycating ability against fructose-induced glycation of goat lens total soluble protein (TSP), α-crystallin from goat lens and a nonlenticular protein bovine serum albumin (BSA). The antiglycating potential of cumin was also investigated by feeding streptozotocin (STZ)-induced diabetic rats with diet containing 0.5% cumin powder. The aqueous extract of cumin prevented *in vitro* glycation of TSP, α-crystallin and BSA. Slit lamp examination revealed that supplementation of cumin delayed progression and maturation of STZ-induced cataract in rats. Cumin was effective in preventing glycation of TSP and α-crystallin in diabetic lens. Interestingly, feeding of cumin to diabetic rats not only prevented loss of chaperone activity but also attenuated the structural changes of α-crystallin in lens. These results indicated that cumin has antiglycating properties that may be attributed to the modulation of chaperone activity of α-crystallin, thus delaying cataract in STZ-induced diabetic rats.

Keywords: α-Crystallin; Antiglycation; Chaperone activity; Cumin; Cataract; Diabetes

1. Introduction

Diabetes is a metabolic disorder characterized by high blood glucose levels resulting from either an absolute or relative deficiency in insulin or due to insulin resistance. Cataractogenesis is one of the earliest secondary complications of diabetes, characterized by opacification of the transparent eye lens. Studies suggest that the long-term pathological sequelae of diabetes are a result of the accumulation of tissue macromolecules that have been progressively modified by advanced glycation end products (AGE) [1,2]. Glycation alters protein conformation and stability [3,4], induces protein aggregation and cross-linking, and increases susceptibility to proteolysis [4–7], which contributes to the pathogenesis of conformational diseases such as cataract. Glycation begins with the nonenzymatic reaction of reducing sugar with the free amino group of the protein (predominantly ε-amino group of lysine, guanidine group of arginine) forming an Amadori

Abbreviations: AGE, Advanced glycation end products; ANS, 8-anilino-1-naphthalene-sulfonic acid; BSA, bovine serum albumin; CML, carboxymethyllysine; G6PD, glucose-6-phosphate dehydrogenase; HMW, high molecular weight; IP, insoluble protein; PTM, posttranslational modifications; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; STZ, streptozotocin; TSP, total soluble protein.

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product [8]. The latter then undergoes a series of complex reactions (oxidative and nonoxidative) resulting in the formation of AGE [9], which are chromophoric, fluorophoric and amino acid cross-linking adducts on proteins [10]. These AGE are shown to be elevated in the lenses and serum of diabetic subjects [11–16].

Structural proteins called crystallins largely determine the transparency of the eye lens. α-Crystallin, a member of the small heat shock protein family, is a major structural element in the protein matrix of the vertebrate eye lens. In addition to its structural role, α-crystallin is shown to exhibit chaperone-like function by preventing the aggregation of several proteins denatured by heat or other stress conditions [17–19]. The chaperoning ability of α-crystallin is believed to be essential for the maintenance of transparency of the lens, thus preventing the formation of cataract. Because of their remarkable longevity, crystallins (including α-crystallin) in the lens are susceptible to several posttranslational modifications (PTM) including nonenzymatic glycation during aging and diabetes. Earlier, we and others reported that *in vitro* glycation of α-crystallin disrupted its structural stability [3,5], resulting in decreased chaperone activity [4,20–22], and this was substantiated by *in vivo* studies [23–25]. Furthermore, various PTM whose formation is accelerated during aging and in diabetes were shown to cause intra- or intermolecular cross-linking of α-crystallin, decreasing the chaperone function as well [26]. Small molecules are reported to modulate the chaperone-like activity of α-crystallin under various circumstances that include *in vitro* glycation and diabetes [25,27–29]. Therefore, it is of pharmaceutical interest to prevent nonenzymatic glycation in order to preserve the chaperone activity of α-crystallin in diabetic condition.

Seeds of *Cuminum cyminum* (cumin) are widely used in Indian cuisine and in Ayurvedic medicine for the treatment of dyspepsia, diarrhea and jaundice [30]. Interestingly, it is also reported that cumin possesses an antihyperglycemic effect [31,32]. In the present study, we demonstrate the antiglycating activity of cumin against *in vitro* glycation. Furthermore, cumin feeding delayed streptozotocin (STZ)-induced diabetic cataract in rats by modulating α-crystallin chaperone activity.

2. Materials and methods

2.1. Materials

Acrylamide, ammonium persulfate, bis-acrylamide, bovine serum albumin (BSA), SDS, TEMED, ANS (8-anilinonaphthalene-1-sulphonic acid), citrate synthase, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, glyoxalic acid, fructose, NADP, *m*-aminophenyl boronic acid-agarose, penicillin, streptomycin, sodium azide, sodium cyanoborohydride and STZ were purchased from Sigma (St. Louis, MO, USA). Molecular weight markers were from Bio-Rad; Sephacryl S-300HR was from Amersham Biosciences (Uppsala, Sweden). Immobilon NC membrane and 0.20-μm filters were from Millipore (Bedford, MA, USA). Glycated hemoglobin (HbA_{1c}) kit was from Biosystems (Barcelona, Spain). Antibody against bovine lens α-crystallin was a gift from Dr. J.S. Zigler (NEI, National Institutes of Health, Bethesda, MD, USA). Fresh cumin seeds were purchased from the local market.

2.2. Preparation of cumin aqueous extract

Fresh and freeze-dried cumin seeds were crushed into fine powder and a 5% aqueous solution was prepared. This was lyophilized to get cumin aqueous extract, which was used for *in vitro* antiglycation studies.

2.3. Production of carboxymethyllysine–BSA antibody

Bovine serum albumin was incubated in 0.2 M sodium phosphate buffer (pH 7.8) containing glyoxalic acid (23 mM) and sodium cyanoborohydride (69 mM) for 24 h at 37°C. After incubation, BSA preparation was dialyzed extensively to remove any unbound glycating agent. Polyclonal antibodies were raised against glycated BSA in New Zealand white rabbits using a standard protocol [33]. Antisera collected from hyperimmunized rabbit were purified by ammonium sulphate precipitation followed by anion exchange on a DE52 chromatography column. The partially purified polyclonal IgG was used in this study.

2.4. Isolation of TSP and α-crystallin from goat lens

Eyeballs were collected from 6-month-old male goats at a local slaughterhouse, and lenses were dissected out. A 10% homogenate of these lenses was prepared in a buffer containing 0.025 M Tris, 0.1 M NaCl, 0.5 mM EDTA and 0.01% NaN_3 (pH 8.0; TNEN buffer), and centrifuged at 10,000×*g* for 30 min at 4°C to collect the supernatant as lens total soluble protein (TSP). TSP was applied onto a Sephacryl S-300 HR preparative column of 100×1.5 cm. The column was equilibrated and crystallins were eluted with TNEN buffer. Fractions corresponding to αH and αL-crystallin were collected separately, and the purity of isolated αL-crystallin was assessed by SDS-PAGE. Protein concentration was determined by the modified Lowry method.

2.5. In vitro glycation

TSP, α-crystallin and BSA were used for *in vitro* glycation. These protein solutions, stock solutions of fructose and cumin extract were filtered through 0.20-μm syringe filters. Each 1 ml incubation mixture contains 10 mg protein, 100 mM fructose, 50 μg of penicillin and streptomycin, and 0.01% sodium azide in 0.2 M sodium phosphate buffer (pH 7.4) for 20 days at 37°C in the absence and presence of 0.1 and 1.0 mg/ml of the material from the 5% cumin aqueous extract. After the incubation period, these protein preparations were extensively dialyzed and used for further analysis. α-Crystallin was isolated from glycated TSP preparations as described above.

2.6. Evaluating the antiglycating activity of cumin

The extent of protein glycation in the absence and presence of aqueous cumin extract was evaluated by monitoring protein cross-linking on SDS-PAGE, AGE-related nontryptophan fluorescence (described below) and immunoblotting.

2.7. Animals and treatment

Two-month-old male Wistar-NIN rats with average body weight of 185 g (obtained from the National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India) were used for this study. All the animals were fed with a semisynthetic AIN-93 diet [34] ad libitum. The control rats (Group I; n=8) received 0.1 M citrate buffer (pH 4.5) as a vehicle, whereas the experimental rats received a single intraperitoneal injection of STZ (32 mg/kg) in the same buffer. After 72 h, fasting blood glucose levels were monitored and animals with blood glucose levels of less than 145 mg/dl were excluded from the experiment and the rest were distributed into two groups. Animals in these groups received either AIN-93 diet only (Group II; n=15) or AIN-93 diet containing 0.5% cumin seed powder (Group III; n=10). Fresh cumin seeds were finely powdered and mixed with AIN-93 diet at 0.5% level and fed to the diabetic rats for 8 weeks. Individual animals were housed in grill-bottomed polypropylene cages and maintained in rooms at a temperature of 22±2°C, 50% humidity and 12-h light/dark cycle. All the animals had free access to water. Food intake was measured daily; body weights and blood glucose were monitored weekly. Animal care and protocols were in accordance with and approved by the institutional animal ethics committee and also in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. Eyes were examined every week using a slit lamp biomicroscope (Kowa Portable, Japan) on dilated pupil, and lenticular opacity was graded as described earlier [35].

At the end of 8 weeks, blood was collected by orbital vein puncture and the animals were sacrificed by CO_2 asphyxiation. Lenses were dissected by the posterior approach and stored at −80°C for further analysis. Plasma glucose was measured by the glucose oxidase–peroxidase method [36], and HbA_{1c} was estimated by the ion exchange resin method [37] using a commercially available kit. TSP and α-crystallin were isolated from rat lens as described above. The pellet obtained after separating the TSP was washed three times with TNEN buffer and constituted in the same buffer which was considered as insoluble protein (IP). Three to five lenses from each group were pooled for various analyses.

2.8. Determination of protein glycation

Quantification of glycated protein in rat TSP was performed by boronate-affinity chromatography. A column (8×1 cm) was packed with immobilized aminophenyl boronic acid agarose equilibrated with 5 column volumes of equilibration buffer (0.25 M ammonium acetate, pH 8.5, containing 0.05 M $MgCl_2$). Rat TSP was applied to the phenyl boronate affinity column. The unbound (non-glycated) protein fraction was eluted with equilibration buffer, whereas glycated protein was bound to the phenyl boronate matrix. The bound glycated protein was eluted with 0.1 M Tris-HCl (pH 7.5) containing 0.2 M sorbitol and read at 280 nm.

2.9. Chaperone activity assays

The chaperone activity of αL-crystallin was assessed using aggregation and nonaggregation assays. Aggregation assays were done by measuring the ability of α-crystallin in suppressing the heat-induced aggregation of βL-crystallin (purified from control rat lenses) at 60°C and citrate synthase at 45°C as a function of time at 360 nm using a Unicam UV 300 spectrophotometer. Aggregation assays with βL-crystallin and citrate synthase were performed as described previously [38]. To represent nonaggregation assay, the residual activity of glucose-6-phosphate dehydrogenase (G6PD) was monitored on heat inactivating G6PD at 42°C in the presence and absence of α-crystallin essentially as described earlier [39].

2.10. Fluorescence measurements

Fluorescence measurements of α-crystallin from eye lens of different groups were performed using a spectroflorometer (FP-6500; JASCO). For all the measurements, 0.15 mg/ml protein in a 20-mM sodium phosphate buffer (pH 7.4) was used. Intrinsic tryptophan fluorescence was recorded by exciting at 280 nm and following the emission between 300 and 400 nm. Nontryptophan AGE fluorescence was recorded by exciting at 370 nm and following the emission from 390 to 480 nm. Fluorescence of 8-anilino-1-naphthalene-sulfonic acid (ANS) bound to α-crystallin was measured by excitation at 390 nm and following the emission between 450 and 550 nm. For this assay, α-crystallin was incubated with 50 μM ANS for 30 min at room temperature and the fluorescence of protein bound dye was measured. The spectra were corrected with appropriate protein and buffer blanks.

2.11. Circular dichroism studies

Far- and near-UV CD spectra were recorded at room temperature using a spectropolarimeter (J-810, JASCO). All spectra were an average of six accumulations and recorded using cells of 0.1 and 0.5 cm path length, respectively, for far- and near-UV CD. Protein concentration used for far- and near-UV CD spectra was 0.15 and 1.5 mg/ml, respectively, in 20 mM sodium phosphate buffer (pH 7.4).

2.12. Other assays

Protein carbonyls were estimated according to the method described earlier [40]. Subunit profile and cross-linking of soluble proteins were analyzed on 12% SDS-PAGE. Presence of α-crystallin in rat IP was detected by immunoblotting.

2.13. Statistical analysis

A proportion test was performed to measure the statistical significance of cataract progression between the groups for a given stage of cataract. One-way analysis of variance was used to test the statistical significance of data between groups for all other parameters. Heterogeneity of variance was tested by the nonparametric Mann–Whitney test. $P<.05$ was considered significant.

3. Results

3.1. In vitro antiglycating effect of cumin

Glycation of TSP with fructose resulted in the loss of native bands on the SDS-PAGE, cross-linking and formation of high molecular weight (HMW) aggregates, some of which could not enter into the resolving gel (Fig. 1A). Interestingly, the presence of aqueous extract of cumin in the *in vitro* glycation mixture prevented the formation of glycation-mediated protein cross-links and reduced HMW aggregates in TSP. Also, there is a recovery of native protein species (Fig. 1A). In the case of BSA, glycation with fructose resulted in the loss of the band corresponding to BSA on SDS-PAGE gel (Fig. 1B), whereas glycation in the presence of the aqueous cumin extract resulted in the preservation of the BSA band in a dose-dependent manner (Fig. 1B). Similarly, increased AGE fluorescence, an indicator of protein glycation, was observed with TSP and α-crystallin

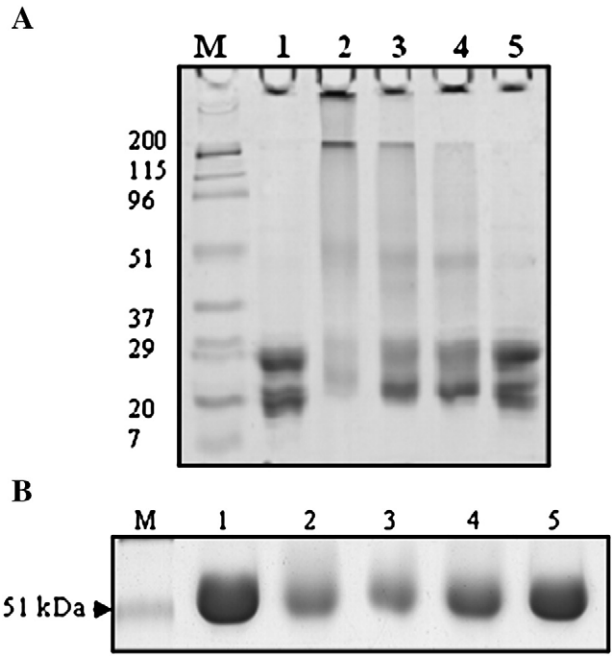

Fig. 1. Effect of cumin on fructose-induced glycation of TSP (A) and BSA (B) in the absence and presence of aqueous extract of cumin by SDS-PAGE analysis. M — molecular weight markers. TSP or BSA (Lane 1), incubated with fructose in the absence (Lane 2) or presence of 0.1 mg/ml (Lane 3) or 1 mg/ml cumin (Lane 4). Lane 5 represents TSP incubated with 1 mg/ml cumin alone.

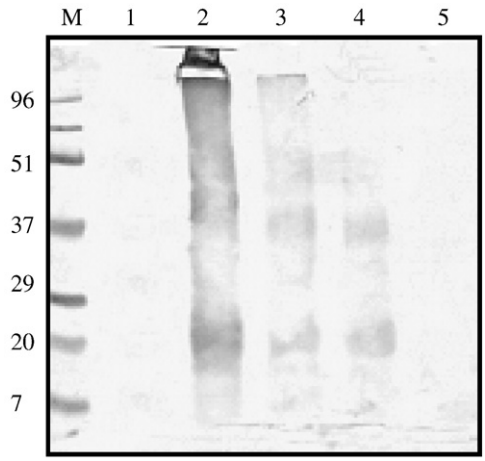

Fig. 2. Immunodetection of CML antigen from *in vitro* glycated TSP with fructose. M — molecular weight markers. Control TSP (Lane 1); TSP incubated with 0.1 M fructose in the absence (Lane 2) or presence of 0.1 mg/ml (Lane 3) or 1 mg/ml cumin (Lane 4). Lane 5 represents TSP incubated with 1 mg/ml cumin.

upon incubation with fructose, while co-incubation with cumin extract resulted in decreased AGE fluorescence in a dose-dependent manner (data not shown). Carboxymethyl-lysine (CML) was the predominant AGE formed in *in vitro* glycation of TSP with fructose, while cumin extract prevented the formation of CML in a dose-dependent manner (Fig. 2). However, we could not find signals that were evident by immunoblotting with other anti-AGE antibodies such as anti-AGE BSA and anti-MGO BSA when TSP was incubated with fructose. α-Crystallin isolated from glycated TSP displayed loss of chaperone activity compared to α-crystallin isolated from the control TSP, and co-incubation with cumin extract had a slight protective

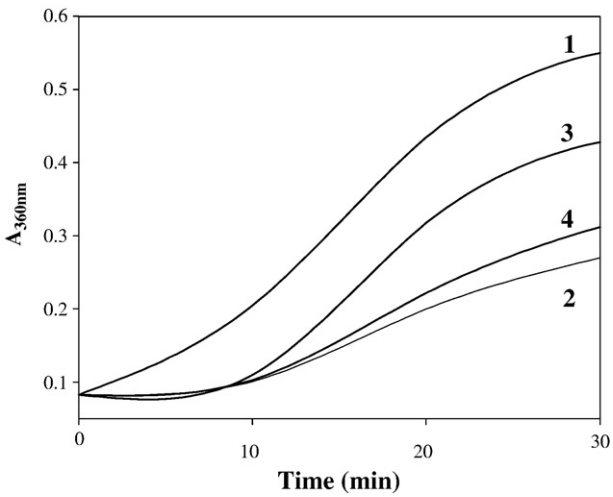

Fig. 3. Chaperone activity of α-crystallin isolated from *in vitro* glycated TSP as assessed by heat-induced aggregation of βL-crystallin (0.2 mg/ml in 50 mM phosphate buffer, pH 7.4) at 60°C. βL-crystallin was incubated in the absence (trace 1) or presence of 0.025 mg/ml of αL-crystallin isolated from control TSP (Trace 2), glycated TSP in the absence (Trace 3) or presence of 1 mg/ml cumin (Trace 4).

Table 1
The effect of cumin on onset of cataract (O) monitored after 4 weeks and maturation of cataract (M) monitored after 8 weeks of STZ administration

	Group I		Group II		Group III	
	O	M	O	M	O	M[a]
Clear	100%	100%	30%	–	50%	
Stage 1	–	–	70%	–	50%	40%
Stage 2	–	–	–	16%	–	60%
Stage 3	–	–	–	84%	–	–

Total lenses in a group are considered as 100% for calculating the incidence of various stages.

[a] Denotes that cumin feeding significantly delayed maturation of cataract compared to Group II diabetic rats ($P \leq .001$).

effect against loss of the chaperone activity of α-crystallin (Fig. 3).

3.2. Effect of cumin on STZ-induced cataract progression

There was an increase in the food intake in untreated and cumin-fed diabetic rats (Groups II and III) compared to control rats (Group I) (data not shown) as observed in our previous studies [25,35,41]. Despite the increased food intake, the body weight of Group II and III animals was decreased (205 and 214 g, respectively), when compared to the controls (325 g). The onset of cataract was observed after 4 weeks in diabetic rats (Groups II and III), whereas all lenses were clear and normal in control rats. In Group II, 30% of lenses were clear and 70% displayed Stage I cataract (Table 1). However, in cumin-treated rats (Group III) 50% of lenses were clear and 50% were in Stage I (Table 1). At the end of 8 weeks, a majority (84%) of the lenses in Group II showed Stage III cataract (Table 1). Remarkably, the lenses in Group III were restricted to Stage II (60%) and Stage I (40%). The data suggest that the progression of cataract due to STZ-induced hyperglycemia from onset through maturation was delayed in Group III rats, whose diet was supplemented with 0.5% cumin.

Table 2
Effect of cumin treatment on protein content of rat lens

	Group I	Group II	Group III
Total protein (mg/g lens)	504±17.4	410±31.8[a]	463±22.3[b]
Soluble protein (mg/g lens)	344±29.8	213±19.3[a]	292±15.5[b]
Soluble protein (%)	68.2	51.9	63

Data are the mean±S.D. for four individual estimations in duplicates ($P \leq .05$).

[a] Significantly different from Group I.

[b] Significantly different from Group II.

3.3. In vivo antiglycating effect of cumin

The effect of cumin on blood glucose, glycated hemoglobin, lens soluble protein content and protein carbonyls (marker of protein oxidation) under hyperglycemic conditions was evaluated. Average fasting glucose levels in control, diabetic and cumin-treated subjects were 80, 290 and 240 mg/dl, respectively. Though the decrease in plasma glucose levels in diabetic rats fed with cumin was significantly lower than that in untreated diabetic rats by statistical means, it was not comparable to control rats. However, cumin significantly decreased the percentage of HbA_{1c} (Group I: 2.5; Group II: 10.3; and Group III: 5.1%) and protein carbonyls (Group I: 4.0; Group II: 8.1; and Group III: 6.3 nmol/mg protein) in diabetic rats. As expected, diabetes resulted in the formation of glycated protein in the TSP of untreated diabetic rats as determined by boronate affinity chromatography, and cumin supplementation notably decreased the amount of glycated protein in diabetic rats (Fig. 4). There was a significant decrease in both total and soluble protein in the diabetic group as compared to the control group (Table 2). However, feeding cumin to diabetic rats significantly improved the total and soluble protein levels (Table 2). Cumin feeding prevented hyperglycemia-mediated HMW cross-links in TSP (Fig. 5).

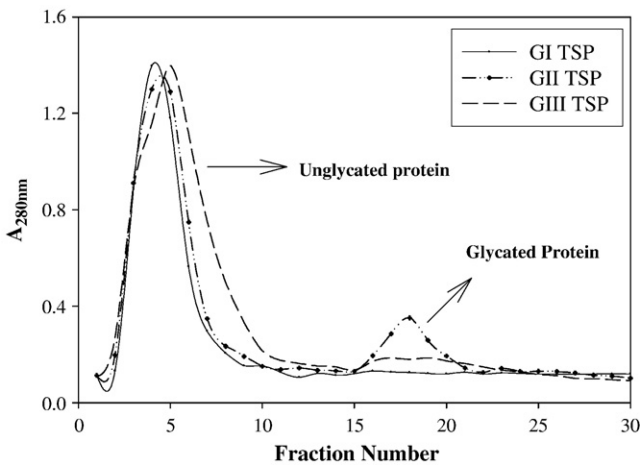

Fig. 4. The effect of cumin on the amount of glycated protein in rat lens TSP analyzed by phenyl boronate affinity chromatography.

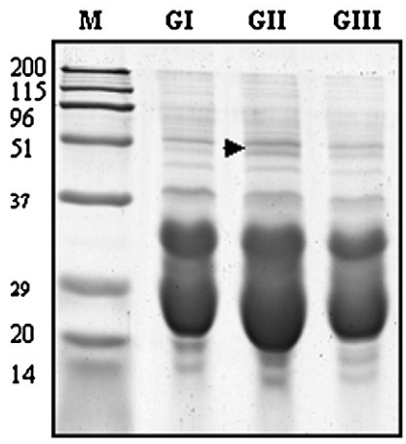

Fig. 5. SDS-PAGE analysis of TSP from control (GI), untreated (GII) and 0.5% cumin-treated (GIII) diabetic rats; M — molecular weight markers. Arrowhead indicates HMW aggregates.

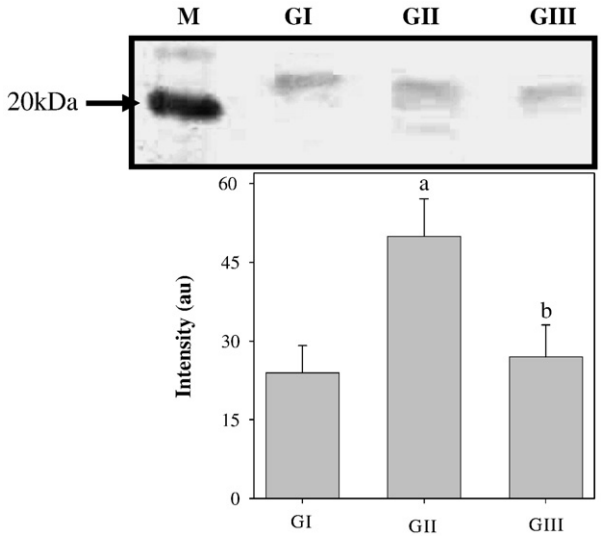

Fig. 6. Immunodetection of α-crystallin in insoluble lens protein from control rats (GI), untreated (GII) and 0.5% cumin-treated (GIII) diabetic rats. Intensity of protein bands was quantified using Bio-Rad quantity one software and expressed as mean±S.D. (n=4) (lower panel). Superscripts 'a' and 'b' indicate significant difference from GI and GII, respectively, at $P \leq .05$.

3.4. Studies with α-crystallin

3.4.1. Distribution of α-crystallin

First and foremost, evaluation by immunoblotting with anti-α-crystallin antibody indicated that there was an increased accumulation of α-crystallin and presence of degraded lower molecular mass product in IP of diabetic rats compared to the control rats (Fig. 6). Feeding cumin to diabetic rats resulted in decreased amount of α-crystallin in IP (Fig. 6), which was comparable to that of control lens. Secondly, there was a marked difference in the relative distribution of α-crystallins in the soluble lens protein between the groups on a preparative size exclusion chromatography column (Fig. 7). Particularly, the αH-crystallin peak was elevated in Group II rat lenses compared to Group I rat lens. Dietary cumin (Group III) normalized the altered crystallin profile in individuals with STZ-induced diabetes (Fig. 7).

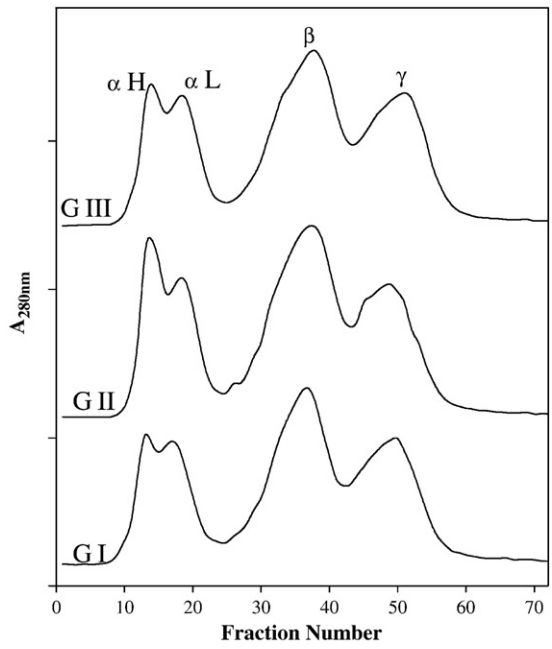

Fig. 7. A representative separation profile of crystallins on a preparative Sephacryl S-300 HR gel filtration column. TSP (100 mg) was loaded onto a column and equilibrated with TNEN buffer. Crystallins were eluted with the same buffer at 0.2 ml/min flow rate and fractions were monitored at 280 nm.

3.4.2. Chaperone activity of α-crystallin

αL-Crystallin from Group II rat lens showed a significant decrease (<50% of control) in chaperone activity in suppressing the heat-induced aggregation of βL-crystallin when compared to Group I (Fig. 8A). Similar results were found in the citrate synthase aggregation assay (Fig. 8B). Interestingly, αL-crystallin from Group III showed improved chaperone-like activity in both assays as compared to Group

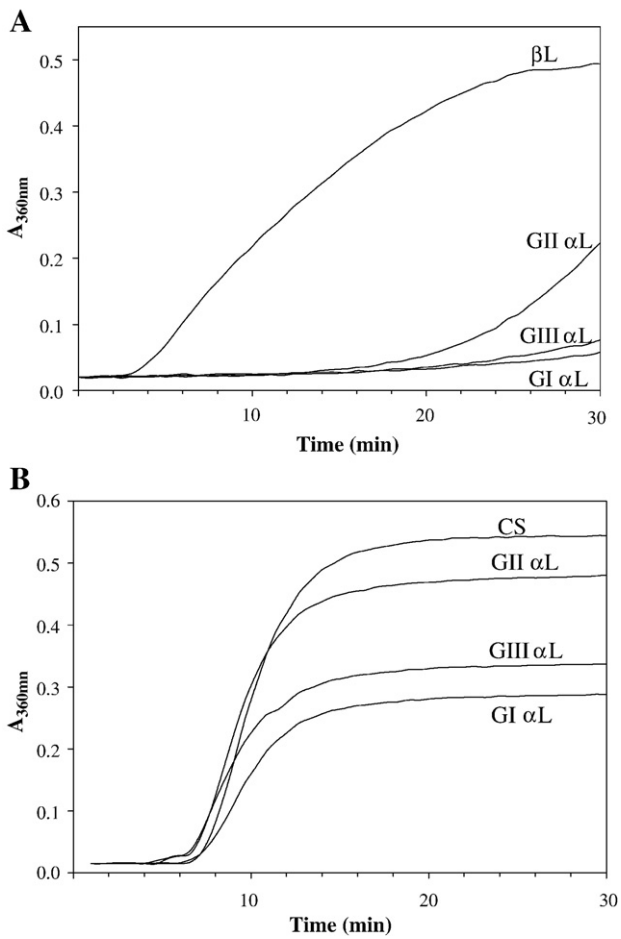

Fig. 8. Chaperone activity of αL-crystallin against heat-induced aggregation of βL-crystallin (0.2 mg/ml in 50 mM phosphate buffer, pH 7.4) at 60°C (A) and CS (0.05 mg/ml in HEPES KOH, pH 8.0) at 45°C (B). βL-Crystallin or CS was incubated in the absence (Trace 1) or presence of 0.025 mg/ml of αL-crystallin from control (Group I), diabetic (Group II) and cumin-treated (Group III) rat lens.

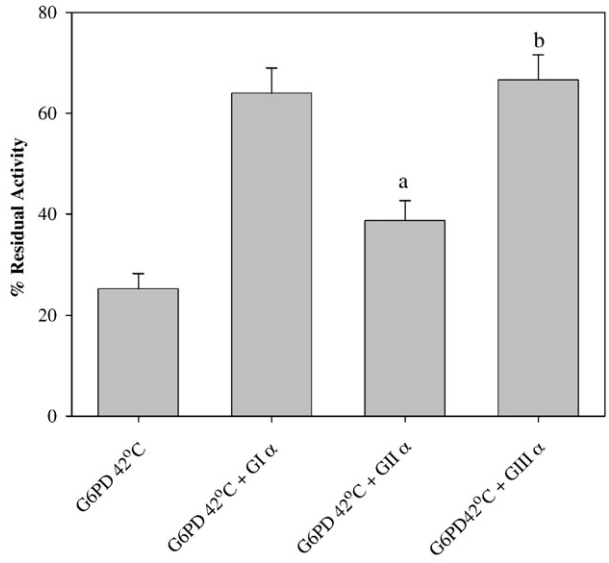

Fig. 9. Chaperone activity of αL-crystallin in G6PD heat inactivation assay at 42°C. Values are expressed as mean±S.D. (n=4). Superscripts 'a' and 'b' indicate significant difference from GI and GII, respectively, at $P \leq .05$.

II rat lenses (Fig. 8). We also assessed the ability of αL-crystallin from control, untreated and cumin-treated diabetic rat lenses to protect heat-induced inactivation of G6PD. Similar to the aggregation assays, the ability of αL-crystallin from Group II in preventing heat-induced inactivation of G6PD declined when compared to Group I (Fig. 9). In contrast to the partially improved chaperone activity in aggregation assays, αL-from cumin-treated rat lens exhibited a remarkable protection of G6PD inactivation (Fig. 9).

3.4.3. Structural alterations

To understand the mechanism of altered chaperone-like function of α-crystallin in diabetic rat lens and its modulation by cumin, we monitored the secondary and tertiary structural states of αL-crystallin by CD and fluorescence spectroscopy. α-Crystallin from Group I had a maximum negative ellipticity of around 217 nm (Fig. 10A), typical of β-sheet structure as reported previously [4,25]. Far-UV CD signal for αL-crystallin isolated from diabetic rat lens decreased, indicating an altered secondary structure. Furthermore, changes in near-UV CD spectra of αL-crystallin from Group II, particularly in the aromatic region (Fig. 10B), also suggested conformational changes at the tertiary structural level due to hyperglycemia. Loss of intensity in tryptophan fluorescence indicated an altered tertiary structure of αL-crystallin due to hyperglycemia (Fig. 11). Cumin feeding attenuated the hyperglycemia-mediated secondary and tertiary structural changes (Figs. 10 and 11).

3.4.4. Hydrophobicity of αL-crystallin

It is evident from several studies that surface-exposed hydrophobic sites on α-crystallin could play a prominent role in chaperone activity [19]. In the present study, we investigated surface hydrophobicity as a function of binding

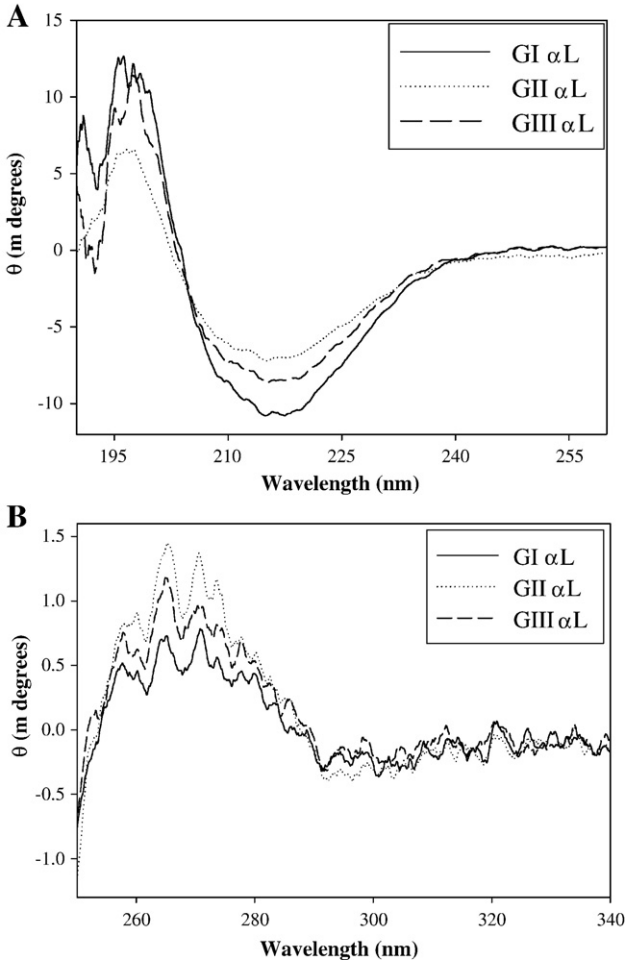

Fig. 10. Secondary (A) and tertiary (B) structure of αL-crystallin from different groups as assessed by far- and near-UV CD spectroscopy using 0.15 and 1.0 mg/ml protein, respectively.

of a hydrophobic probe, ANS, to α-crystallin. αL-Crystallin from Group II showed lesser ANS binding than that from Group I (Fig. 12), which correlated well with the decreased chaperone activity in Group II. Furthermore, improved

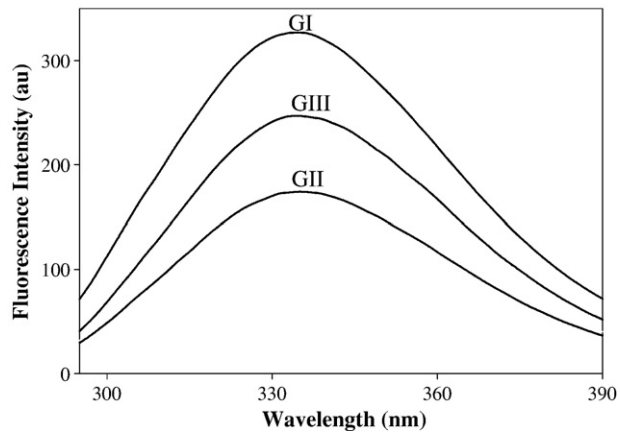

Fig. 11. Intrinsic tryptophan fluorescence spectra of αL-crystallin from different experimental groups.

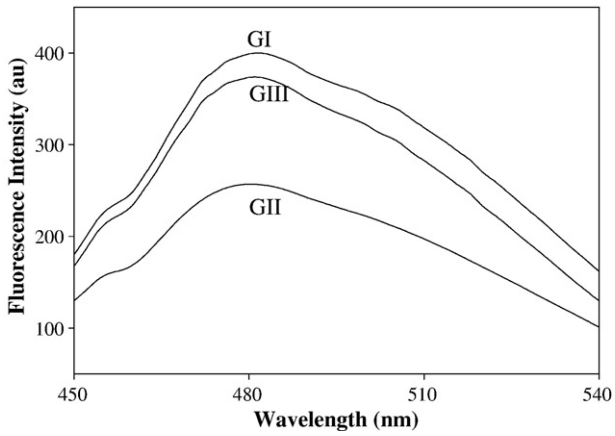

Fig. 12. Hydrophobicity of αL-crystallin as assessed by protein bound ANS fluorescence.

chaperone activity of αL-crystallin from Group III was reflected in increased ANS binding compared to that of αL-crystallin from Group II (Fig. 12).

4. Discussion

Cataract is the leading cause of blindness worldwide [42]. Both the Blue Mountains Eye Study [43] and the Barbados Eye Study [44] state that diabetes has a harmful effect on the lens and it is associated with cortical and posterior subcapsular cataracts. Globally, the number of people with diabetes is projected to rise from 171 million in 2000 to 330 million in 2030 [45]. It has been estimated that India will have the largest number of diabetic subjects in the world by the year 2025 [46]. Taking these projections into account, diabetes could become a major threat to public health and the management of cataract blindness. Several mechanisms have been proposed to explain accelerated cataract formation due to diabetes [47]. Glycation-induced structural damage to lens proteins resulting in the formation of light scattering aggregates and the aldose reductase-mediated polyol osmotic theory have both been considered as predominant factors. It has been previously found that curcumin, an aldose reductase inhibitor and an antioxidant, delays diabetic cataract in rats [35], and it had been demonstrated that modulation of α-crystallin chaperone activity by curcumin might be involved in delaying diabetic cataract [25]. In continuation of our quest towards developing potential agents to delay or prevent diabetic cataract, we have screened several plant and spice sources for their antiglycation potential. In this report, we demonstrated that cumin effectively acted as an antiglycating agent against fructose-induced glycation of not only TSP, but also of nonlenticular proteins like BSA. Concomitantly, we investigated the antiglycating effect of cumin in experimental diabetic rats as well as its ability to modulate the chaperone activity of α-crystallin and to delay the diabetic cataract.

In this study, we first demonstrated that the aqueous extract of cumin shows antiglycating activity in terms of preventing protein cross-linking, HMW aggregates and AGE formation upon fructose-mediated *in vitro* glycation of TSP, BSA and isolated α-crystallin. The significance of this observation was further investigated in an STZ-induced diabetic cataract rat model. We assessed the degree of glycation by a series of parameters; some are complimentary to each other. CML indicates levels of early glycated product, and phenyl boronate affinity chromatography measures total glycated protein moieties (both early and late). Protein carbonyls and cross-linking profiles represented glycation-mediated changes. Though there was a significant reduction in HbA_{1c}, the decrease in glucose levels upon feeding of cumin to diabetic rats was not that remarkable. Nevertheless, reduction of markers of protein glycation such as nontryptophan AGE fluorescence, glycated protein levels, CML formation and protein cross-links indicates the antiglycating ability of cumin under hyperglycemic conditions. Formation of protein carbonyls indicates oxidative damage to the lens protein. Cumin supplementation resulted in the attenuation of protein carbonyl formation. This suggests that cumin may be inhibiting the formation of AGE by trapping or scavenging the free radicals and their oxidation products generated by hyperglycemia.

Glycation is known to affect the structure and chaperone-like activity of α-crystallin [3–5,20–22], which in turn could influence the lens opacity [23–25]. Prevention of glycation-mediated loss of α-crystallin chaperone function and structural alteration both under *in vitro* and *in vivo* conditions supports the antiglycating effect of cumin. Usually, αH-crystallin has been treated as a modified version of αL-crystallin due to various insults and is intermediate between soluble and insoluble lens protein. Cumin treatment also resulted in a normalization of elevated αH-fraction in diabetic conditions and attenuated accumulation of α-crystallin into insoluble portion in diabetic rat lenses. Modulation of α-crystallin chaperone activity by cumin was well correlated with its improved hydrophobicity. The level of cumin used in this study was based on a pilot study in which we were unable to observe more pronounced effects with higher doses (2.5%). However, it may be possible to observe more pronounced effects with intermediate doses. Since the level of cumin fed to these animals in this study (0.5%) was many folds higher than that of the aqueous extract used in *in vitro* glycation studies, the pharmacokinetics has to be investigated to understand its absorption and tissue distribution.

It should be noted that α-crystallin from both diabetic rat and human lenses has shown a substantial loss in its chaperone function [23–25]. Furthermore, impaired α-crystallin chaperone activity has also been found in galactosemic rat lenses [48]. These studies imply that the impaired chaperone function of α-crystallin may be involved in the formation of diabetic cataract. Therefore, it is essential to investigate the ways and means by which we can maintain

or prevent the loss of the chaperone potential of α-crystallin under diabetic conditions. Cumin treatment contributed to a modest lowering of blood glucose levels in diabetic rats. Though there was a decrease in plasma glucose levels in cumin-fed diabetic rats, the levels were still higher than 150 mg/dl, which in Wistar-NIN rats causes cataract after 4–5 weeks [25,35,41]. Therefore, it should be noted that even though there is a partial lowering of blood glucose, cumin feeding resulted in a delay of cataract progression, which could be attributed at least partly to the modulation of structure and function of α-crystallin. Thus, it is believed that the antiglycating nature of cumin could modulate α-crystallin chaperone activity under STZ-induced diabetic conditions.

Activation of polyol pathway, alternatively or in parallel, has been implicated in the development of diabetic cataract, particularly in rats. Thus, delay of diabetic cataract in rats by cumin through modulation of osmotic stress cannot be ruled out. The main constituent and important compound present in cumin is cuminaldehyde, and substituted pyrazines are the other compounds. Although a study reported that cuminaldehyde inhibits rat lens aldose reductase [49], we did not find such inhibition with either rat lens aldose reductase or recombinant human aldose reductase (data not shown). Secondly, cuminaldehyde did not inhibit protein glycation under *in vitro* conditions up to 10 mM concentration (data not shown). Studies are underway to investigate other component(s) of cumin responsible for its antiglycating action and delay of diabetic cataract. In conclusion, cumin at low doses of 0.5% in the diet was able to delay diabetes-caused cataract progression and maturation, probably through modulation of the α-crystallin chaperone-like function.

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