The reaction of methylglyoxal with human and bovine lens proteins

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Abstract

Methylglyoxal is an endogenous metabolite that increases in diabetes and has been implicated in some of its long-term complications such as retinopathy, neuropathy and cataract. We investigated the reaction of methylglyoxal with isolated human and bovine lens crystallins (α, β_H, β_L and γ). After 7 days incubation at 37°C and pH 6.9, the reaction of methylglyoxal with lens proteins yielded stable adducts that exhibited fluorescent properties. SDS-polyacrylamide gel electrophoresis was used to monitor aggregation and crosslinking of the modified protein and autoradiography showed that [^{14}C]methylglyoxal was incorporated into all the protein bands. Bovine γ-crystallin was the most reactive towards methylglyoxal. Reaction of methylglyoxal with bovine γII-crystallin, which is found mainly in the lens nucleus, could alter the charge surface network of the molecule, resulting in aggregation, increased light scattering and hence cataract. Modification of γII-crystallin by methylglyoxal produced an overall loss of positive charge and an increase in molecular weight and non-disulfide covalent crosslinking. Amino acid analysis of the modified γII-crystallin showed a loss of 47% of arginine residues.

1. Introduction

Methylglyoxal (2-oxopropanal) is a biological metabolite formed by the non-enzymatic and enzymatic degradation of triose phosphates, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Other sources include aminoacetone and hydroxyacetone, products of threonine catabolism and acetone metabolism respectively [1]. The glyoxalase system, using reduced glutathione as a cofactor, catalyzes the conversion of methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione.

Thornalley et al. have shown that the system is modified in hyperglycemia, which may be linked to the development of diabetic complications [2]. The concentration of methylglyoxal increased in cultured human red blood cells during hyperglycemia and increased levels of methylglyoxal were found in blood from diabetic patients [3] and in the lens of rats made diabetic by injection of streptozotocin [4].

Methylglyoxal is a reactive compound which covalently binds to lysozyme, introducing crosslinking and fluorescence similar to the Maillard reaction (non-enzymic browning) [5]. Van der Jagt showed that glucose and methylglyoxal reacted with albumin to produce modified proteins with new absorption and emission bands that were spectrally similar and that methylglyoxal was much more reactive than glucose [6]. Methylglyoxal has also been shown to inhibit the activity of red blood cell Na,K-ATPase [7] and of some glycolytic enzymes [8]. It reacted mainly with arginine and lysine residues of proteins [9–11].

Diabetes is a powerful risk factor for cataract, especially in females [12]. The cataracts in older diabetic patients do not appear to have any specific morphology but seem to be part of a multifactorial mechanism leading to lens opacification [13]. Human lens contains, by weight, about 35% protein [14], the bulk of this being crystallin structural protein [15]. The rate of protein turnover in lens fibre cells is very low and thus the proteins are susceptible to environmental insults over decades. Post-translational modification of lens protein has been shown to result in conformational changes, crosslinking and aggregation of proteins leading to lens opacification [15]. This is the first report on the in vitro binding of methylglyoxal to isolated human and bovine lens crystallins (α, β_H, β_L and γ). Modification by methylglyoxal resulted in the formation of

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stable adducts which showed new fluorescent properties. Crosslinking of protein was monitored by SDS-polyacrylamide gel electrophoresis.

Bovine γ-crystallin was the most reactive towards methylglyoxal. A detailed study of the reaction of methylglioxal with one of the γ-crystallins, bovine γII-crystallin, which is found mainly in the lens nucleus, was undertaken. The complete amino acid sequence of γII-crystallin is known [16,17] and its crystal structure has been determined [18]. The surface of γII is largely polar and hydrophillic and contains several arginine residues. By binding to arginine residues on the surface of the molecule, methylglyoxal may affect the overall charge distribution of the molecule, causing altered interaction with adjacent molecules and aggregate formation.

2. Materials and methods

2.1. Chemicals

Methylglyoxal was provided by Dr. Thornalley, prepared by methods described [19]. The concentration of the aqueous solution was determined by endpoint assay involving conversion to S-D-lactoylglutathione with glyoxalase I and hydrolysis catalyzed by glyoxalase II following the method of MacLellan et al. [20]. [2-^{14}C]Methylglyoxal was from Dr. Thornalley, prepared by methods described [21]. Sephacryl S300 High Resolution was from Pharmacia (Milton Keynes, Bucks., UK). Hyperfilm-MP autoradiography film was from Amersham (Little Chalfont, Bucks.). Unless otherwise indicated, reagents were of the highest quality obtainable from Sigma (Poole, Dorset, UK) or BDH (Poole, Dorset, UK).

2.2. Lenses

Bovine eyes were obtained fresh from the local abattoir. The lenses were dissected out and stored at $-20°C$ until use. Intact human cataract lenses were provided by Dr. R.M. Broekhuyse (University of Nijmegen, The Netherlands). 32 lenses (5 grade I cataractous, and 27 grade II, according to the Pirie grading system [22]), were used to isolate the human crystallins.

2.3. Isolation of human and bovine lens crystallins

Seven bovine lenses and 32 human cataractous lenses were separated by gel filtration on a Sephacryl S300HR column (97 cm × 7 cm i.d.) according to the method of Slingsby and Bateman [23]. Fractions corresponding to each protein peak (α, β_H, β_L and γ) were dialysed against four changes of distilled water, freeze-dried and stored at $-20°C$. Protein purity was assayed using SDS-polyacrylamide gel electrophoresis using the method given below.

2.4. Incorporation of [^{14}C]methylglyoxal into isolated bovine and human crystallins

Unless otherwise indicated all incubations were carried out in 0.1 M sodium phosphate buffer, pH 6.9, containing 0.05% NaN_3 to prevent bacterial growth. To determine the rate of incorporation of methylglyoxal into isolated bovine crystallins, incubations were set-up containing final concentrations of 2 mg ml^{-1} of α, β_H, β_L or γ-crystallin respectively and 5 mM methylglyoxal (4.5 μCi of [^{14}C]methylglyoxal diluted with the unlabelled compound) in a total volume of 3 ml. For the human crystallins, incubations were set-up containing final concentrations of 2 mg ml^{-1} of α, β_H, β_L or γ-crystallin respectively and 5 mM methylglyoxal (1.0 μCi of [^{14}C]methylglyoxal diluted with the unlabelled compound) in a total volume of 3 ml. Control incubations were set up containing the isolated human or bovine crystallins (α, β_H, β_L and γ) in buffer alone. Another control incubation contained 5 mM methylglyoxal in buffer. In a separate experiment, incubations were set-up to monitor incorporation into bovine γ-crystallin (2 mg ml^{-1}) at lower concentrations of methylglyoxal (3 mM and 0.3 mM).

The solutions were filtered through 0.2 μm-pore size Nalgene filters into sterile vials and incubated at 37°C in a shaking water bath for 7 days. Aliquots (3 × 20 μl) were removed at zero time using a sterile needle and syringe to estimate the total radioactivity in the incubation. Every 24 h, 3 × 50 μl aliquots were removed and incorporation of label into trichloroacetic acid precipitable protein was determined as described by Crompton et al. [24]. Results were calculated as moles of methylglyoxal bound per mole of polypeptide (taking into account the dilution by unlabelled methylglyoxal and assuming an average polypeptide mass of 20 kDa for the protein). Aliquots were removed after 0 and 7 days incubation for SDS-polyacrylamide gel electrophoresis.

2.5. Addition of sodium borohydride to modified isolated human crystallins

Sodium borohydride, which reduces and thus stabilises Schiff bases, was added to aliquots of the incubating solution to ascertain whether any methylglyoxal was bound as a Schiff-base to the protein. 1 h before the end of the 7 day incubation, 3 × 50 μl aliquots were removed from the incubating solutions of human crystallins and placed in Eppendorf tubes. Sodium borohydride was dissolved in 0.1 M sodium phosphate buffer (pH 6.9, 0.05% NaN_3) to make a 215 mM solution, from which 50 μl was immediately removed and added to each Eppendorf tube. After 1 h the reaction was stopped by the addition of 1 ml of 10% (w/v) trichloroacetic acid. The tubes were left overnight at 4°C. The number of mol of methylglyoxal bound per mole of polypeptide was determined.

2.6. Aggregation of isolated bovine and human crystallins monitored by SDS-polyacrylamide gel electrophoresis

After 7 days of incubation of the bovine and human crystallins with methylglyoxal, 0.2 ml of each solution was centrifuged in a Millipore Ultra-filter (molecular cut-off; 10 kDa) at 2800 × g for 10 min until the protein solution was reduced to approx. 40 μl. 100 μl of distilled water was added to the Ultra-filters and the tubes were centrifuged for a further 7 min. This served to concentrate the protein and remove any salts and unreacted methylglyoxal. 100 μl of loading buffer was added and the solutions were vortexed and stored at −20°C until they were run on a 12.5% gel. The same procedure was followed for the control incubations (isolated bovine and human crystallins incubated in buffer alone and for crystallins dissolved in buffer that had not been incubated.

2.7. SDS-polyacrylamide gel electrophoresis

This method is based on that of Laemmli [25]. The protein samples were boiled in the loading buffer (63 mM Tris buffer, pH 6.8, containing 2% (w/v) sodium dodecyl-sulfate, 5% (v/v) mercaptoethanol, 10% (w/v) glycerol and 0.01% (w/v) Bromophenol blue) for 5 min prior to electrophoresis. SDS-polyacrylamide gel electrophoresis was performed using an LKB 2052 Midget Electrophoresis unit, 12.5% slab gels and 5% stacking gels. Standard proteins were run with each gel; bovine serum albumin 66 kDa, egg albumin 45 kDa, thermolysin 37.5 kDa, carbonic anhydrase 29 kDa and lysozyme 14 kDa. The gels were run at 40 mA constant current. Protein bands were visualized by Coomassie Brilliant Blue staining (1.25%) and destained in methanol/acetic acid/water (50:10:40, v/v).

2.8. Autoradiography

The gel containing [^{14}C]methylglyoxal-modified bovine crystallins was dried onto filter paper using a LKB 2003 slab gel drier unit. Autoradiography was carried out using autoradiography film (Hyperfilm MP) with a 21 day exposure.

2.9. Spectrofluorimetric analysis

Experimental incubations were set-up containing final concentrations of 2 mg ml^{-1} of bovine α, β_H, β_L or γ-crystallin with 5 mM methylglyoxal in a final volume of 3 ml. Control incubations were set-up without methylglyoxal. After 7 days the solutions were dialysed against two changes of distilled water (2 × 2 l) and 2 changes of incubation buffer (2 × 1.5 l) to remove any unreacted methylglyoxal. The protein concentrations of the dialysed solutions were determined using the Bradford method. The solutions were diluted to a protein concentration of 0.25

mg ml^{-1} with incubation buffer for spectrofluorimetric analysis.

Analysis of tryptophan fluorescence used an exciting wavelength of 280 nm, recording emission spectra from 300 to 500 nm. Non-tryptophan fluorescence used an exciting wavelength of 320 nm, recording emission from 340–500 nm. Emission spectra were obtained using a Perkin Elmer Luminescence Spectrometer LS50 (emission and excitation slit widths 5.0 nm; scan speed 240 nm min^{-1}).

2.10. Isolation of γ-crystallin

Bulk preparation of γ-crystallin was carried out using a Sephadex G-50 column (fine beaded) according to the method of Martin and Harding [26]. γ-Crystallin purity was tested by SDS-polyacrylamide gel electrophoresis (single band at 20 kDa).

2.11. Isolation of γII-crystallin

The γ-crystallins were separated according to the method of Björk [28], except a SP-Sephadex C-50 column (84 cm × 1.8 cm i.d.) was used instead of a SE-Sephadex C-50 column. The γII-crystallin obtained gave a single band by SDS-polyacrylamide gel electrophoresis and a single peak on HPLC under conditions given below.

2.12. Modification of γII-crystallin by methylglyoxal and elution from a cation exchange column

γII-Crystallin (17.1 mg) was dissolved in the incubation buffer containing 5 mM methylglyoxal to a final concentration of 4 mg ml^{-1}. The solution was sterile filtered into a sterile vial and incubated at 37°C in a shaking water bath for 24 h. The reaction was stopped by dialysis against three changes of distilled water (2 l) and three changes of 0.2 M sodium acetate buffer pH 5.0 (1.5 l) over 48 h. The protein solution was then eluted from the same cation exchange column as described previously. The methylglyoxal modified protein eluted as a single peak. The fractions corresponding to this peak were pooled and extensively dialysed against distilled water (seven changes including one overnight dialysis) then freeze-dried. 12.5 mg of an off-white solid was recovered. This product was analysed by HPLC and SDS-polyacrylamide gel electrophoresis.

2.13. Aggregation monitored by HPLC

The methylglyoxal-modified γII-crystallin (2 mg ml^{-1}) and a sample of unmodified γII-crystallin (2 mg ml^{-1}) were dissolved in elution buffer (0.1 M sodium phosphate, pH 6.6, containing 0.1 M sodium sulfate). The samples were filtered through 0.22 μm Millipore filter units. 10 μl samples were injected onto a TSK G3000 SWXL size

exclusion column and eluted with elution buffer (which had been filtered and degassed) at a flow rate of 1 ml/min. The absorbance was measured at 280 nm on a Gilson UV detector. The column was calibrated with standards.

2.14. Aggregation monitored by SDS-polyacrylamide gel electrophoresis

The methylglyoxal-modified γII-crystallin (2 mg ml^{-1}) and a sample of unmodified γII-crystallin (2 mg ml^{-1}) were boiled in loading buffer before being run on a 12.5% SDS-polyacrylamide gel as described previously.

2.15. Amino acid analysis

The methylglyoxal-modified γII- (2 mg ml^{-1}) and unmodified γII-crystallin (2 mg ml^{-1}) were dissolved in 6 M HCl and hydrolysed in vacuo for 18 h at 108–110°C. The acid was removed by rotary evaporation and the hydrosylate was analysed using the Waters Pico.Tag method (Millipore Corporation, Waters Chromatography Division, MA, USA) based on a method by Heinrikson and Meredith [27]. The hydrolysed protein samples were derivatised with phenylisothiocyanate then separated by reverse phase HPLC using an octadecylsilane (ODS) column. The phenylthiocarbamyl amino acids eluted were detected at 254 nm on a Gilson UV detector over 25 min.

3. Results

3.1. Incorporation of [^{14}C]methylglyoxal into isolated bovine and human crystallins

The incorporation of label was monitored for seven days. Fig. 1A and B shows the incorporation of 5 mM [^{14}C]methylglyoxal into bovine and human crystallins. Bovine crystallins appear more susceptible to modification than human crystallins, but all the major crystallin fractions of both species react with methylglyoxal. Fig. 1C shows the incorporation of lower concentrations of [^{14}C]methylglyoxal (3 mM and 0.3 mM) into bovine γ-crystallin.

3.2. Effect of sodium borohydride on methylglyoxal-modified human crystallins

Sodium borohydride reduces and stabilises Schiff-base adducts. There was no significant change in the number of mol of bound methylglyoxal to human α, β$_H$ or β$_L$-crystallin after addition of NaBH$_4$. The number of moles of methylglyoxal bound to human γ-crystallin increased by 15.7% after reduction with sodium borohydride ($P < 0.05$, Student's t-test).

3.3. Aggregation of bovine lens proteins

Fig. 2A shows the SDS-polyacrylamide gel of bovine crystallins (α, β$_H$, β$_L$ and γ) isolated from the Sephacryl S300 HR column. No change was observed after incubation of the crystallins in buffer for 7 days (Fig. 2B). Fig. 2C shows the results obtained after bovine crystallins (α, β$_H$, β$_L$ and γ) had been incubated in 5 mM methylglyoxal for 7 days. The bands of the modified crystallins all appear more diffuse. Both α- and γ-crystallins show an apparent increase in molecular mass from approx. 20 to 29 kDa. Aggregated bands are present with each modified crystallin with an apparent molecular mass of approx. 45 to 55 kDa. Bands are also present between the stacking gel and the running gel with the modified α, β$_H$ and β$_L$-crystallins due to precipitated protein. Similar results were obtained for the human lens proteins except for the absence of an aggregated γ-crystallin band after modification by methylglyoxal (Fig. 2D, E and F).
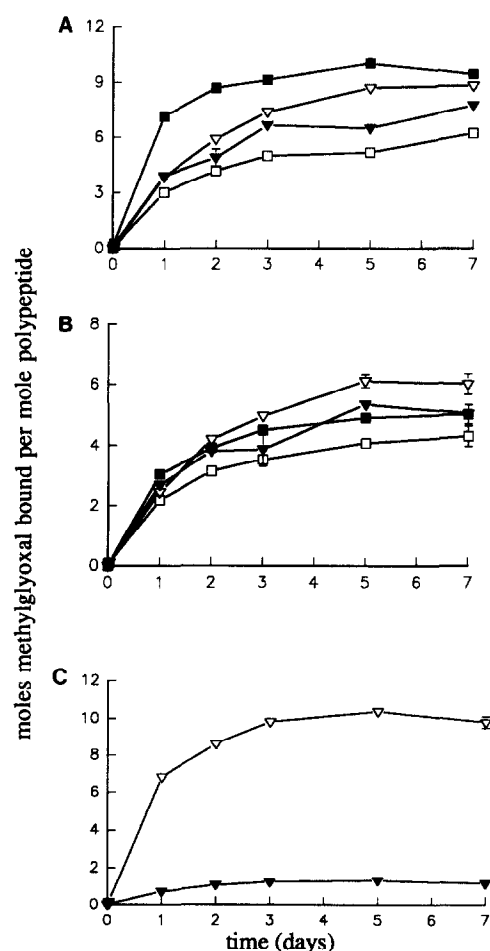

Fig. 1. Incorporation of [^{14}C]methylglyoxal into isolated (A) bovine lens crystallins and (B) human crystallins: ■, γ; ▽, α; ▼ β$_H$; □, β$_L$; (C) incorporation of 3 mM (▽) and 0.3 mM (▼) [^{14}C]methylglyoxal into bovine γ-crystallins. All points were calculated from the mean of triplicate determinations ± S.D. Error bars for the lowest curve are too small to be seen.
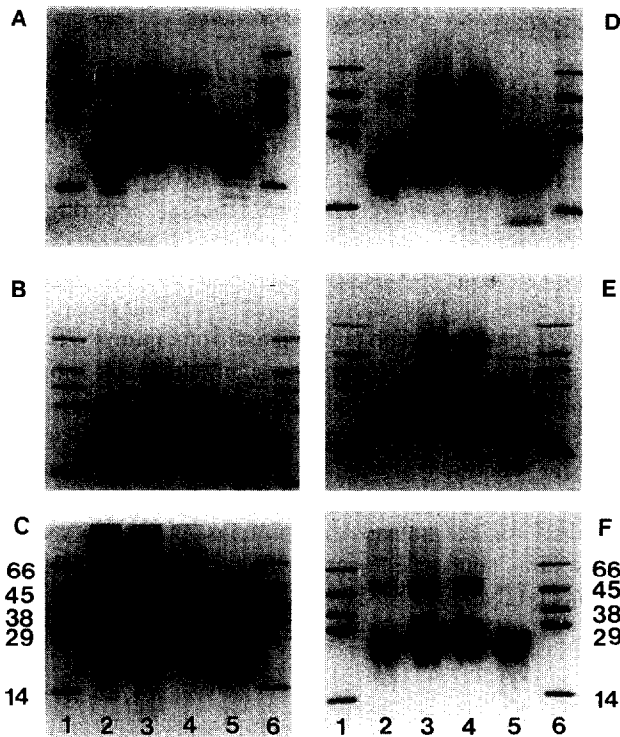
Fig. 2. SDS-PAGE of (A) unmodified bovine crystallins, (B) bovine crystallins incubated in buffer for 7 days, (C) bovine crystallins incubated in 5 mM methylglyoxal for 7 days, (D) unmodified human crystallins, (E) human crystallins incubated in buffer for 7 days, (F) human crystallins incubated in methylglyoxal for 7 days. Lanes: (2) α, (3) β_H, (4) β_L, (5) γ. Molecular weight standards were run in lanes (1) and (6) and were the same in each gel.

3.4. Autoradiography

Autoradiography of the dried gel containing bovine crystallins, modified with [^{14}C]methylglyoxal showed that the label had been incorporated into all the crystallin fractions and also into the aggregated bands (Fig. 3).

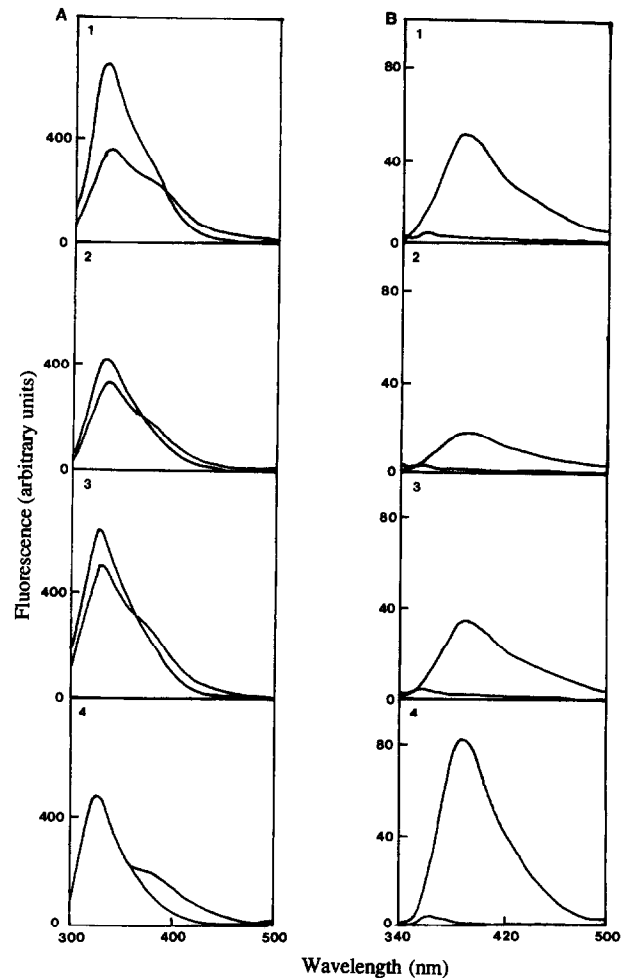Fig. 3. Autoradiography of the gel shown in Fig. 2C, corresponding to lanes 2 to 5.

Fig. 4. Tryptophan fluorescence spectra (ex. 280 nm, em. 300–500 nm) of (1) α, (2) β_H, (3) β_L, (4) γ-crystallin. Spectra are presented for crystallins incubated in 5 mM methylglyoxal for 7 days (lower curve) and crystallins incubated in buffer alone (upper curve). (B) Non-tryptophan fluorescence spectra (ex. 320 nm, em. 340–500 nm) of (1) α, (2) β_H, (3) β_L, (4) γ-crystallin. Spectra are presented for crystallins incubated in 5 mM methylglyoxal for 7 days (upper curve) and crystallins incubated in buffer alone (lower curve).

3.5. Changes in fluorescence after modification by methylglyoxal

Excitation of isolated lens proteins at 280 nm yielded tryptophan fluorescence with an emission maximum at approx. 335 nm which did not change significantly after 7 days incubation. Incubation of the isolated crystallins with methylglyoxal resulted in a decrease in tryptophan fluorescence with α-, β_H- and β_L-crystallin (Fig. 4A). Decreased quantum yields have usually been associated with the chromophore being in a more polar environment, e.g., more surface exposed. When excited at 280 nm, methylglyoxal-modified γ-crystallin yielded a second emission maximum at 385 nm. Methylglyoxal incubated alone as a control produced little fluorescence after excitation at 280 nm.
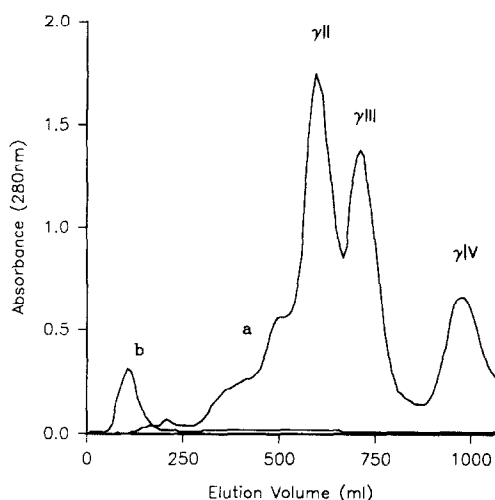
Fig. 5. Elution profiles from a SP-Sephadex C-50 cation exchange column (a) of γ-crystallins (γII to γIV), (b) of methylglyoxal-modified γII-crystallin.
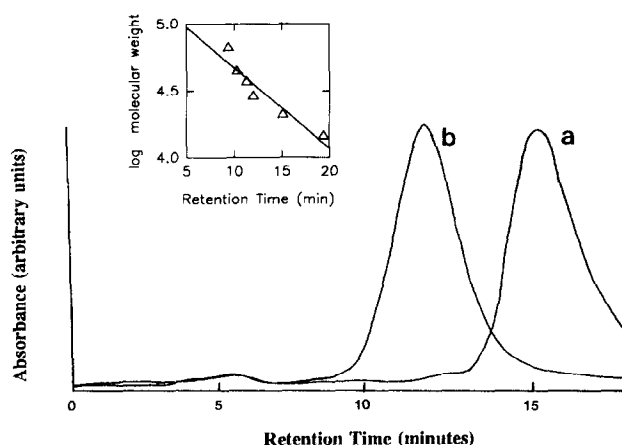

Fig. 6. HPLC chromatogram of (a) unmodified bovine γII-crystallin, (b) methylglyoxal-modified γII-crystallin. (Inset) Calibration standard curve using molecular weight markers; bovine serum albumin 66 kDa, egg albumin 45 kDa, thermolysin 37.5 kDa, carbonic anhydrase 29 kDa, γII-crystallin 21 kDa and lysozyme 14 kDa.

Incubation of the individual crystallins with methylglyoxal resulted in non-tryptophan fluorescence in all cases (ex. 320 nm; em. 340–500 nm) with an emission maximum at approx. 390 nm (Fig. 4B). The greatest increase in non-tryptophan fluorescence was produced by methylglyoxal modified γ-crystallin. Lens crystallins (α, β_H, β_L and γ) incubated alone and methylglyoxal incubated alone produced little fluorescence after excitation at 320 nm.

3.6. Isolation of γII-crystallin

The Sephadex G-50 elution profile for bovine lens soluble protein gave two main peaks and was similar to that obtained by Martin and Harding [26]. SDS-polyacrylamide gel electrophoresis showed the first peak to be a mixture of α- and β-crystallins and the second peak to be γ-crystallin (gel not shown).

The SP-Sephadex C-50 cation exchange column allows separation of the γ-crystallins (γII to γIV). The elution profile is shown in Fig. 5 (elution profile a) and is similar to that obtained by Björk [28]. The three fractions were run on an SDS-polyacrylamide gel and corresponded to a molecular mass of about 20 kDa (gel not shown). The fractions were analysed by electrospray mass spectrometry. The ESMS of II-crystallin gave a mass of 20969 kDa which agrees exactly with the sequence [17] and that obtained previously by ESMS [29]. The methylglyoxal-modified γII-crystallin eluted from the same cation-exchange column in the void volume, as a single peak (Fig. 5, elution profile b).

3.7. Aggregation of methylglyoxal-modified γII-crystallin monitored by HPLC

To study the crosslinking and aggregation, γII-crystallin was incubated with 5 mM methylglyoxal for 24 h. At

this time 76% of the maximum reaction should have occurred (Fig. 1A). Chromatograms of methylglyoxal-modified γII-crystallin and unmodified γII-crystallin are shown in Fig. 6. γII-Crystallin eluted as a single peak after 15.1 min (elution profile a). The modified sample eluted after 11.9 min (elution profile b), indicating that methylglyoxal had caused aggregation of all the protein. The molecular weight of the modified protein was estimated at 35.5 kDa from the calibration curve (Fig. 6, inset).

3.8. Aggregation of methylglyoxal-modified γII-crystallin monitored by SDS-polyacrylamide gel electrophoresis

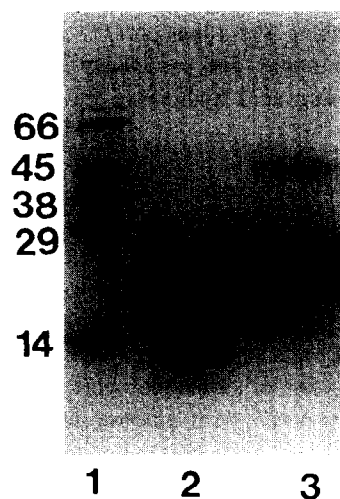Fig. 7 shows an SDS-polyacrylamide gel of methylglyoxal-modified γII-crystallin and of unmodified γII-crys-

Fig. 7. SDS-PAGE. Lanes: (2) unmodified γII (20 μg), (3) methylglyoxal-modified γII (20 μg). Molecular weight standards were run in lane (1).

Table 1

Amino acid	Amino acid composition (residues/molecule)		
	calculated	γII-crystallin	methylglyoxal-modified γII-crystallin
Asp	19	12.8	15.6
Glu	19	17.5	21.2
Ser	13	15.0	15.8
Gly	14	17.2	17.1
His	5	5.4	5.4
Thr	5	5.6	5.8
Arg	20	20.1 [a]	9.3
Ala	2		2.7
Pro	8	8.7	8.9
Tyr	15	15.5	14.2
Val	6	6.1	6.3
Met	7	7.5	9.9
Ile	6	8.4	8.2
Leu	13	12.7	12.7
Phe	9	9.0	8.7
Lys	2	1.9	1.7

Results shown are the mean of duplicate determinations.

[a] Arg and Ala residues coeluted.

tallin. For the modified sample there is an apparent increase in molecular weight of the major band from 20 kDa to approx. 25 kDa and the appearance of a faint aggregated band at approx. 48 kDa. This faint band is due to non-disulfide covalent cross-linking, since mercaptoethanol (which breaks disulfide bonds) was present in the loading buffer. Comparing Figs. 6 and 7 it appears that dimerization of all the protein (as seen in Fig. 6) is due to non-covalent binding because the SDS-gel indicates that only a small proportion of the protein is covalently crosslinked.

3.9. Determination of γII-crystallin residues modified by methylglyoxal

The results obtained from amino acid analysis of γII-crystallin are similar to the theoretical values calculated from the published sequence [17]. After modification by methylglyoxal there was a loss of 47% of arginine residues (Table 1). Tryptophan and cysteine were not determined due to loss during acid hydrolysis and derivitization, respectively.

4. Discussion

Diabetes is an established risk factor for cataract. Work has been published suggesting that non-enzymic glycosylation (glycation) of lens proteins may be involved in cataractogenesis and other diabetic complications [30]. Reaction of lens proteins with glucose and glucose-6-phosphate was first reported by Stevens et al. [31]. The authors suggested that glycation altered the conformation of proteins thus promoting disulfide crosslinking and aggregation.

Methylglyoxal also increases in diabetes and reacts much more rapidly than glucose with proteins [6], so its impact in vivo might be greater than its low concentration would suggest. The concentrations of methylglyoxal used in our experiments were much higher than those found physiologically [20], but cataract develops over years or decades. Methylglyoxal has been shown to bind to plasma proteins under physiological conditions (1 μM) [11].

Our experiments show that methylglyoxal will readily form adducts with the major human and bovine lens proteins, resulting in a possible alteration in the tertiary structure of the protein, detected by a decrease in tryptophan fluorescence of the modified samples compared with the controls. The adducts formed exhibit non-tryptophan fluorescence (exc. 320 nm; em. 340–500 nm). Similar changes were observed for glycated α-crystallin compared with non-glycated α-crystallin, isolated from human diabetic lenses [32].

Reaction with methylglyoxal resulted in aggregation and non-disulfide covalent crosslinking of lens crystallins similar to changes that occur during the formation of cataract in man [13]. Reduction of the modified bovine crystallins with sodium borohydride indicated that only γ-crystallin had reacted to form a Schiff-base with methylglyoxal and this accounted for only 13.5% of the total amount of methylglyoxal bound. The γ-crystallins have a free terminal α-amino group which, in γII-crystallin, has been shown to react with fructose [33]. However, since the human crystallins were isolated from grade I and grade II cataractous lenses, it is likely that they had already suffered some post-translational modifications.

An overall loss of positive charge of methylglyoxal-modified γII-crystallin was demonstrated by its increased elution rate from a cation exchange column. Amino acid analysis of modified γII-crystallin showed arginine residues to be the major site of reaction. Modification of an arginine residue would result in the loss of a positive charge, which could disrupt the surface network of charges of γII-crystallin and destabilize its native conformation. The charge network is thought to be very important for stability [34]. Aggregation and crosslinking of modified protein was shown by HPLC and SDS-polyacrylamide gel electrophoresis.

Many of these changes that have been shown to occur in vitro with glucose, fructose, glucose-6-phosphate, glucosamine and ribose [13]. During cataractogenesis it is possible to envisage several metabolites with reactive aldehyde groups acting in an additive or synergistic manner. Diabetic cataracts probably have multifactorial origins and non-enzymic post-translational modification of proteins most likely is involved in cataractogenesis.

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