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PIGMENTATION AND ENZYMIC INHIBITION BY CARBONYL COMPOUNDS IN THE RABBIT LENS*

THOMAS G. SCHARFF and OCIA L. MONTGOMERY

Departments of Pharmacology and Ophthalmology, University of Louisville, Schools of Dentistry and Medicine, Health Sciences Center, Louisville, Ky. 40201, U.S.A.

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Abstract—When certain α-hydroxycarbonyl or dicarbonyl compounds were added to cultured rabbit lenses, changes occurred in enzymic activity, color and fluorescence. These changes resemble the decreased metabolism and the development of color and fluorescence that is seen in some aged human lenses. One representative compound, glycolaldehyde, inhibited activity of at least one enzyme (aldolase). When added to lysine, to muscle aldolase, to dialyzed lens homogenates or to intact cultured rabbit lenses, glycolaldehyde elicited U.V.-fluorescence and color, the latter ranging progressively from yellow to brown or even to black. Glycolaldehyde in solution with lysine at pH 8 formed a 1–1 adduct. Through subsequent reactions of the Maillard type there occurred further changes yielding a progression of color similar to that seen in glycolaldehyde-treated lenses or in colored human lenses. Other α-hydroxycarbonyl compounds or dicarbonyls which are inhibitory to metabolism in the lens (T. G. Scharff and O. L. Montgomery, *Proc. Soc. exp. Biol. Med.* **134,** 658 1970) also probably underwent Maillard-type reactions with amino acids in solution to form similarly colored, fluorescent products with muscle aldolase, dialyzed lens homogenates or intact lenses. It is possible that colored Maillard-type complexes between unidentified carbonyl compounds and free or protein-incorporated amino acids may be involved in reduced metabolism and in color development in the aged human lens or other pigmented, aging tissues.

VARIOUS tissues are known to become progressively more pigmented as they age,[1–3] with color development ranging from yellow to dark brown. More specifically, lenses from a number of vertebrates exhibit increased coloration and decreased metabolism during aging[4,5] or as a result of the introduction of certain poisons.[6–8] Most of these lens pigments have not been identified, primarily because they are present in such small quantities.[9]

Recently we found[10] that incubation of young rabbit lenses in a meduim containing glycolaldehyde led to reduced glycolysis rates and reduced content of high-energy phosphate compounds. Methylglyoxal, glyoxal, and DL-glyceraldehyde also inhibited glycolysis.

In the present report we describe the appearance and progression of color and fluorescence in rabbit lens systems exposed to the above inhibitory carbonyl compounds. Similarities are seen between carbonyl-treated rabbit lens systems and pigmentation developed in aged human lenses. Evidence will be presented that the complex of reactions called the "browning" reaction (or "Maillard" reaction)[11,12] may be implicated in the carbonyl-treated rabbit lens and possibly in the aging human

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lens. The Maillard reaction is really a complex of reactions in which initial formation of a 1–1 adduct between amino groups and certain carbonyl groups is followed by a number of ill-defined rearrangements, unsaturations, cyclizations, cleavages and polymerizations.[11–13]

Most of the experiments presented in this report were done using glycolaldehyde as a prototype.

METHODS

Experiments were done on: (a) intact cultured rabbit lenses,[10] (b) rabbit lens homogenates prepared from frozen lenses (Pel-Freez, Inc., Rogers, Ark.) thawed and homogenized in glass and used as described earlier,[10] (c) purified crystalline rabbit muscle aldolase (Sigma Chemical Co.), and (d) amino acid-carbonyl compounds in solution. Studies on homogenates and enzymes were carried out at 30° or 37° in 0·154 M KCl–0·154 M $NaHCO_3$ under 5% CO_2–95% N_2 or in buffers under air. Intact lenses were incubated in rabbit Ringer's solution.[10]

$Ba(OH)_2$–$ZnSO_4$ extracts of lens homogenates were used (14 lenses weighing 200–350 mg/lens in 4 ml of KCl–$NaHCO_3$ solution) in the determination of lactic acid via a chemical method.[14]

Dialyzed lens homogenates were used in the determinations of lens aldolase activity and color and were prepared by homogenizing four lenses in 2 ml of KCl–$NaHCO_3$ and dialyzing in the cold against 0·85% saline.

Lens or muscle aldolase activity (fructose-1,6-diphosphate substrate) was measured through the coupled oxidation of reduced nicotinamide dinucleotide (NADH)* in excess α-glycerophosphate dehydrogenase (Sigma Chemical Co.). In order to preclude further glycolysis of triosephosphate formed in the presence of aldolase in homogenates, aldolase activity was measured after incubation of homogenate in 1 mM iodoacetate for 30 min. In the test of aldolase activity the coupled glycerophosphate dehydrogenase activity did not become rate-limiting in the presence of glycolaldehyde, propionaldehyde or iodoacetate. Glycolaldehyde could reduce oxidized NAD^+ in the presence of α-glycerophosphate dehydrogenase and affect slightly the net conversion of NADH to NAD^+ in the measurement of aldolase activity. Correction was made for this.

Osmolarity changes were measured in a Fiske osmometer. Loss of amino groups was measured by change in pH.[15] Other details of procedure will be seen in the text and illustrations.

RESULTS

Inhibition of glycolysis by glycolaldehyde in lens homogenates

To determine where in the glycolytic pathway glycolaldehyde (as a representative) might be active, two glycolytic intermediates were used in inhibition tests. Results are seen in Table 1. Inhibition of glycolysis of HDP by 20 mM glycolaldehyde was about 62 per cent. Glycolysis of 3-PGA was not inhibited but showed a 62 per cent stimulation in the presence of glycolaldehyde. This stimulation was due to the above-mentioned ability of glycolaldehyde to recycle NAD^+ in the presence of α-glycero-

* Additional abbreviations used: NAD^+, nicotinamide adenine dinucleotide; dHAP, dihydroxyacetone phosphate; α-GP, α-glycerophosphate; HDP, fructose-1,6-diphosphate; 3-PGA, 3-phosphoglycerate; G-3-P, glyceraldehyde-3-phosphate.

TABLE 1. EFFECTS OF GLYCOLALDEHYDE ON LACTIC ACID PRODUCTION FROM DIFFERENT SUBSTRATES IN RABBIT LENS HOMOGENATES*

Substrate	Lactic acid formed (μmoles/ml homogenate)	Inhibition by inhibitor (%)
0·005 M fructose-1,6-diphosphate	2·76 ± 0·23† (16)‡	
0·005 M fructose-1,6-diphosphate plus 0·02 M glycolaldehyde	1·06 ± 0·09 (16)	+ 62
0·010 M 3-phosphoglyceric acid	1·04 ± 0·09 (9)	
0·010 M 3-phosphoglyceric acid plus 0·02 M glycolaldehyde	1·68 ± 0·26 (9)	− 62

* Samples for lactic acid analyses were taken upon addition of substrate and again after incubation at pH 7·8–8·2 and 37° for 4 hr under 5% CO_2–95% N_2.

† Standard error of the mean.

‡ Numbers in parentheses indicate number of homogenates tested.

phosphate dehydrogenase. Although the stimulatory effect of glycolaldehyde might be masking a direct inhibitory effect of this compound in glycolysis, the higher rate of lactic acid production from 3-PGA (1·68 μmoles/ml) than from HDP (1·06 μmoles/ml) in the presence of glycolaldehyde indicated that some of the inhibition by glycolaldehyde occurred in the region of the aldolase and 3-phosphoglycerokinase reactions.

Inhibition by glycolaldehyde of crystalline rabbit muscle aldolase and of aldolase activity in lens homogenates

Rabbit muscle aldolase contains in its active centers lysine residues which form Schiff bases with substrates.[16] Carbonyl substances might inhibit this and other enzymes which form Schiff bases[17] by interfering with formation of the normal Schiff base. Aldolase activity therefore was tested in the presence and absence of glycolaldehyde. Figure 1 shows that muscle aldolase was inhibited 51 per cent (tangents to steepest portion of curves) in the presence of 10 mM glycolaldehyde. Similarly, lens aldolase activity in homogenates was inhibited 46 per cent by glycolaldehyde. Propionaldehyde also slightly inhibited muscle aldolase (data not shown). (Spolter *et al.*[18] found propionaldehyde under different conditions to be noninhibitory to aldolase.)

Tests of the reversibility of the inhibition of muscle and lens aldolases by glycolaldehyde were run on aliquots of the two preparations preincubated and tested in 10 mM glycolaldehyde. At zero time HDP was added in each case to a concentration of 0·2 mM, and activity was measured until constant rate was reached (Figs. 2 and 3). HDP was then again added in small volume to give final concentrations as shown. Figure 2 shows that 0·2 mM HDP (no increase) and 0·3 mM HDP (50 per cent increase) resulted in little or no increase in aldolase activity; with HDP added to 1·0 mM, however, the rate was increased 2·3 times to essentially the uninhibited rate. Similarly, in homogenates (Fig. 3), increase of HDP to 1·0 mM increased the rate to 2·4 times the inhibited rate. We concluded that the early inhibition of both lens and muscle aldolases by glycolaldehyde was reversible.

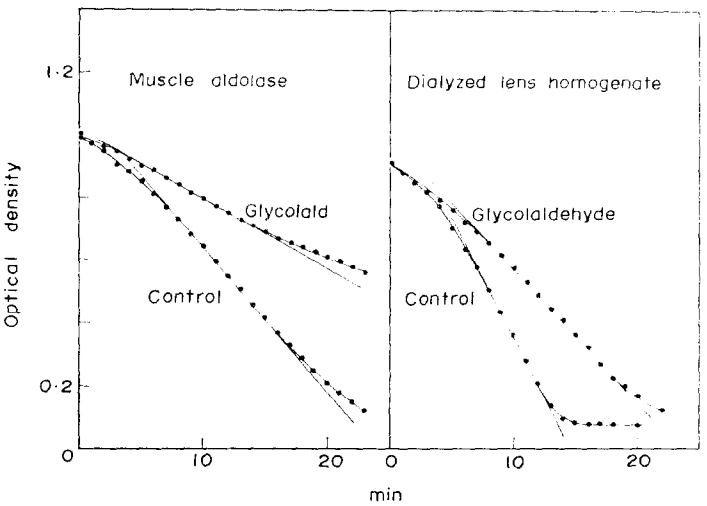

FIG. 1. Inhibition of muscle and lens aldolases by glycolaldehyde. Each reaction mixture contained in a total volume of 2·3 ml the following: 0·2 mM HDP, 0 or 10 mM glycolaldehyde, 0·2 mg of α-glycerophosphate dehydrogenase (approximately 95 units/mg), 0·5 mg DPNH, 1 μg rabbit muscle aldolase (or 0·2 ml of 25 × diluted dialyzed homogenate in 1 mM iodoacetate) in 0·15 M KCl–0·15 M $NaHCO_3$. Substrate was added at zero time. Where glycolaldehyde was used, the enzyme or homogenate was preincubated with the inhibitor 15 min prior to addition of HDP.

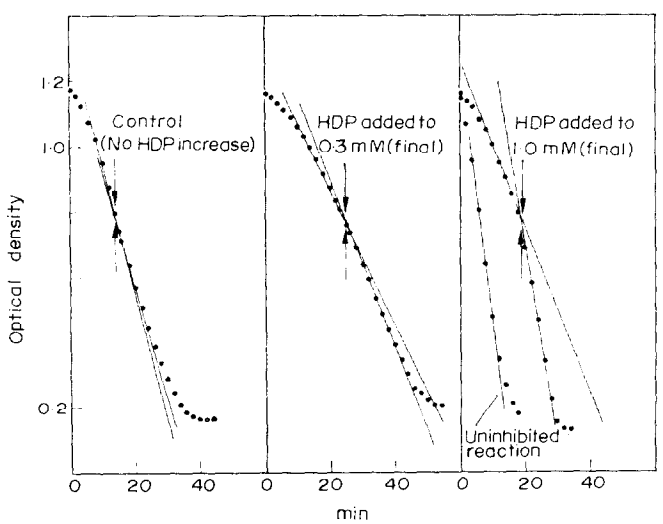

FIG. 2. Reversibility of the inhibition of purified rabbit muscle aldolase. Conditions were those given in legend to Fig. 1, except that at optical density of 0·80, HDP in one twenty-fourth of the volume was added to give the final concentrations shown in the figure. Glycolaldehyde (10 mM) was present in all reactions.

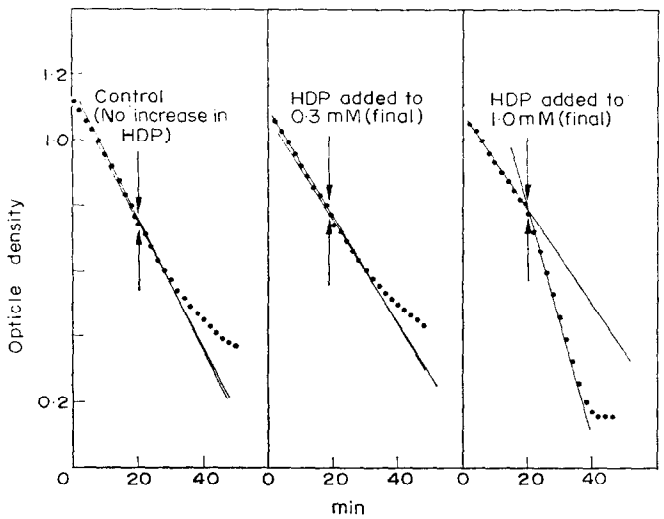

FIG. 3. Reversibility of the inhibition of aldolase in dialyzed rabbit lens homogenates. Conditions were those shown in Fig. 2, except that homogenate and 1 mM iodoacetate replaced purified muscle aldolase.

Color reactions and formation of carbonyl-amino complexes

In addition to its inhibitory effects on metabolism[10] and on aldolase activity, glycolaldehyde produced color in intact lenses, lens homogenates and purified rabbit muscle aldolase preparations. Since it is known that (a) the ε-amino group of lysine at the active centers of aldolase forms a Schiff base with substrate,[16] (b) glycolaldehyde (from the present study) inhibits and colors aldolase and (c) carbonyl compounds may produce color when reacted with protein or amino acids in solution,[13] it appeared that lysine–glycolaldehyde mixtures in solution might lead to color formation. This indeed occurred. Results follow on all three preparations treated with carbonyl compounds—lens homogenates, purified muscle aldolase and amino acids.

Aldehyde-amino acid reaction in solution—evidence for Maillard-type reactions. Although we did not find reports of a specific glycolaldehyde–lysine reaction in the literature, amino acids and α-hydroxycarbonyl or dicarbonyl compounds are known to undergo the Maillard complex of reactions,[11,12] forming initially colorless glycosylamines, the latter then undergoing further reactions leading to development of color and fluorescence to U.V.-light. Using the glycolaldehyde–lysine reaction as prototype, we obtained the following evidence that the reaction is of the Maillard type: (1) Incubation of glycolaldehyde and lysine together resulted in the appearance of color ranging from pale yellow to rust brown or even to black (Figs. 4 and 5); the Maillard reaction shows a similar progression of color.[11] In part a of Fig. 4 is shown the visible absorption spectrum for the lysine–glycolaldehyde solution. The solution was colorless initially, but with increasing time, absorption increased, especially in the blue–violet region. The data are replotted in Fig. 4b to show rough comparative rates of change of absorbance at different wavelengths. The rate of increase in absorption with time at 400 nm was roughly 100 times that seen at 700 nm. The actual color of

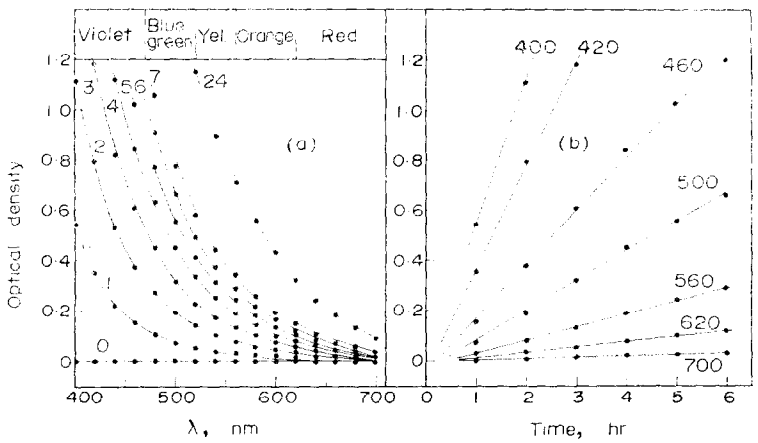

FIG. 4. Change in absorbance at different wavelengths and times for lysine–glycolaldehyde. Conditions: 12 mM glycolaldehyde–12 mM lysine, incubated at pH 8·0 and 37°. (a) Numbers in graph refer to times (in hr) of incubation; (b) Data are replotted for particular wavelengths. Numbers refer to wavelengths. Approximate ranges of wavelengths for visible colors are seen above the graph of A. Light path, 1·6 cm.

the transmitted light was shifted with time toward the red end of the spectrum. This shift could account for the typical Maillard-type progressive change from water-clear to yellow to brown to black. Lysine or glycolaldehyde incubated separately under the same conditions showed no optical density after 24 hr. In addition, incubated mixtures of the two substances produced U.V.-fluorescent spots when the solutions were dried on filter paper. Figure 5 shows the development of color in 24-hr reactions. (2) A musky odor was detected after incubation of glycolaldehyde with

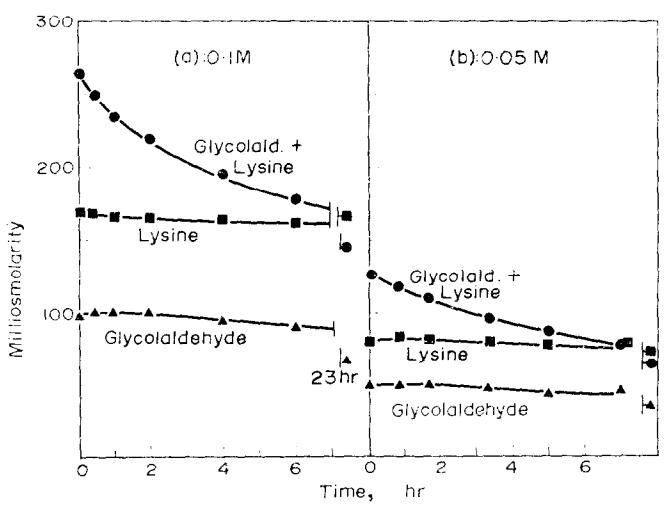

FIG. 6. Osmolarity changes vs. time in glycolaldehyde–lysine mixtures. Reactions were carried out at pH 8·0 and 37° in 200 mOsM KCl–$NaHCO_3$. (a) Glycolaldehyde and lysine each present at 0·1 M; (b) glycolaldehyde and lysine each present at 0·05 M.

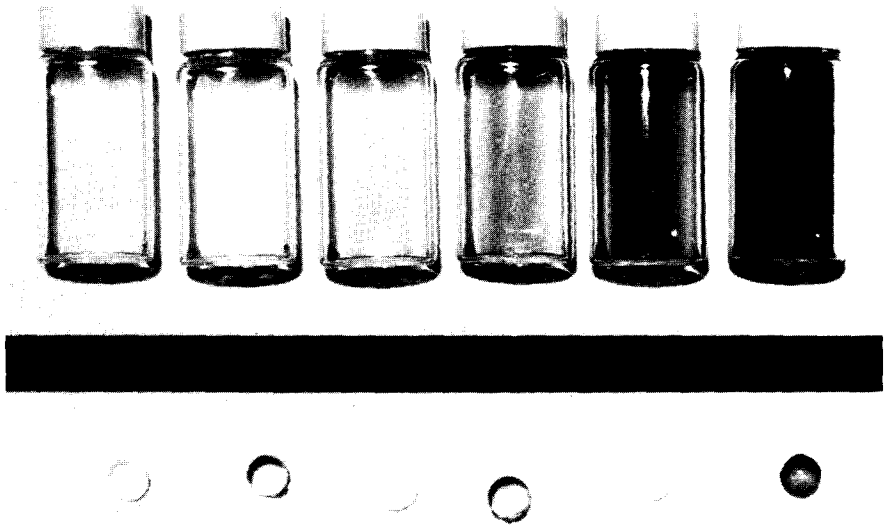

FIG. 5. Color development resulting from incubation of glycolaldehyde with lysine or with intact rabbit lenses. Incubations were carried out at 37° for 24 hr. Color development in lysine–glycolaldehyde vials is seen (viewing from right to left starting with 0·2 M lysine and 0·2 M glycolaldehyde). Succeeding vials were 4-fold dilutions of the reactants in vials. Darkest vial at right was red-brown, nearly black. Dilutions progressed to brown, straw, yellow and water clear. In the bottom portion of the figure are three pairs of rabbit lenses incubated for 8 days in rabbit Ringer's and 5 mM glycose under 5% CO_2–95% nitrogen. Left pair: control lens in rabbit Ringer's solution; lens incubated in 25 mM glycolaldehyde. Middle pair: lens incubated in 40 mM proprionaldehyde; lens incubated in 40 mM glycolaldehyde. Right pair: control lens; lens incubated in 180 mM glycolaldehyde added in 20 or 40 mM daily increments. Volume of incubating medium: 5 ml. Lens on right was dark brown after incubation.

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lysine. The Maillard reaction leads to production of odor.[19] (3) In the Maillard reaction an initial 1–1 condensation takes place between aldose and amine.[11] Further evidence of Maillard reactions between glycolaldehyde and lysine was derived from osmolarity experiments shown in Fig. 6. Total decreases in osmolarity after mixing were approximately 100 and 50 mOsM in parts a and b of the figure, the decline expected in formation of a 1–1 adduct. Slower decreases in osmolarity were seen for propionaldehyde–lysine mixtures, without appearance of color or U.V.-fluorescence. This indicated a condensation of the latter two, but with no further reaction leading to color or fluorescence. (4) In the Maillard reaction the carbonyl-amino adduct is colorless or only slightly colored, color only developing later.[11] Similarly, combination

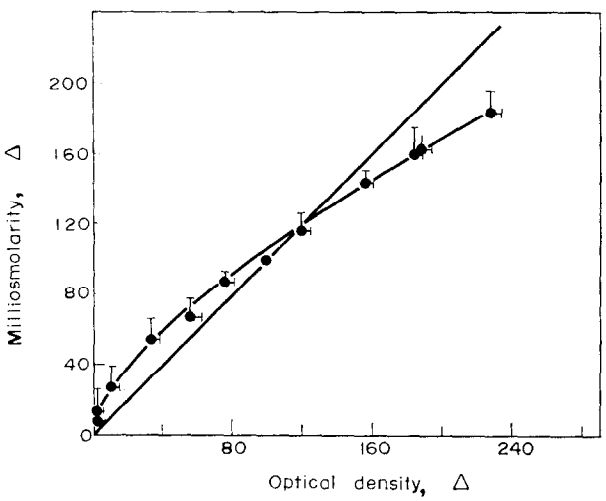

FIG. 7. Changes in osmolarity vs. changes in optical density for glycolaldehyde–lysine mixtures. Reaction mixtures of 100 mM glycolaldehyde and 100 mM lysine were incubated at 30°. Simultaneous measurements of absorbance at 560 nm and of osmolarity decrease were made at intervals of time. All absorbance and osmolarity changes were expressed as percentages of the values at 150 min arbitrarily assigned a value of 100. Incubation medium contained 0·154 M KCl–0·154 M $NaHCO_3$. Straight line is the line of identity between color change and combination.

preceded color development in our experiments, since osmolarity changes preceded color changes (Fig. 7). (5) The Maillard reaction progresses more rapidly at higher pH values, reaction rates below pH 6 being minimal.[11] Table 2 shows similar effects of pH on both combination and color change at different pH values. No appreciable combination or color change occurred at or below pH 4. (6) The Maillard reaction requires both carbonyl and amino groups.[11] In our experiments both the carbonyl group of glycolaldehyde and the amino groups of lysine were involved in the reaction (Table 3). The two reactants failed to combine or form color when the carbonyl-reducing agent, sodium borohydride, was added. Participation of the amino groups in the lysine–glycolaldehyde reaction was shown by the loss of basicity after incubation. Both the α- and the ε-amino groups were probably a part of the lysine–glycolaldehyde reaction, since loss of basicity occurred after incubation of glycolaldehyde with α-amino caproate or ε-amino caproate. (7) Color could not be removed completely when it

TABLE 2. EFFECTS OF pH ON COMBINATION AND COLOR DEVELOPMENT IN THE LYSINE–GLYCOLALDEHYDE PAIR*

	Change in osmolarity (mosM) at av. pH of				Increase in O.D. (400 nm)† at av. pH of			
Time (hr)	4·0	5·8	8·0	10·0	4·0	5·8	8·0	10·0
0								
1	+ 30‡	+2 4	− 22	− 36	0·002	0·010	0·076	0·082
3	+ 30	+ 6	− 48	− 65	0·001	0·104	0·252	0·286
6	+ 27	− 14	− 74	− 75	0·016	0·227	0·388	0·540

* Temperature, 30°. Initial concentration of glycolaldehyde and lysine was 100 mM. All values are averages of two experiments. Buffers: pH 10: 0·125 M borate (pH 8·5 after 6 hr of incubation); pH 8: 0·15 M KCl–0·15 M $NaHCO_3$ (pH 6·78 after 6 hr); pH 5·8: 0·2 M acetate (pH 5·30 after 6 hr); and pH 4: 0·2 M acetate (pH 3·8 after 6 hr).

† Samples diluted 20-fold for measurement of optical density.

‡ Increased osmolarity probably resulted from breakdown of slightly polymerized glycolaldehyde, since the same increase in osmolarity was seen for glycolaldehyde without added lysine.

TABLE 3. INVOLVEMENT OF CARBONYL AND AMINO GROUPS IN THE LYSINE–GLYCOLALDEHYDE REACTIONS

Solution	Milliosmolarity 0 hr	Milliosmolarity 24 hr	Change in milliosmolarity
A. Carbonyl group*			
Lysine + glycolaldehyde	261	139	− 121
Lysine + glycolaldehyde + sodium borohydride	264	255	− 9†

Solution	Average change in pH
B. Amino groups‡	
Lysine	0
Glycolaldehyde	0
Lysine + glycolaldehyde	− 0·98
ε-Aminocaproic acid + glycolaldehyde	− 1·06
α-Aminocaproic acid§ + glycolaldehyde	− 0·38

* Initial concentration of each reactant: 100 mM. Temperature, 30°. Borohydride was added at zero time in solid form to make 100 mM. All values are corrected for 295 mOsM medium of $NaHCO_3$ and KCl in equal concentrations.

† No color change seen.

‡ Each reactant was present initially in 0·1 M solution at pH 8·0 in 0·15 M KCl–0·15 M $NaHCO_3$. Atmosphere: air. Solutions were incubated at 37° for 5 hr. Results are averages of two experiments.

§ Because of difficulty in solubilizing α-aminocaproic acid at pH 8, this compound and glycolaldehyde were mixed in final concentration of 50 mM each.

was once formed in the reactions between glycolaldehyde and lysine, even after treatment with sodium bisulfite or sodium borohydride. This irreversibility was reported earlier for Maillard-type reactions.[11]

On the basis of the foregoing findings we concluded that the lysine–glycolaldehyde reaction was of the Maillard type.

Studies were made of the interactions between other carbonyl compounds and other amino compounds. Table 4 shows that, using glycolaldehyde, the most chromogenic reactions occurred with lysine, glutathione, glycine, alanine, arginine, and tryptophase. Lysine and glutathione were tested for color development (Table 5) with other carbonyl compounds. Most of the α-hydroxy-trioses and dioses tested showed greater chromogenicity than did the hexoses. Xylose and arabinose in high concentration showed color with lysine after 74 hr. Dihydroxyacetone and dihydroxyacetone phosphate yielded little color.

TABLE 4. COLOR PRODUCED BY VARIOUS AMINO ACID–GLYCOLALDEHYDE COMBINATIONS*

Exp. No.	Amino acid or peptide used with glycolaldehyde	Incubation time (hr)	O.D. (400 nm)
1			
Glycolaldehyde (10 mM)	+10 mM Glycine	20	1·15
(10 mM)	+10 mM Alanine	20	1·0
(10 mM)	+10 mM Glutathione	20	1·2
(10 mM)	+10 mM Aspartate	20	0·08
(10 mM)	+10 mM Cysteine	20	0·03
(10 mM)	+10 mM Lysine	20	1·8
(10 mM)	+10 mM Serine	20	0·16
(10 mM)	+10 mM Arginine	20	1·0
2	+10 mM Tryptophane	24	1·0
Glycolaldehyde (10 mM)	+10 mM Threonine	24	0·02
(10 mM)	+10 mM Aspartate	24	0·05
(10 mM)	+10 mM Histidine	24	0·07
(10 mM)	+10 mM Methionine	24	0·62
(10 mM)	+10 mM Proline	24	0·03
(10 mM)	+10 mM Lysine	24	1·45
(10 mM)	+10 mM Asparagine	24	0·01
(10 mM)	+10 mM Leucine	24	0·25

* All experiments were run at 37 ± 2° and approximately pH 8 in 0·15 M KCl–0·15 M $NaHCO_3$ (initial concentrations). Mixtures were gassed with 5% CO_2–95% N_2.

Glycolaldehyde–aldolase reactions. Color of the Maillard type also developed when glycolaldehyde was incubated with muscle aldolase. In Fig. 8b is seen the absorption spectrum for the yellow "solution" obtained after incubating lysine-containing muscle aldolase with glycolaldehyde. The curve resembles that of the lysine–glycolaldehyde reaction, and again, the preparation fluoresced under U.V.-light. Because of the limitation in molar concentration of rabbit muscle aldolase, osmolarity measurements were not practicable, nor could color beyond yellow be obtained. The absorption curves (Fig. 8a and b) were very similar, however, and the enzyme-carbonyl reaction was typical of the Maillard type.

TABLE 5. COLOR DEVELOPMENT BETWEEN CARBONYL COMPOUNDS WITH LYSINE OR REDUCED GLUTATHIONE IN SOLUTION*

Exp. No.	Carbonyl compound	Time of incubation (hr)	O.D. (400 nm)
1			
(10 mM lysine)	DL-Glyceraldehyde	19	1·4
	Glycolaldehyde	19	1·9
	Dihydroxyacetone	19	0·15
	Dihydroxyacetone phosphate	19	0·0
	Methylglyoxal	19	0·87
2			
(10 mM reduced glutathione)	Glycolaldehyde	24	1·28
	D-Glyceraldehyde	24	1·06
	Glyoxal	24	0·64
	Methylglyoxal	24	0·24
	Dihydroxyacetone	24	0·10
3			
(10 mM lysine)	Fructose (100 mM)	72	0·09†
	Galactose (100 mM)	72	0·25
	Glucose (100 mM)	72	0·05
	Xylose (100 mM)	72	1·25
	l-Arabinose (100 mM)	72	1·05
4			
(10 mM lysine)	Glycolaldehyde	24	1·45
	DL-Glyceraldehyde	24	1·40
	Diacetyl‡	24	0·49
5			
(10 mM lysine)	dHAP	20	0·0§
	F-6-P	20	0·04
	HDP	20	0·0
	Glycolaldehyde	20	1·0

* Each reactant, with exception of pentose and hexoses, was used in final concentration of 10 mM with lysine or glutathione. Initial pH was 8·0. Each solution containing 0·075 to 0·15 M KCl–0·075 to 0·15 M $NaHCO_3$ was bubbled with 5% CO_2–95% N_2 for 1 min prior to incubation at 37°.

† Only negligible changes in osmolarity (+ 2 to − 10 mOsM) were seen after 72 hr of incubation of lysine with the sugars of Exp. 3 of this table.

‡ This compound showed initial color before admixture with lysine.

§ Failure to show an increase in optical density does not necessarily indicate failure of any two compounds to combine.

Once the glycolaldehyde–aldolase color developed, it could not be removed from the protein by dialysis. We concluded that, once color developed, the bond between glycolaldehyde and aldolase was a tight, essentially irreversible one. Proteins interact with sugars in a Maillard-type reaction and form colored, nondialyzable irreversible complexes, primarily through carbonyl interaction with ϵ-amino groups of lysine residues.[12]

Figure 8C shows the spectrum of the yellow-reaction products of lens homogenates and glycolaldehyde. The spectrum resembles those of glycolaldehyde-muscle aldolase and of glycolaldehyde-lysine. The glycolaldehyde-homogenate product again fluo-

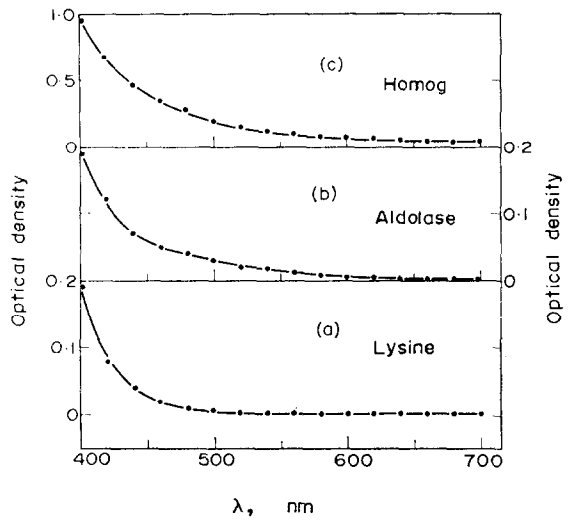

FIG. 8. Visible light difference spectra of glycolaldehyde with lysine, muscle aldolase or dialyzed lens homogenates. All three pairs were prepared in 0·15 M KCl–0·15 M $NaHCO_3$ and incubated at 37°. (a) 10^{-4} M lysine with 10 mM glycolaldehyde, 22 hr; (b) 2·5 mg/ml rabbit muscle aldolase with 10 mM glycolaldehyde, 18 hr; (c) dialyzed homogenate (1 lens/2 ml) with 10 mM glycolaldehyde, 18 hr. Light paths: 0·7 cm for b and c, 1·6 cm for a. All reactions under 5% CO_2–95% N_2.

resced under U.V.-light. It was concluded that all three pairs (Fig. 8a, b, and c) probably were undergoing similar, Maillard-type reactions.

Glycolaldehyde–intact lens reactions. Rabbit lenses cultured with glycolaldehyde showed varying degrees of color, ranging from yellow to dark rust brown, the color dependent in part on the amount of glycolaldehyde (Fig. 5). Treated lenses also fluoresced. Propionaldehyde under the same conditions elicited no color or fluorescence. In other experiments not shown here, and in which no effort was made to obtain deeply colored lenses, glyceraldehyde (reported earlier in reference 7) and methylglyoxal produced yellow or brown discoloration of rabbit lenses. Hence, it is seen that several carbonyl compounds which form color with amino acids in solution (Table 5) also color lenses.

The color and fluorescent characteristics of the products formed by glycolaldehyde with lysine, aldolases or intact lenses resembled the color developed[4,6] in aged human lenses.

Glycolaldehyde-exposed lenses exhibited color primarily in the cortical region. The cortex was hard and inelastic—similar in consistency to brown-colored nuclear sclerotic cataracts in human lenses.[4,5] When the lenses were resuspended in new medium without glycolaldehyde, no appreciable leakage of color from the lens was seen (Table 6). When the lenses were homogenized and dialyzed, several ninhydrin-positive spots were seen after chromatography of the dialysate, indicating the passage of small amounts of amino compounds through the dialysis membrane. Decrease in color of protein was negligible, however. We concluded that (a) color was formed between protein and glycolaldehyde, and to a lesser extent between free amino compounds of low molecular weight and glycolaldehyde; (b) the lens was poorly permeable to both the colored proteins and the smaller colored compounds.

TABLE 6. PERMEABILITY OF LENSES AND OF DIALYSIS MEMBRANES TO COLORED MATERIAL IN GLYCOLALDEHYDE-TREATED LENSES*

A. Optical density (400 nm) of medium to which pretreated glycolaldehyde and control lenses were added

Time (hr)	Control	Glycolaldehyde-pretreated
0	0·002	0·002
3	0·000	0·004
7	0·001	0·010
96	0·025	0·025

B. Optical density (400 nm) in 22 hr dialysate from above lenses (after homogenization):

Control	0·021
Glycolaldehyde-treated	0·039

* Lenses were incubated for 2 days in 5 mM glucose at 37° in rabbit Ringer's under 95% N_2–5% CO_2. Values listed are averages for two control and two glycolaldehyde-treated lenses. Glycolaldehyde was added hourly in small (1/50th) volumes to give 40 mM increases in final concentration in a 5-ml volume of medium. Total concentration added was 120 mM. Lenses became brown after 2 days of glycolaldehyde. All four lenses were transferred to glycolaldehyde-free medium. Samples were taken periodically from the medium for measurement of optical density. The lenses were later homogenized and dialyzed against an equal volume of 0·15 M $NaHCO_3$–0·15 M KCl.

DISCUSSION

The ability of of α-hydroxy-carbonyl and dicarbonyl compounds to inhibit glycolysis,[10] to inhibit at least one enzyme (aldolase) and to develop progressive coloration and fluorescence under U.V.-light in the rabbit lens parallels the decreased metabolic rate,[4] decreased activity of enzymes,[4] and the development of coloration and increased U.V. fluorescence[5] which are seen in aging and some senile cataractous human lenses. Lenses treated with carbonyl compounds in this study also showed hardening in the colored portion.

The inhibitory effects of reactive carbonyl compounds in the lens probably are not limited to aldolase. Transaldolase, another enzyme which contains lysine at its active centers,[20] may also be a target. In addition, other enzymes containing lysine residues or which form Schiff bases[17] may be susceptible.

The precise mechanism by which glycolaldehyde inhibits Schiff base formation in enzyme-substrate catalysis remains to be shown. It appears that the early reversal of glycolaldehyde inhibition of aldolase with increasing HDP concentration results from the displacement of glycolaldehyde by HDP from a relatively loose glycolaldehyde-lysine complex at the active centers. With time, however, a tighter, less reversible, perhaps covalent, combination appears to be formed between glycolaldehyde and the enzyme. At this stage HDP no longer would be able to reverse the inhibition by glycolaldehyde (as prototype).

Little doubt remains that the nonenzymic browning or Maillard reaction occurs

between glycolaldehyde and lysine in solution. Since other amino acids to varying degrees also form progressively similarly colored, fluorescent products with glycolaldehyde and related α-hydroxycarbonyl or dicarbonyl compounds, and since lenses, lens homogenates and purified muscle aldolase also show the typical yellow–brown–black (extent of coloration depending on such factors as time, reactant concentration and reactant species) coloration and the development of fluorescence, the Maillard reaction is implicated in these cases also. Furthermore, the similar color changes in aging human lenses may also be the result of Maillard-type reactions. The Maillard reaction has already been implicated in the yellowing of teeth.[1] It is possible that formation of carbonyl compounds occurs in the lens (and other pigmenting, aging tissues), and that these compounds react with amino groups of various proteins and free amino acids; the presence in the lens of such carbonyl compounds may be difficult to demonstrate, however, since they would be formed slowly and are highly reactive, yielding extremely low concentrations of unreacted carbonyl compounds in the lens tissue.

Zigman[21] recently postulated that photo-oxidation of amino acids may lead to cataract formation through insolubilization of lens proteins. We found that the browning reactions observed by us occurred independently of light and are not the exact light-requiring reactions of Zigman. However, the possibility exists that the Maillard reactions may also be important in cataractogenesis. This topic will be dealt with in a later communication.

Carbonyl compounds of the type studied in this paper may become important as tools in studying the aging process (if the Maillard reaction is proven to occur in aging tissue). Such compounds could be used perhaps to accelerate and mimic the pigmentation process occurring during natural aging.

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