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DIABETES AFFECTS α-CRYSTALLIN CHAPERONE FUNCTION

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SUMMARY *In vitro* glycation was previously shown to influence α-crystallin chaperone function. In the present study we show that this function is compromised in diabetes. The α_H, α_L and the total α fractions were isolated by gel permeation chromatography from the water - soluble protein of streptozotocin - diabetic as well as age-matched normal rats. Based on the β_L-crystallin thermal denaturation assay the chaperone function was significantly decreased in the diabetic rats.

α-Crystallin of the eye lens is constituted of two major closely related subunits namely α_A and α_B. These are independent proteins with 57% sequence identity and each having a molecular mass of 20 kDa (1-3). α-Crystallin, α_B in particular, is expressed widely outside the lens (3-6). Ingolia and Craig (1) discovered sequence similarities between small heat-shock proteins of *Drosophila* and α-crystallin. α-Crystallin is believed to be a major structural element in the highly ordered and highly concentrated protein matrix which is essential to maintain the transparency and refractive properties of the lens. Recent studies have shown that α-crystallin having a chaperone - like property (7-9) which is advantageous for the protection of other lens proteins from denaturation and aggregation.

Lens protein aggregation and insolubilization are known to increase during aging and during the progression of diabetes (10,11) and this may, at least in part, due to the α-crystallin loosing its ability to protect other lenticular proteins from denaturation. In fact, recent studies by others (8) and by us (12) have established that α-crystallin from aged lenses has decreased chaperone property. We have also shown in the above study that *in vitro* oxidation and glycation

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can also lead to significant loses in chaperone function as determined by the ability to protect thermal denaturation of β-crystallin. Since diabetic lenses are believed to undergo more oxidation and glycation than aged-matched control lenses we hypothesized that α-crystallin from diabetic lenses will have chaperone function further compromised. To test this we isolated the high molecular weight α (α_H), low molecular weight α (α_L) and the total α (α_H + α_L) fractions from the lens water-soluble proteins of streptozotocin-diabetic as well as normal control rats to examine their chaperone function.

MATERIALS AND METHODS

Animals: Diabetes was induced in 1 month old Sprague Dawley rats by a single injection of streptozotocin (65 mg Kg^{-1}) through tail vein as detailed in an earlier report (11) while the control rats were injected with saline. Progression of diabetes was evaluated by enzymatic determination of fasting plasma glucose (13) with a Sigma kit and by boronate affinity chromatographic determination of glycated hemoglobin (GHb) (14) using the Glyc-Affin system

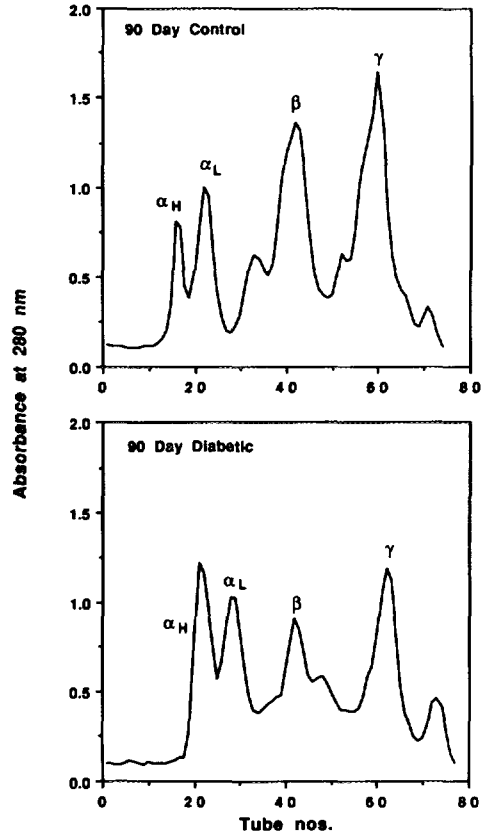

Fig. 1. Sephacryl S-300-HR gel permeation chromatographic separation of water-soluble crystallins from 90-day control and 90-day diabetic rats.

from Isolab, Inc. Glycated lens proteins were also quantified by the Glyc-Affin system as described earlier (11). The following three groups of rats were used for the present study: 1) Diabetics 90 days postinjection (90-day diabetic); 2) normal controls 90 days postinjection (90-day control); 3) untreated 1 month old rats (0-day control). Development of lens opacity was assessed by Scheimflug slit-image analysis (15).

Preparation of crystallins: Five rats from each group were killed to collect their blood and lenses. Blood samples were used to determine plasma glucose and GHb. Lenses were homogenized in 50 mM phosphate buffer pH 7.0 containing 0.1% NaN_3 and 0.2 mM PMSF. After centrifugation at 10,000 g for 1 hour at 4°C, supernatant water-soluble fractions were collected. Newborn calf lenses were homogenized and the soluble fraction prepared in a similar manner. Various crystallin fractions were isolated by preparative Sephacryl S-300-HR gel permeation chromatography. About 30 mg soluble protein was applied to a 100 x 1.5 cm column and developed isocratically with the phosphate buffer (the homogenization buffer). The α_H, α_L, and the total α (α_H + α_L) fractions (Fig.1) from control and diabetic rats and the β_L fraction from calf lenses were concentrated by ultrafiltration in a Diaflo apparatus and their purity checked SDS-PAGE done according to Laemmli (16) using a Bio-Rad Mini-Protean II system. Protein concentrations were determined by the method of Bradford (17).

Determination of molecular chaperone property of α-crystallin from normal and diabetes rats: Heat-induced denaturation and aggregation of calf β_L-crystallin (used as the target protein) in the presence and absence of α-crystallin was performed according to Horwitz (7) to assess the protective effect of the α_L, α_H and total α fractions on β_L aggregation. Forty micrograms of the rat α-crystallin fraction were mixed with 400 μg of calf β_L-crystallin in a cuvette and made up to a final volume of 1 ml with 50 mM phosphate buffer pH 7.0. The cuvette was heated at 55°C in a Shimadzu model UV160 spectrophotometer equipped with a temperature regulated cell holder. Light scattering which was caused by β_L denaturation was scanned over a time period of 3000 seconds at 360 nm absorbance. The various α fractions from 0-day controls, 90-day controls and 90-day diabetics were examined in this manner.

RESULTS

Levels of plasma glucose, glycated proteins, and α_H in the diabetic and control rats: Table I summarizes the levels of fasting plasma glucose, GHb, glycated lens proteins and the α_H fraction. By 90 days, fasting plasma glucose level rose about 8-fold to a mean value of 770 mg/dl and GHb and glycated lens protein increased 4-5 fold to 14.8% and 15.4%, respectively while the α_H fraction showed a 3-fold increase to about 50% of the total α-crystallin. Similar changes were seen during earlier studies of streptozotocin-diabetic rats (11).

Molecular chaperone property of α-crystallin from diabetic and control rats: Three different types of α-crystallin preparations, namely α_L, α_H, and α_L + α_H fractions from 0-day control, 90-day control, and 90-day diabetic rats were used for β_L-crystallin aggregation studies. The heat-induced denaturation and aggregation of calf β_L-crystallin was measured in the absence and in the presence of the various α crystallin preparations. β_L alone underwent complete thermal

Table I

*Levels of plasma glucose, glycated protein and α_H in streptozotocin-diabetic and normal control rats

	0-Day Control	90-Day Control	90-Day Diabetic
Plasma glucose (mg/dl)	102	100	770
GHb (%)	3.2	5.1	14.8
Glycated lens protein (%)	4.1	5.3	15.4
α_H (%)**	15.5	20.3	45.6

*Mean of the results from 5 different pairs of lenses
**Percentage of the total α (α_H + α_L)

denaturation and aggregation whereas in the presence of α-crystallin (α_L + α_H) from the 0-day and 90-day control rats β_L-crystallin aggregation was reduced substantially (Fig.2). However, when α-crystallin from the 90-day diabetic rats was used about 50% loss of β_L aggregation was observed. The α_L and α_H fractions were also used in the chaperone assay. These studies were expected to show whether the decreased chaperone activity of the α-crystallin from the 90-day diabetic rats is due to the α_L, α_H or both having altered function. As compared to the α_L fraction from the 0-day control, α_L from 90-day control and 90-day diabetic rats had decreased

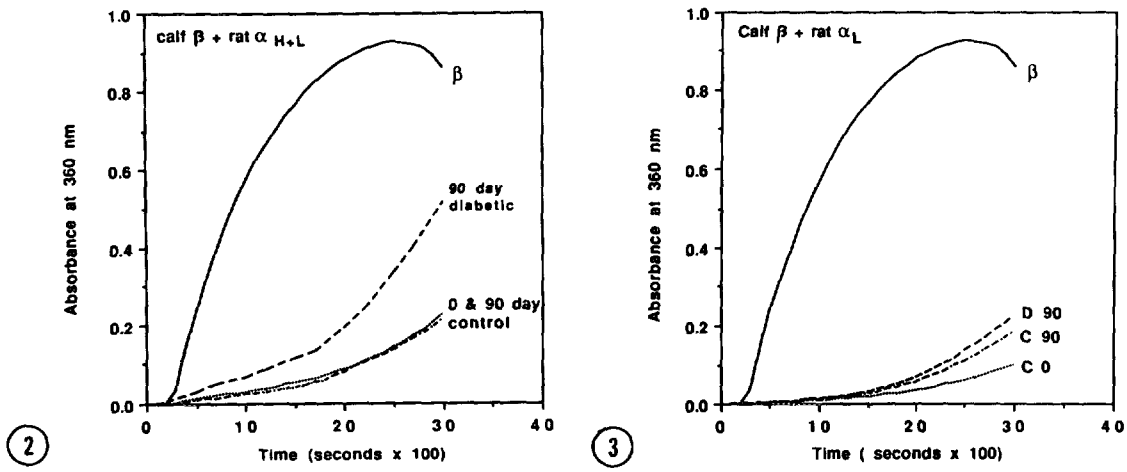

Fig. 2. Calf β_L-crystallin thermal denaturation and aggregation in the absence and presence of α-crystallin (α_H + α_L) from 0-day control, 90-day control and 90-day diabetic rats.

Fig. 3. Thermal denaturations of calf β_L in the absence and presence of α_L from 0-day control (C0), 90-day control (C90) and 90-day diabetic (D90) rats.

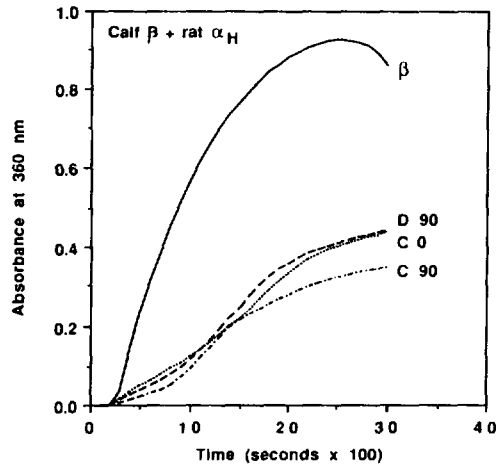

Fig. 4. Thermal denaturation of calf β_L in the absence and presence of α_H from 0-day control (C0), 90-day control (C90) and 90-day diabetic (D90) rats.

chaperone activity, α_L from the diabetic rats showing the largest change (Fig.3). When the α_H fractions were used in the chaperone assay all the three groups of rats showed nearly 50% loss of chaperone activity (Fig.4).

DISCUSSION

The present data show that there is an overall decrease in the chaperone like property of α-crystallin due to diabetes. Since we have shown recently that *in vitro* glycation decreases α-crystallin chaperone activity (12) and due to the fact that 3-4 fold increase in glycation of lens crystallins occurs in diabetic rats (11; Table I) the most likely cause for this change in chaperone function is glycation of α-crystallin . Increased level of α_H also seems to influence the chaperone activity of α-crystallin from the diabetic rats. Since α_H itself has significantly decreased function (Fig. 4) increased level of this aggregated α fraction is expected to lower the chaperone activity of α-crystallin. It is noteworthy that the α_H fraction has altered function independent of the fact whether it was isolated from the diabetic or control rats. Working with α_H and α_L fractions from bovine lenses Takemoto and Boyle (18) have noticed α_H fraction having decreased ability to protect heat-induced denaturation and aggregation of γ-crystallin and alcohol dehydrogenase. According to Boyle *et al* (19) the decreased chaperone activity of α_H fraction may be the result of β-and/or γ-crystallin being bound to the central region of the α-

crystallin particles. The other possibility is that intrinsic differences in α-crystallin particles due to posttranslational modifications like glycation and oxidation do exist in the α_H fraction. The unique aspect of our present finding is that even the α_L fraction from the diabetic as well as the age-matched control rats behaved differently from the α_L of 1 month old normal rats. This may suggest that further aggregation of α_L to α_H is not an obligatory step before a modified function can be noticed. This observation has similarity to our recent finding that the α_L fraction from aged human lenses had significantly decreased chaperone function (12). Subsequent studies of the α_H fraction from the same human lenses also showed that α_H from both young and aged lenses had chaperone function significantly decreased (Cherian and Abraham, unpublished).

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