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Glycation of human lens proteins from diabetic and (nondiabetic) senile cataract patients

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Glycation (nonenzymatic glycosylation) in the human lens (cortex and nucleus) in senile (nondiabetic) and diabetic cataracts was studied by measuring the extent of early and late glycation products, the content of free ϵ-amino groups and the formation of disulfide bonds in the soluble lens proteins. There was a significant ($p < 0.001$) increase in early and late glycation in the lens nucleus compared to the cortex in both the senile and diabetic groups. Overall these changes were much larger in the diabetic group. The concentration of free ϵ-amino groups was decreased in the senile nucleus as well as in the diabetic nucleus when compared with the senile and diabetic cortex ($p < 0.001$). Disulfide bond content was in the order of diabetic nucleus > diabetic cortex > senile nucleus > senile cortex. Glycation of the lens proteins is a generalized feature which is enhanced in the diabetic lens compared to senile lens proteins and is associated with a decrease in free ϵ-amino groups and an increase in disulfide bonds formation in the lens proteins.

Keywords: glycation, lens proteins, diabetes, ageing, cataract

Introduction

Senile cataract, a major cause of blindness worldwide, is an-age associated condition [1, 2]. The term senile refers to the fact that no specific ophthalmic or metabolic diseases are known to precede or to be involved in this type of cataract [3]. On the other hand, diabetes is also considered a significant risk factor accelerating cataract formation [4, 5].

Several mechanisms have been postulated to explain the role of diabetes in accelerating cataractogenesis. However, these mechanisms are still far from understood. Increasing experimental evidences suggests that glycation of lens proteins is involved in cataract formation [6–9].

Glycation of lens proteins, whereby glucose or other reducing sugars react with the ϵ-amino group of lysine residues or amino termini of proteins resulting in the formation of a Schiff base (SB). The SB undergoes an Amadori rearrangement via the Maillard reaction giving rise to a more stable ketoamine or Amadori product (early glycation products). At a later stage, the Amadori products undergo dehydration and rearrangement to form cross-links between adjacent proteins, resulting in protein aggregates or advanced glycation products (AGEs) [10, 11].

Since the lens proteins are long-lived, they are highly susceptible to post-translational modification such as glycation. Glycation is believed to enhance protein unfolding, changing not only the physiochemical properties of lens proteins, but also their functional properties [12, 14].

In a previous work we have shown that age-related changes can be observed successfully using the long-lived lens proteins [15] which undergo minimal or no turnover [16]. To detect changes during protein synthesis, the lens cortex protein may be studied, since they have been relatively newly synthesized. However, changes due to long exposure to glycation may be best observed utilizing the older proteins such as the lens nuclear proteins.

In an attempt to understand the role of glycation in the cataractous lens, soluble proteins from the cortex and nucleus of lens from senile and diabetic individuals have been used for the determination of early and late glycation, free ϵ-amino group content and levels of disulfide bonds.

Materials and methods

The lens cortex and the nucleus were collected from operated senile and diabetic individuals undergoing eye

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surgery at King Abdulaziz Hospital, King Saud University, Riyadh, Saudi Arabia. Twelve patients known to be diabetic with non insulin-dependent diabetes mellitus were used in this study. All were treated with diet alone. Twelve cases of senile cataract with no history of diabetes were also selected for the study. The age range of all 24 cases was between 55–75 years.

The cortical portion was homogenized separately using a Teflon homogenizer. The homogenized cortex was centrifuged at 10000 × **g** for 20 min. The supernatant of the cortex was concentrated using an Amicon apparatus fitted with a PLCC cellulose membrane (cutoff point 5000 kDa). The concentrated cortex preparation was diluted with 20 mM sodium phosphate buffer, pH 7.2 and concentrated again and stored at −20 °C until further analysis.

The nuclei from diabetic and senile cataract cases were collected separately and after recording their weight and colour, they were each homogenized in a 20 mM sodium phosphate buffer, pH 7.2 using Teflon homogenizer. The homogenized nucleus was centrifuged at 10000 × **g** for 20 min. The supernatant was decanted and stored at −20 °C until further use.

Quantification of early glycation products

Early glycation of the proteins from cortex and nucleus was determined colorimetrically using the periodate assay originally described by Gallop *et al.* [17], later modified by Ahmed and Furth [18]. Glycated protein reacts with periodate to release formaldehyde, which reacts with acetylacetone and ammonia to give 3,5-diacetyl 1,4-dihydrolutidine (DDL) a chromophore, which can be detected at 405 nm. Its concentration can be calculated from its molar extinction coefficient value of 7780. Early glycation products are expressed as nmol DDL per mg protein.

Quantification of advanced glycation products

Advanced glycation end-products (AGEs) or late glycation products were measured by their fluorescence at a wavelength of 440 nm (excitation at 370 nm) using a Perkin-Elmer 204-A spectro-fluorimeter, according to Van Boekel and Hoenders [19]. AGEs are expressed as fluorescence at 370 nm/440 nm per mg protein.

Determination of the ϵ-amino groups

The concentration of ϵ-amino groups in the soluble proteins of the cortex and the nucleus was determined according to the method of Eklund [20] using a 2, 4, 6-trinitro benzene sulfonic acid reagent (TNBS). The free ϵ-amino group content was calculated using the molar extinction coefficient value of 1.46×10^4 and the values are expressed as nmol per mg protein.

Determination of free and total sulfhydryl groups

Levels of free and total sulfhydryl groups in the soluble proteins of the cortex and the nucleus of the lens were determined according to the method of Hum and Augusteyn [21]. The disulfide bonds are calculated from the difference between total and free sulfhydryl groups.

Protein assays

Protein concentration was measured by Bradford method [22] using lyophilized human lens cortex or nucleus protein as standard.

Results

Ageing of the human lens, as characterized by an opacification associated with lens coloration [23], has been confirmed in our study and is also enhanced in diabetes. The colour of the lens was found to increase gradually from pale yellow to dark yellow in senile cataracts and from brown to dark brown in diabetic cataract. The extent of early and late glycation, free ϵ-amino groups content and disulfide content in the soluble proteins of the cortex and nucleus from senile and diabetic cataract lens is presented in Table 1.

The extent of formation of early glycation products in the lens proteins was determined by the DDL level. The

Table 1. Early and late glycation products, free ϵ-amino groups and disulfide bond concentration in the lenses of human senile and diabetic cataracts.

Group	*DDL content (nmol per mg protein)*	*AGE content (fluorescence per mg protein)*	*ϵ-amino group content (nmol per mg protein)*	*Disulfide bond content (nmol per mg protein)*
Senile cortex	3.44 ± 0.15	4.19 ± 1.60	651.0 ± 10.1	16.7 ± 0.88
Senile nucleus	5.73 ± 0.20	9.06 ± 3.23	480.2 ± 10.6	19.8 ± 1.26
Diabetic cortex	4.54 ± 0.42	7.13 ± 3.62	621.9 ± 18.7	25.0 ± 1.51
Diabetic nucleus	16.0 ± 0.20	37.5 ± 12.8	474.2 ± 14.0	28.2 ± 2.54

All the values are expressed as mean ± SD.
The following statistical comparisons were made using the student's t test; senile cortex vs senile nucleus, diabetic cortex versus diabetic nucleus, senile cortex versus diabetic cortex, and senile nucleus versus diabetic nucleus. All these comparisons were significant at a p value < 0.001 except for the AGE content of the senile and diabetic cortices where the p value < 0.01 and for the ϵ amino group content of the senile and diabetic nuclei where the p value > 0.05.
The age range of the senile ($n = 12$) and diabetic ($n = 12$) individuals was 55–75 years.

DDL content of cortex soluble proteins of the diabetic lens was increased when compared to the senile cortex ($p < 0.001$). Glycation of nuclear proteins in the diabetic lens was also greater than glycation of senile nuclear protein ($p < 0.001$). This was nearly a three-fold change.

The fluorescence of the nuclear proteins of the senile lens revealed a two-fold increase compared to the fluorescence from the senile cortex proteins ($p < 0.001$). Whereas, the nuclear protein of diabetic lens showed a five-fold increase compared to the diabetic cortex protein ($p < 0.001$).

The difference in fluorescence between the senile and the diabetic protein fractions is more pronounced (four-fold) when nuclear proteins are compared ($p < 0.001$). However, a smaller but still significant increase was observed in the fluorescence of the diabetic cortex fraction compared to the senile cortex fraction ($p < 0.01$).

The free ϵ-amino group content of senile nuclear proteins was significantly decreased compared to the senile cortex proteins ($p < 0.001$). Similarly, the free ϵ-amino group content in diabetic nuclear proteins was decreased when compared to the diabetic cortex proteins. However, the difference in the ϵ-amino group content of cortex or nuclear proteins was small when senile and diabetic individuals were compared.

The disulfide bonds formed in the soluble proteins of the nucleus were slightly increased compared to the cortex for both the senile and diabetic groups ($p < 0.001$). Whereas, the disulfide bond formation was much greater in the diabetic groups compared to their corresponding senile groups ($p < 0.001$).

Discussion

A possible role for glycation of lens proteins has been implicated in the increase in coloration of lens from the pale yellow lenses of the senile cataract patients to the dark brown lenses of diabetic cataract patients. This change in colour may reflect a higher level of fluorescence of proteins caused by glycation in senile and diabetic lens. Glycation of long-lived proteins such as collagen and lens proteins in diabetes and ageing has been recognized as a major post-translational modification process, leading to the cross-linking, aggregation and insolubilization of such proteins [23–26].

Cataract etiology and pathogenesis are still unclear. High levels of early as well as late glycated proteins in the nucleus of the senile lens, and to greater extent in the nucleus of diabetic lens suggest that glycation of lens proteins is age-related in senile cataract, and age and disease-related in diabetic cataract. These observations suggest that glycation may accelerate the development of cataract in diabetic, and to lesser extent in senile cataract patients, which is in agreement with the report of Abraham *et al.* [26].

Since glycation is a reaction between reducing sugars and free amino groups, a decrease in the ϵ-amino groups in the nucleus compared to the cortex in senile and diabetic individuals is probably related to the extent of glycation. Apart from glycation, other post-translation modifications such as deamination, oxidation and carbamylation may be involved in decreasing free ϵ-amino groups in the lens.

The involvement of disulfide bonds in the protein's cross-linking and the formation of high molecular weight protein aggregates in cataractous lens is well established [26, 27]. A gradual increase in disulfide bond formation in the cortex and nucleus of senile and diabetic cataract individuals, supports the hypothesis that glycation initiates protein conformational changes and unfolding, exposing free sulfhydryl groups to oxidation and leading to the formation of disulfide-linked aggregation [6]. Glycation may be a major factor leading to cataractogenesis [19, 28]. Further investigation of glycation in the human lens is needed in order to devise prevention strategies against cataract formation in man.

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