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Research Article

We have examined the nonenzymatic glycation of human lens crystallin, an extremely long-lived protein, from 16 normal human ocular lenses 0.2-99 yr of age, and from 11 diabetic lenses 52-82-yr-old. The glucitol-lysine (Glc-Lys) content of soluble and insoluble crystallin was determined after reduction with H-borohydride followed by acid hydrolysis, boronic acid affinity chromatography, and high pressure cation exchange chromatography. Normal lens crystallin, soluble and insoluble, had 0.028 +/- 0.011 nanomoles Glc-Lys per nanomole crystallin monomer. Soluble and insoluble crystallins had equivalent levels of glycation. The content of Glc-Lys in normal lens crystallin increased with age in a linear fashion. Thus, the nonenzymatic glycation of nondiabetic lens crystallin may be regarded as a biological clock. The diabetic lens crystallin samples (n = 11) had a higher content of Glc-Lys (0.070 +/- 0.034 nmol/nmol monomer). Over an age range comparable to that of the control samples, the diabetic crystallin samples contained about twice as much Glc-Lys. The Glc-Lys content of the diabetic lens crystallin samples did not increase with lens age.



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Nonenzymatic Glycation of Human Lens Crystallin

Effect of Aging and Diabetes Mellitus

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Introduction

The nonenzymatic glycation of a protein proceeds by the bimolecular condensation of a monosaccharide, usually glucose, with certain amino groups on the protein. The initial labile Schiff base adduct slowly rearranges to the stable ketoamine form (2-4). In the Maillard scheme of nonenzymatic glycation or browning, the glucose-amino acid product proceeds beyond the ketoamine to products that may include covalently cross-linked amino acids (4-5). The extent of nonenzymatic glycation either in vitro or in vivo is dependent upon the number of potentially reactive amino groups on the protein that are exposed to the solvent, the concentration of glucose in the surrounding medium, and the life span of the protein.

Among the many proteins that undergo nonenzymatic glycation, human hemoglobin (Hb)³ has received the most thorough scrutiny (5–10). This protein undergoes continuous modification during the red cell's 120-d lifespan (11), and is \sim 7.5% glycated (12, 13). The levels of glycated Hb are elevated in diabetics, and reflect the degree of control of blood sugar over the preceeding 120 d (14, 15). In contrast, human serum albumin (HSA) has a half-life of 19 d (16) and in normal individuals is \sim 7–12% glycated (17, 18). The levels of glycated HSA are also elevated in diabetics (17).

Human lens crystallin has a much longer life span than other proteins in the body. It is synthesized within the epithelial cells on the anterior and equatorial surfaces of the ocular lens which then divide, lose their nuclei and other organelles, and elongate, forming the fiber cells. Approximately 90% of the

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^{1.} The Joint Commission on Biochemical Nomenclature of the International Union of Biochemistry (IUB) and the International Union of Pure and Applied Chemistry (IUPAC) suggests the term "glycation" rather than "glycosylation" or "glucosylation" for the nonenzymatic reaction between glucose or other sugars, and free amino groups of proteins (1).

^{2.} The borohydride-reduced product of lysino-1-deoxyfructose (glucosyllysine) is a mixture of lysino-1-deoxysorbitol and lysino-1-deoxymannitol. The Durrum DC-6A cation exchange column does not separate these two epimers; they elute in one symmetrical peak. For simplicity, we refer to the mixture of these epimeric forms as glucitol-lysine throughout this paper. In like manner, the borohydride-reduced product of valyl-1-deoxyfructose (glucosyl-valine) is a mixture of valyl-1-deoxysorbitol and valyl-1-deoxymannitol, which also elutes as one peak on our column and is called glucitol-valine.

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^{3.} Abbreviations used in this paper: Hb, hemoglobin; HSA, human serum albumin; Glc-Lys, glucitol-lysine; Glc-Val, glucitol-valine.

soluble protein within the fiber cells is crystallin. The lens grows throughout life, building up layer upon layer of fiber cells around a central core, and never shedding the cells. The cells at the center, or nucleus, of the lens are thought to be as old as the organism itself. The proteins in the fiber cells do not turn over (or do so exceedingly slowly), so that some protein at the center of the lens is there for the lifetime of the individual (19–21).

In the present study we have accurately quantified the extent of nonenzymatic glycation of the long-lived lens crystallin, have identified lysine as the favored residue for glycation, and have examined the effects of aging and of diabetes mellitus.

Methods

27 human ocular lenses were obtained from the National Diabetic Research Interchange (Philadelphia, PA) from 11 diabetic and 16 nondiabetic donors (aged 0.2–99 yr). The diagnosis of diabetes was clearly established in the diabetic group. The lenses were decapsulated and homogenized in 3.0 ml of 0.1 M potassium phosphate, pH 8.70. The homogenates were stored at -20° C before analysis. Because the lenses were received frozen, it was not possible to define the presence and/or degree of cataract formation.

The frozen lens homogenates were thawed on ice, and an aliquot from each specimen was centrifuged at 10,000 g for 20 min at 4°C. The soluble protein fraction was transferred to another tube, and the insoluble lens fraction was washed three times with ice-cold 0.1 M potassium phosphate, pH 9.0, followed by centrifugation at 10,000 g for 20 min. The protein concentration of each soluble and insoluble sample was determined as described (22), using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a protein standard.

2 mg of each soluble and insoluble lens crystallin sample was dissolved in 0.5 ml of 0.1 M potassium phosphate, pH 9.0, and immersed in boiling water for 5 min to denature proteolytic enzymes. The samples were made 10 M in urea (Pierce Sequanal grade) which had been deionized by treatment with Rexyn 300 (Fisher Scientific Co., Medford, MA) mixed-bed ion exchange resin, and then diluted to 6 M in urea with a final potassium phosphate concentration of 0.1 M, pH 9.0. The protein samples were incubated for 24 h, 4°C, with a 100-fold molar excess of Na-3H-borohydride (New England Nuclear, Boston, MA), originally 340 mCi/mmol, which had been diluted with nonradioactive NaBH₄ (Sigma Chemical Co.) to a specific radioactivity of 100 mCi/mmol. A polypeptide chain molecular weight of 20,000 was assumed for the lens crystallin monomers (19-21). A sample containing 1.0 mg of human Hb fraction A1c was also reacted with ³H-borohydride under the same conditions (molecular weight of Hb A_{1c} dimer equals 31,000). The Hb A_{1c} had been prepared by BioRex 70 (Bio-Rad Laboratories, Richmond, CA) cation exchange chromatography followed by Glyco-Gel B boronic acid affinity chromatography (13). The specific reducing equivalence of the ³H-borohydride solution was quantified by stoichiometric reduction of a molar excess of nicotinamide adenine dinucleotide (NAD+, Sigma Chemical Co.) and was monitored by absorbance at 340 nm (23). The ³H-NADH formed was isolated by chromatography on DEAE-Sephadex (24), and the specific activity was determined from analysis of its absorbance and ³H-radioactivity.

The borohydride reaction was stopped by acidification with 2 ml of 2 M sodium acetate, pH 5.0. The samples were dialyzed versus

numerous changes of deionized water at 4° C for 3 d and lyophilized. The lyophilized ³H-borohydride-labeled protein samples were resuspended in 2.0 ml deionized water, and two 0.1-ml aliquots were removed from each sample for analysis of the protein concentration (22). To the remainder of each protein sample was added 1.8 ml Ultrex brand ultrapure concentrated HCl (J. T. Baker Chemical Co., Phillipsburg, NJ), and the samples were subjected to hydrolysis at 105°C for 24 h in vacuo, after which the hydrolysates were lyophilized.

The dried hydrolysates were resuspended in 5.0 ml deionized water and passed through 0.2- μ m filters. A small aliquot of filtrate was taken for radioactivity analysis, and the remainder was subjected to boronic acid affinity chromatography on 1 \times 12-cm columns of Affi-Gel 601 (Bio-Rad Laboratories, Richmond, CA) essentially as described (13, 18, 25). The amino acids were applied in 0.25 M ammonium acetate, pH 9.0, and the nonglycated amino acids were eluted from the column in this buffer. The bound glycated amino acids were eluted with 0.5 M acetic acid. The columns were run at 11 ml/h at room temperature. The glycated amino acid fractions from Affi-Gel 601 were dried under nitrogen, and stored at -20° C.

The glycated amino acids were dissolved in 0.2 M pyridine-acetate, pH 3.1, and were pooled into the following fractions: experiment 1: glycated amino acids from soluble nondiabetic crystallin; glycated amino acids from insoluble nondiabetic crystallin; glycated amino acids from diabetic soluble crystallin; and glycated amino acids from diabetic insoluble crystallin; experiment 2: glycated amino acids from soluble nondiabetic crystallin; and glycated amino acids from insoluble nondiabetic crystallin. In experiment 2 the glycated amino acids from the soluble and insoluble crystallin of one nondiabetic 99-vr-old donor were not pooled with samples from the younger donors, but were chromatographed individually. An aliquot from each pool was subjected to Durrum DC-6A (Dionex, Sunnyvale, CA) high pressure cation exchange chromatography as described (13, 18). The elution positions of glucitol-valine (Glc-Val) and Glc-Lys were determined with the use of radiolabeled standards that were prepared in this laboratory (13, 18). The structure of the Glc-Lys standard was verified by mass spectrometry.

Results

The specific radioactivities of the hydrolysates of lens crystallin samples are shown in Table I. An aliquot of each hydrolysate was analyzed by Affi-Gel 601 boronate affinity chromatography (Fig. 1). The percentage of ³H-radioactivity recovered from each chromatographic run approached 100%. Unbound and bound fractions from Affi-Gel 601 were dried under N₂, resuspended in starting buffer, and rechromatographed on this affinity gel. For the unbound fraction, 95.9–100% of the radioactivity remained unbound upon rechromatography (mean = 98.8%, n = 4). For the bound fraction, 94.4–97.6% of the radioactivity was again bound upon rechromatography (mean = 96.2, n = 3).

In order to convert CPM/mg to nanomoles Glc-Lys per nanomole lens protein, the following assumptions were made: the average molecular weight of the lens crystallin polypeptides is 20,000 (19–21); the ketone functions of glucosyl-valine in Hb A_{1c} and glucosyl-lysine in crystallin label with ³H-BH₄ with equal efficiency. The reducing capacity of the unlabeled borohydride was $98\pm2\%$ as measured colorimetrically by the

Sample*	Age and sex	Soluble or insoluble	Exp. #	Specific radioactivity (cpm/mg)‡	Percentage of radioactivity bound to Affi-Gel 601	Nanomoles glucitol- lysine per nanomole crystallin monomer
NI	0.2, M	I	2	106,000	13.4	0.016
N1	0.2, M	S	2	138,000	7.3	0.009
N2	18, F	I	2	174,000	9.3	0.018
N2	18, F	S	2	129,000	19.6	0.023
N3	19. M	I	2	199.000	8.9	0.020
N3	19, M	S	2	138,000	21.1	0.026
N4	20 F	T	2	191.000	12.1	0.026
N4	20, F	S	2	135,000	16.2	0.020
N5	21 F	T	2	158.000	13.6	0.024
N5	21, T 21, F	S	2	98,700	9.9	0.009
N6	22 M	T	2	83 400	13.0	0.013
N6	22, M 22, M	S	2	134,000	15.0	0.013
N/7	, 66 M	Ţ	1	60,600	26 4	0.022
N7 N7	50, M 56 M	I S	1	53.000	30.4 49.6	0.025
	<i></i>			75,000	22.7	0.010
N8 N8	66, M 66, M	l S	1	75,900	22.1 AA 7	0.018
140	00, 141	5	1	41,500		0.010
N9	68, F	I	1	66,900	44.9	0.032
INY	08, F	3	I	00,800	30.2	0.030
N10	69, M	I	1	88,300	35.3	0.033
N10	69, M	8	1	63,600	40.3	0.025
N11	70, F	Ι	1	70,700	36.9	0.028
N11	70, F	S	1	80,500	49.2	0.039
N12	71, F	I	1	122,000	30.7	0.040
N12	71, F	S	1	109,000	36.3	0.039
N13	73, F	I	1	73,200	45.0	0.035
N13	73, F	S	1	76,400	52.4	0.040
N14	76, F	I	1	122,000	44.3	0.057
N14	76, F	S	1	71,000	47.5	0.033
N15	83, F	I	1	106,000	33.1	0.038
N15	83, F	S	1	74,100	47.1	0.034
N16	99. M	I	2	129,000	31.5	0.047
N16	99, M	S	2	129,000	28.2	0.037
וח	52. M	I	1	65,700	47.3	0.039
DI	52, M	S	1	67,600	33.0	0.028
D2	52 M	T	1	127.000	33.0	0.053
D2	52, M	S	1	78,500	41.0	0.040
D3	57 M	T	1	175.000	59.0	0.130
D3	57, M	S	1	130,000	60.6	0.100
ри	66 M	T	1	144.000	54.3	0.100
D4	66, M	S	1	133,000	69.4	0.116

Table I. Glycated Amino Acid Content of Crystallin

Table I.	(Continued)
I GOIC I.	(Comment)

Sample*	Age and sex	Soluble or insoluble	Exp. #	Specific radioactivity (<i>cpm/mg</i>)‡	Percentage of radioactivity bound to Affi-Gel 601	Nanomoles glucitol- lysine per nanomole crystallin monomer
D5	68, M	I	1	121,000	48.7	0.075
D5	68, M	S	1	86,400	57.0	0.061
D6	69, M	I	1	123,000	47.6	0.074
D6	69, M	S	1	95,700	66.3	0.079
D7	70, F	I	1	70,700	36.0	0.032
D7	70, F	S	1	54,700	46.9	0.032
D8	71, F	I	1	156,000	59.2	0.117
D8	71, F	S	1	109,000	72.1	0.098
D9	73, F	I	1	80,500	30.8	0.031
D9	73, F	S	1	52,100	35.1	0.023
D10	76, M	I	1	85,400	51.9	0.055
D10	76, M	S	1	73,700	56.9	0.052
DH	82, F	I	1	151,000	57.5	0.111
D11	82, F	S	1	117,000	69.7	0.102

* The designation N indicates nondiabetic lens crystallin samples; D indicates diabetic samples. ‡ The specific radioactivities of the lens crystallin hydrolysates were determined in the following manner. The protein concentration of an aliquot of lens crystallin was determined before hydrolyzing the sample in the same test tube. After hydrolysis and filtration, an aliquot was taken for radioactivity analysis. The specific radioactivity of each sample represents the radioactivity of each hydrolysate divided by the protein concentration, in milligrams, of the original crystallin sample.

reduction of NAD⁺ to NADH. In contrast, the reducing capacity of the ³H-labeled borohydride was only 60–70% measured as the specific radioactivity of purified ³H-NADH. These data indicate that the specific radioactivity of the ³H-borohydride was lower than specified, possibly due to degradation or hydrolysis of the ³H-borohydride during storage or during the labeling reaction, contamination with other ³H-labeled compounds, or to an isotope effect. For this reason we used Hb A_{1c} as an internal standard for the ³H-borohydride reduction of lens crystallin. 1 mg Hb A_{1c} contains 32.2 nmol dimer and thus 32.2 nmol Glc-Val.

The specific radioactivities of the Hb A_{1c} hydrolysates from two BH₄ reductions were 807,000 cpm/mg and 1,060,000 cpm/mg, respectively. When acid hydrolysates from the two Hb A_{1c} samples were analyzed by Affi-Gel 601 boronic acid affinity chromatography, the adherence of radioactivity was 53 and 39%, respectively. Thus, the specific radioactivities of the glycated amino acids in the Hb A_{1c} hydrolysates were 426,000 cpm/mg and 418,000 cpm/mg. When the portion of each Hb A_{1c} hydrolysate which adhered to Affi-Gel 601 was subjected to high pressure cation exchange chromatography on Durrum DC-6A resin, 93.8 and 98.2% of the radioactivity was recovered, and 80.6 and 84.6% of the counts eluted as Glc-Val (Fig. 2). Thus the corrected specific radioactivity of the first sample of Hb A_{1c} was 426,000 cpm/mg \times 0.806 or 340,000 cpm/mg; the radioactivity due to Glc-Val was 10,700 cpm/nmol Glc-Val. The corresponding value for the second sample of Hb A_{1c} was 11,000 cpm/nmol Glc-Val.

To help insure that the glycated amino acids were accessible to ${}^{3}\text{H-BH}_{4}$, we denatured and solubilized the crystallin samples in 10 M urea, and then diluted this to 6 M in urea before the borohydride reaction. In addition, the intra- and inter-chain disulfide linkages in lens crystallin would be reduced to sulfhydryl groups by the borohydride (26).

The fractions of the lens crystallin hydrolysates which adhered to Affi-Gel 601 were also chromatographed on the DC-6A cation exchange column (Fig. 3). Since there was not enough radioactivity in each sample to individually chromatograph the samples on Durrum DC-6A, the samples were pooled as indicated in Methods. In eight analyses, 89-103%of the radioactivity was recovered (average equals $95.1\pm4.7\%$). In four pooled samples of boronate-bound amino acids from insoluble crystallin $62.6\pm3.9\%$ of the recovered radioactivity was Glc-Lys. The corresponding value for four pooled samples of boronate-bound amino acids from soluble crystallin was $56.6\pm6.5\%$. As shown in Fig. 3, Glc-Lys was the major labeled peak. A considerably smaller peak eluted in the column void volume. The percentage of radioactivity in each pool which



Figure 1. Boronic acid affinity chromatography of an acid hydrolysate of ³Hborohydride-reduced crystallin on Affi-Gel 601. The acid hydrolysates were applied to a 1×12 -cm column of Affi-Gel 601 in 0.25 M ammonium acetate. pH 9.0, and the nonadherent amino acids were eluted in this starting buffer. The adherent fraction, which contained the glycated amino acids, was eluted with 0.5 M acetic acid

eluted as Glc-Lys was multiplied times the specific radioactivity of the lens crystallin hydrolysate which was boronate bound to give the specific radioactivity, in CPM per milligram lens hydrolysate due to Glc-Lys.

The specific radioactivities due to Glc-Lys for the crystallin hydrolysates (cpm/mg) were multiplied by 0.02 to give cpm/ nmol lens crystallin due to Glc-Lys. The values for cpm/nmol due to Glc-Lys (in lens crystallin) were then divided by cpm/ nmol Glc-Val in Hb A_{1c} (10,700 cpm/nmol in experiment 1 and 11,000 cpm/nmol Glc-Val in experiment 2). These values for nmoles Glc-Lys per nanomole lens crystallin monomer are shown in Table I. They range from 0.009–0.057 nmol Glc-Lys per nanomole crystallin monomer in the normal lens crystallin samples, and 0.023–0.130 nmol Glc-Lys per nanomole diabetic lens crystallin monomer. In Fig. 4, nanomoles Glc-



Figure 2. Analysis of the boronate-adherent amino acids from an acid hydrolysate of ³H-borohydride-reduced Hb A_{1c}. The acid hydrolysate was first chromatographed on Affi-Gel 601, and the adherent amino acids were chromatographed on a 0.9 \times 20-cm column of Durrum DC-6A cation exchange resin using a gradient of 0.2 M pyridinium acetate, pH 3.1, to 1.5 M pyridinium acetate, pH 5.0, at 55°C and 67 ml/h. The elution positions at Glc-Lys and Glc-Val were deter-

mined by chromatographing synthetic standards that were prepared in this laboratory. Of the ³H-radioactivity that was applied to the column, 98% was recovered.



Lys/nmol lens crystallin monomer is plotted versus lens age. For soluble plus insoluble lens crystallin from normal individuals there is a positive slope (Y = 0.00029X + 0.0130) with a correlation coefficient of 0.77, which indicates that the content of Glc-Lys in lens crystallin increases with age. There was no significant difference in Glc-Lys content between soluble and insoluble crystallin samples. The Glc-Lys content of diabetic samples was approximately twofold higher than that of age-



Figure 4. Content of Glc-Lys in crystallin samples versus age of the lens. The content of Glc-Lys in crystallin hydrolysates, as nanomoles Glc-Lys per nanomole crystallin monomer, is plotted vs. age (in years) of each lens. A molecular weight of 20,000 was assumed for the crystallin monomer in order to make the calculations. Linear regression analysis was performed on all four sets of samples. The line that is drawn represents linear regression analysis of hydrolysates from soluble and insoluble crystallin from normal lenses. The formula that describes this line is F(X) = 0.00029X + 0.013, with a coefficient of correlation equal to 0.766. The formula for just the soluble samples is F(X) = 0.00027X + 0.013, with r = 0.790. The formula for insoluble samples only is F(X) = 0.00031X + 0.013, r = 0.751.

matched groups of normal samples. In contrast to the normal samples, we found no significant correlation between the Glc-Lys content of diabetic samples with age (r = 0.178).

Discussion

Because of its remarkable longevity, human lens crystallin undergoes a number of age-related, post-translational modifications in vivo (19–21). Among these are formation of disulfide bonds and other covalent crosslinks; accumulation of high molecular weight aggregates; deamidation of asparagine and glutamine residues; racemization of aspartic acid residues; partial proteolysis of polypeptide chains at characteristic sites; photo-oxidation of tryptophan; and nonenzymatic glycation of lysine (19–21, 27). Some of these post-translational events contribute to the increasing amount of insoluble lens crystallin during aging. In human lenses over 60 yr of age, 40-55% of the crystallin is in the insoluble fraction (19–21, 28).

Our study provides an unambiguous demonstration of nonenzymatic glycation of human lens crystallins and permits accurate quantitation of the extent of this modification as affected by aging and diabetes. Previous analyses of human (29) and rat (30) lens crystallins were hampered by inadequate resolution of glycated amino acids and lack of stoichiometric quantitation. Among the nondiabetic samples that we analyzed, the average amount of glycation was 0.028 nmol Glc-Lys per nanomole crystallin monomer. According to linear regression analysis in Fig. 4, the content of crystallin monomers containing Glc-Lys increases from 1.3% in an infant to 2.7% at 50 yr of age, and to 4.2% at 100 yr of age. An age dependent increase in nonenzymatic glycation has also been demonstrated in human skin collagen (31), which is another protein that turns over very slowly.

The amount of glycation of crystallin from diabetic lenses was twofold higher than that of normals. Qualitative data have been reported previously for diabetic human (29, 32, 33) and rat (30) crystallins. In the present study, the content of glucosyl-lysine in diabetic lenses ranged from 2.3-13.0% of crystallin monomers and average 7.0%. That a number of diabetics had unexpectedly high levels of glycated crystallin (four times age matched controls) may reflect alteration in the folding of the protein which leads to enhanced exposure of lysine residues to glucose.

There is no correlation between Glc-Lys content and lens age for the diabetic samples. This is not surprising, in view of the fact that the patients have had diabetes for varying lengths of time, and have had varying levels of control of blood sugar. The inability to detect an age effect of glycation in the diabetic lens crystallin may also be due to a relatively small age range (52-82 y) in our study.

Human crystallin accumulates during the lifetime of the individual, and yet contains only 2.7% Glc-Lys after 50 yr of glucose exposure. In contrast, HSA has a half-life of circulation of 19 d (16) and is 7-12\% glycated (17, 18), while human Hb

has a lifespan of 120 d and is 7.5% glycated (12, 13). This very low rate of glycation can be viewed as molecular adaptation. If crystallin had lysine residues as reactive as those of Hb or albumin they would be fully glycated, with probable adverse functional consequences.

A number of factors may contribute to the remarkably slow rate of nonenzymatic glycation of crystallin. Like the red cell, the ocular lens does not require insulin for the inward transport of glucose. The interior of the lens has a glucose concentration of 1 mM in normal individuals (34) which is approximately one-sixth that of the blood plasma or inside the red cell (35). Whereas human Hb and serum albumin are globular proteins that contain a high content of alpha helical structure, the lens crystallins contain almost no alpha helix but have a high content of beta pleated sheet structure (36-42). Human Hb and serum albumin are free to rotate in their respective fluid media, while evidence from Raman spectroscopy suggests that crystallin may be packed in a more structured array, with the β -sheets oriented orthogonal to the lens optic axis, and thus lacking free rotational or translational movement (43). Therefore, the tightness of packing and the constraint placed on its movement may make crystallin less available for nonenzymatic glycation. A third factor that lowers the rate of nonenzymatic glycation of crystallins is the fact that the lysine content of alpha, beta, and gamma crystallins is lower than that of either human Hb or HSA (19, 44-49). Gamma crystallin contains 1.2% lysine (two lysine residues per 165 total amino acids) (47), the α crystallin A and B chains have 4.1 and 5.7% lysine (44, 45), and the crystallin β -chain has 6.4% lysine (46). In contrast, human Hb has 7.6% lysine (48), and HSA is 10.1% lysine (49). Furthermore, in glycation of Hb and albumin, certain lysine residues are favored over others (18, 50). Hemoglobin β -Lys-66 and α -Lys-61, and serum albumin Lys-525, which are the principal lysine residues modified, are all the second or carboxyl lysines of Lys-Lys pairs. The sites of nonenzymatic glycation of crystallin chains are not yet known. However, the B subunit of α crystallin has one Lys-Lys pair at the -COOH terminus (45), while the α crystallin A chain lacks a Lys-Lys pair (44). While the human beta and gamma crystallin chains have not been sequenced, the bovine gamma chain lacks a Lys-Lys pair (47), while the bovine beta chain has one Lys-Lys pair near the middle of the molecule (46).

In the nondiabetic and diabetic samples, the Glc-Lys content is approximately the same in soluble and insoluble lens protein fractions. This finding is consistent with that of Pande et al. (29), who used ³H-borohydride reduction to show no difference in ketoamine-linked hexose in soluble and insoluble protein fractions from normal human lenses, as well as from senile cataractous lenses with varying degrees of opacity. These findings are in contrast to human skin collagen in which more ketoamine-linked glucose is bound to the insoluble than the soluble protein fraction (31).

It is unknown at present how or if nonenzymatic glycation

contributes to senile or diabetic cataract formation. Perhaps the development of senile or diabetic cataracts is not dependent upon the accumulation of ketoamine-linked glucose residues, but may depend in part upon glycation-induced cross-links. Monnier and Cerami (51) have incubated bovine lens crystallin for 10 mo with either glucose or glucose-6-phosphate under physiological conditions and have produced covalently crosslinked crystallin which contains fluorescent vellow-brown pigments. The fluorescence spectrum of these glycated samples matched that of digests of human cataractous lens crystallin (51). These non-disulfide bonded cross-links are thought to be due to mellanoidins, which are yellow-brown and probably result from the condensation of protein amino groups with reactive carbonyl compounds from products of the Maillard reaction scheme (51, 52). In a simpler model system, ribonuclease A was found to polymerize when incubated with glucose in vitro (53). The polymerization was caused by the condensation of a glycated amino acid on the RNase molecule with a free amino group on the other.

In order to properly quantify the entire amount of nonenzymatic glycation of human lens crystallin, the Maillard products will first have to be isolated, identified, and quantified. Because of its extreme longevity in vivo, crystallin is an ideal protein with which to investigate the formation of Maillard products in vivo.

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