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Formation of Cross-Links and Fluorescence in Polylysine, Soluble Proteins and Membrane Proteins by Reaction with 1-Butanal

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The reaction of polylysine and 1-butanal afforded 2-ethyl-2-hexenal, an aldol condensation and dehydration product of 1-butanal. 1-Butanal and 2-ethyl-2-hexenal were able to form fluorescence in polylysine, bovine serum albumin and erythrocyte membrane proteins. They were able to cross-link polylysine, hemoglobin, erythrocyte membrane proteins and isolated spectrin. Formation of cross-links by these aldehydes depended on the kind of protein. The ability of the proteins to form cross-links may reflect the contents of functional groups responsible and the quaternary structures of the proteins. 1-Butanal and 2-ethyl-2-hexenal may participate in the formation of cross-links and fluorescence by interaction with amino groups in the proteins.

Keywords——1-butanal; 2-ethyl-2-hexenal; polylysine; bovine serum albumin; hemoglobin; erythrocyte membrane protein; spectrin; cross-link; fluorescence

Proteins in biomembranes are susceptible to chemical modification during lipid oxidation. Induction of lipid oxidation in erythrocyte membrane resulted in the fomation of cross-links,[1-7] fluorophores[6] and borohydride-reducible functions[7] in the membrane proteins. It is suggested that a great diversity of aldehydes such as malonaldehyde, alkanals, 2-alkenals, 2,4-alkadienals and 4-hydroxyalkenals are generated as a result of lipid oxidation.[8,9] Malonaldehyde has been shown to produce cross-links,[1,2,10] fluorescence[11-13] and borohydride-reducible functions[7,10] in the proteins. The reaction products of proteins and malonaldehyde have been elucidated.[12,13] Monofunctional aldehydes also produce cross-links,[6,14,15] fluorescence[6,14,16] and borohydride-reducible functions.[7] Fluorescence spectra of the proteins modified with monofunctional aldehydes are similar to those of proteins modified during lipid oxidation.[6,12-14] Thus, it is important to elucidate the characteristics of the formation of cross-links and fluorescence by monofunctional aldehydes, because they may be the active species in the deterioration of protein during lipid oxidation.

Alkanals have the simplest structures among monofunctional aldehydes. Nevertheless, studies of the chemistry of alkanals are complicated by their tendency to undergo aldol condensation to give branched chain 2-alkenals.[17,18] We have shown that the reaction of 1-butanal with methylamine gave 2-ethyl-2-hexenal, and that several fluorophores were formed by the reaction of 1-butanal and/or 2-ethyl-2-hexenal.[19] This time, we investigated the reaction of polylysine, soluble proteins and membrane proteins with 1-butanal with respect to the formation of cross-links and fluorescence.

Experimental

Materials——1-Butanal and 2-ethyl-2-hexenal were the products of Wako Pure Chemical Industries, Ltd., Osaka, Japan and Tokyo Kasei Kogyo Company, Ltd., Tokyo, Japan, respectively. Poly-DL-lysine hydrobromide (type VIIB; degree of polymerization, 170) and bovine serum albumin were the products of Sigma Chemical Company, St. Louis, MO. Malonaldehyde was prepared by acid hydrolysis of tetramethoxypropane (Tokyo Kasei Kogyo Company, Ltd.).[20] An acidic solution of 1 M malonaldehyde was diluted with water and adjusted to pH 7.5

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with 1 N NaOH to prepare neutral 400 mM malonaldehyde solution for use.

The CO-liganded form of hemoglobin was purified from human erythrocyte lysate by carboxymethyl (CM) Sephadex C-50 ion exchange column chromatography as described previously.[21] Hemoglobin cross-linked between the β-subunits with bis(3,5-dibromosalicyl)fumarate[21] was used as a reference standard of subunit dimer in the electrophoresis. The concentration of the hemoglobin was determined spectrophotometrically.[21]

Erythrocyte ghosts were isolated according to the method of Steck[22] and stored at −20°C. Spectrin was prepared from erythrocyte ghosts according to the methods previously described.[23,24] Thus, ghosts (25 mg protein) were washed with 5 mM phosphate buffer (pH 8.0)–1 mM ethylenediaminetetraacetic acid (EDTA)–0.03 mM phenylmethanesulfonyl fluoride (PMSF) three times, and resuspended in 5 ml of the solution. Spectrin was extracted at 37 °C for 20 min with 20 ml of the extraction buffer [0.1 mM EDTA–0.5 mM 2-mercaptoethanol–0.03 mM PMSF (pH 9.0)]. The mixture was centrifuged at 20 °C and 30000 rpm for 60 min. After the volume of the supernatant had been reduced in a Collodion bag (Schleicher and Schuell, Inc., Keene, New Hampshire) under vacuum, the supernatant was passed through a Sepharose CL-4B column.[24] The fraction containing spectrin was concentrated at 0 °C, and dialyzed against 0.1 M phosphate buffer (pH 7.0)–0.03 mM PMSF. It was concentrated again to give 2 ml of spectrin solution (1 mg/ml).

Analytical Methods——Fluorescence spectra and intensities were measured by the use of a Hitachi 650-60 fluorescence spectrophotometer; the solvent background was subtracted automatically. Fluorescence intensities were determined relative to 0.1 μM quinine sulfate in 0.1 N sulfuric acid measured at 350 nm (excitation) and 450 nm (emission). Protein concentrations were determined by the method of Lowry *et al.*[25]

Gas chromatography was done by the use of a Hitachi 263-30 gas chromatograph equipped with a column (0.3 × 200 cm) of silicone SE 30. Nitrogen gas was used as a carrier gas at a flow rate of 30 ml/min. The chromatograph was operated at 120 °C (column temperature) and 150 °C (injection temperature).

Gel filtration was performed on a column (1.1 × 63 cm) of LKB Ultrogel AcA 44 (fractionation range from 10000 to 130000 Da) equilibrated with 0.1 M phosphate buffer (pH 7.0) containing 1.0% sodium chloride. The column was first calibrated by using molecular weight standards: bovine serum albumin (67000 Da), ovalbumin (43000 Da) (Pharmacia Fine Chemicals, Sweden), and lysozyme (14300 Da) (Wako Pure Chemical Industries, Ltd.). The void volume of the column was determined with blue dextran (2000000 Da).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was done in the discontinuous buffer system of Laemmli[26] with 7.5% (for bovine serum albumin and ghost proteins) or 15% (for hemoglobin) separating gel and 4% stacking gel. Samples were prepared by solubilization in the sample buffer containing 5% 2-mercaptoethanol and 1 mM PMSF, and the mixture was incubated at 37 °C for 1 h. Gel bands were visualized by Coomassie Brilliant Blue R-250 staining. Major protein bands from erythrocyte membrane are indicated according to the numbering of Steck.[27]

Detection of 2-Ethyl-2-hexenal in the Reaction of Polylysine and 1-Butanal——A mixture of polylysine (10 mg) and 1-butanal (100 μmol) in 10 ml of 0.1 M phosphate buffer (pH 7.5) was incubated at 37 °C for 24 h. The reaction mixture was extracted with 10 ml of chloroform. Gas chromatography of the extract showed two peaks corresponding to 1-butanal (retention time: 2.0 min) and to 2-ethyl-2-hexenal (retention time: 5.5 min). The amount of 2-ethyl-2-hexenal was estimated to be 7 μmol by comparison with the peak area of the standard solution of 2-ethyl-2-hexenal.

Results

1-Butanal undergoes aldol condensation and dehydration to give 2-ethyl-2-hexenal in the presence of a catalytic amount of methylamine.[19] This conversion also took place in the presence of polylysine. Thus, a mixture of polylysine (10 mg) and 1-butanal (100 μmol) was incubated at pH 7.5 and 37 °C for 24 h. Gas chromatography of the reaction mixture revealed two peaks corresponding to 1-butanal and 2-ethyl-2-hexenal, and the yield of 2-ethyl-2-hexenal was found to be 14%. It is suggested that 1-butanal is partially converted into 2-ethyl-2-hexenal in the reaction with proteins.

Polylysine (10 mg) was allowed to react with 1-butanal (100 μmol) or 2-ethyl-2-hexenal (50 μmol) at pH 7.5 and 37 °C. The reaction mixtures of 1-butanal and 2-ethyl-2-hexenal showed similar fluorescence with excitation maxima at 340—350 nm and emission maxima at 420—430 nm. The time course of the increase in fluorescence intensities of the reaction mixtures was followed (Fig. 1A). The rate of increase in the fluorescence intensities of the 2-ethyl-2-hexenal reaction mixture was much higher than that of the 1-butanal reaction mixture. Formation of the fluorescence from 1-butanal and 2-ethyl-2-hexenal was only slightly inhibited when oxygen was removed from the reaction mixtures. The fluorescence spectra and

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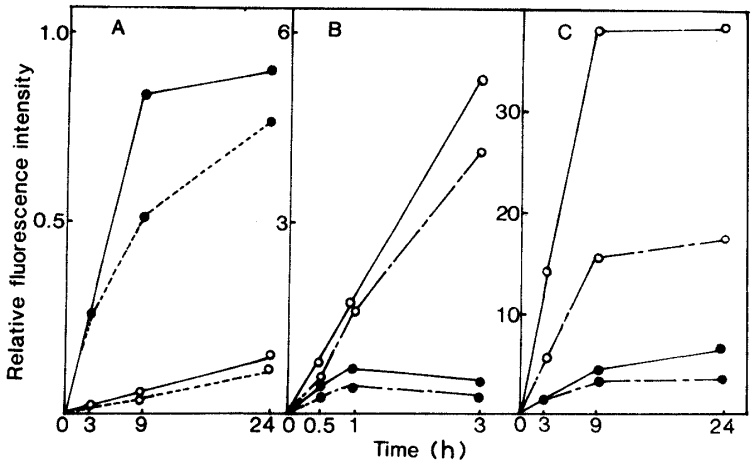

Fig. 1. Time Course of Fluorescence Intensities of the Reaction Mixtures of Polylysine (A), Bovine Serum Albumin (B) and Ghost Proteins (C) with 1-Butanol (○) and 2-Ethyl-2-hexenal (●)

A: A mixture of polylysine (10 mg) and 1-butanal (100 μmol) or 2-ethyl-2-hexenal (50 μmol) in 10 ml of 0.1 M phosphate buffer (pH 7.5) was incubated at 37 °C in the presence (——) or absence (------) of oxygen. The reaction mixtures of 1-butanal showed an excitation maximum at 340 nm, and an emission maximum at 420 nm. The reaction mixtures of 2-ethyl-2-hexenal showed an excitation maximum at 350 nm and an emission maximum at 425 nm. Fluorescence intensities of the reaction mixtures relative to 0.1 μM quinine sulfate are shown.

B: A mixture of bovine serum albumin (10 mg) and 1-butanal (400 μmol) or 2-ethyl-2-hexenal (200 μmol) in 2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated at 37 °C (——). Reaction mixtures prepared similarly were treated with 300 μmol of sodium borohydride (— - —), and an equal volume of 8% SDS was added to obtain a clear solution. The reaction mixtures of 1-butanal showed an excitation maximum at 350 nm and an emission maximum at 420 nm. The reaction mixtures of 2-ethyl-2-hexenal showed an excitation maximum at 355 nm and an emission maximum at 440 nm. The spectra were not changed after the borohydride treatment. Fluorescence intensities for the bovine serum albumin concentration of 10 mg/ml are expressed relative to 0.1 μM quinine sulfate.

C: A mixture of ghosts (5 mg protein) and 1-butanal (400 μmol) or 2-ethyl-2-hexenal (200 μmol) in 2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated at 37 °C. The recovered ghosts were resuspended in 1 ml of the buffer (——). Suspensions prepared similarly were treated with 20 μmol of sodium borohydride (— - —). Proteins in the recovered ghosts were freed from lipids by extraction with chloroform–methanol (2 : 1), and they were dissolved in 5% sodium dodecyl sulfate to measure fluorescence. The protein fraction modified with 1-butanal showed an excitation maximum at 359 nm and an emission maximum at 446 nm. The protein fraction modified with 2-ethyl-2-hexenal showed an excitation maximum at 359 nm and an emission maximum at 449 nm. Spectra were not changed on treatment with borohydride. Fluorescence intensities for the ghost protein concentration of 10 mg/ml are expressed relative to 0.1 μM quinine sulfate.

intensities were little changed on treatment with borohydride.

Polylysine modified with 1-butanal for 24 h was fractionated by gel filtration (Fig. 2A). Two fluorescent peaks were detected: the first was eluted at the position of 40000—70000 Da and the second at the position of unmodified polylysine (5000—20000 Da). The first peak corresponded to the dimer or trimer of polylysine. The cross-linked polylysine was also produced in the absence of oxygen. The modified polylysine treated with borohydride revealed two similar peaks, although the elution of the cross-linked polylysine was slightly delayed. The reaction of polylysine with 1-butanal gave fluorophores and stable cross-links, probably along with Schiff base and pyridinium salt,[19] at the ε- and N-terminal amino groups.

Polylysine modified with 2-ethyl-2-hexenal gave two fluorescent peaks (Fig. 2B), both peaks being eluted slightly faster than those of polylysine modified with 1-butanal. Modification with 2-ethyl-2-hexenal afforded the higher-molecular-weight cross-linked polylysine. The cross-linked polylysine was formed in the absence of oxygen. Upon reduction with

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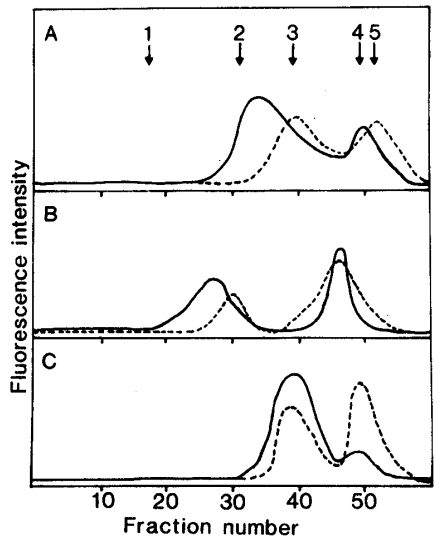

Fig. 2. Gel Filtration of Polylysine Modified with 1-Butanal (A), 2-Ethyl-2-hexenal (B) and Malonaldehyde (C)

A: A mixture of polylysine (10 mg) and 1-butanal (100 μmol) in 0.1 M phosphate buffer (pH 7.5) was incubated at 37 °C for 24 h and the reaction mixture was dialyzed against water (——). The same reaction mixture was treated with 600 μmol of sodium borohydride and dialyzed (– – – –).

B: A mixture of polylysine (10 mg) and 2-ethyl-2-hexenal (50 μmol) in 10 ml of the buffer was similarly incubated and dialyzed (——). The same reaction mixture was treated with sodium borohydride and dialyzed (– – – –).

C: A mixture of polylysine (25 mg) and malonaldehyde (40 μmol) in 1.2 ml of the buffer was incubated at 37 °C for 24 h and the reaction mixture was dialyzed (——). The same reaction mixture was treated with 100 μmol of sodium borohydride and dialyzed (– – – –).

An aliquot of each solution was applied to a column of Ultrogel AcA 44 and eluted with 0.1 M phosphate buffer (pH 7.0) containing 1.0% sodium chloride; fractions of 1.5 ml were collected. Fluorescence intensities of the peaks were monitored with excitation at 340 nm and emission at 420 nm (A and B), or with excitation at 400 nm and emission at 465 nm (C). The arrows in the figures indicate the elution position of molecular weight standards; 1, blue dextran; 2, bovine serum albumin; 3, ovalbumin; 4, unmodified polylysine; 5, lyzozyme. Standards 2, 3 and 5 were detected at 280 nm, and 4 was detected after the fractions had been treated with 0.1 ml of 400 mM malonaldehyde at pH 7 and 37 °C overnight.

borohydride the modified polylysine gave two similar peaks.

Polylysine thus modified (Fig. 2A and B) was compared with that modified with malonaldehyde (Fig. 2C). It has been shown that malonaldehyde modifies polylysine to produce 1,4-dihydropyridine-3,5-dicarbaldehyde fluorophore, cross-links due to the conjugated Schiff base, and aminopropenal.[14] Reduction of the fluorophore, the conjugated Schiff bases, and the aminopropenal with borohydride results in conversion into the much less fluorescent 1,4-dihydropyridine-3-hydroxymethyl-5-carbaldehyde,[28] into the diaminopropane, and into the aminopropanol, respectively. Polylysine modified with malonaldehyde and its reduced form revealed two similar fluorescent peaks. The 1-butanal or 2-ethyl-2-hexenal modifications of polylysine provided stable cross-links similar to those in malonaldehyde-modified polylysine.

When bovine serum albumin (10 mg) was incubated with 1-butanal (400 μmol) or 2-ethyl-2-hexenal (200 μmol), a time-dependent increase in the fluorescence intensities of the reaction mixtures was observed (Fig. 1B). The rate of increase in the fluorescence intensities of the 1-butanal reaction was higher than that of the 2-ethyl-2-hexenal reaction. Treatment of the reaction mixtures with borohydride resulted in a partial decrease in fluorescence intensities. Bovine serum albumin modified for 24 h was recovered by dialysis against water and analyzed by SDS–PAGE. Only a single protein band corresponding to the intact bovine serum albumin was observed, and no intermolecularly cross-linked protein band was seen on the electrophoretogram. Thus, the reaction of bovine serum albumin with 1-butanal or 2-ethyl-2-hexenal resulted in the formation of fluorescence but no intermolecular cross-links.

Hemoglobin, composed of 2α- and 2β-subunits,[29] was treated with 1-butanal or 2-ethyl-2-hexenal. Large amounts of precipitates were produced in the reaction mixtures. SDS–PAGE of the supernatants and the precipitates was carried out (Fig. 3). While the subunits in the supernatants remained uncross-linked (lanes 1 and 3), the subunits in the precipitates were

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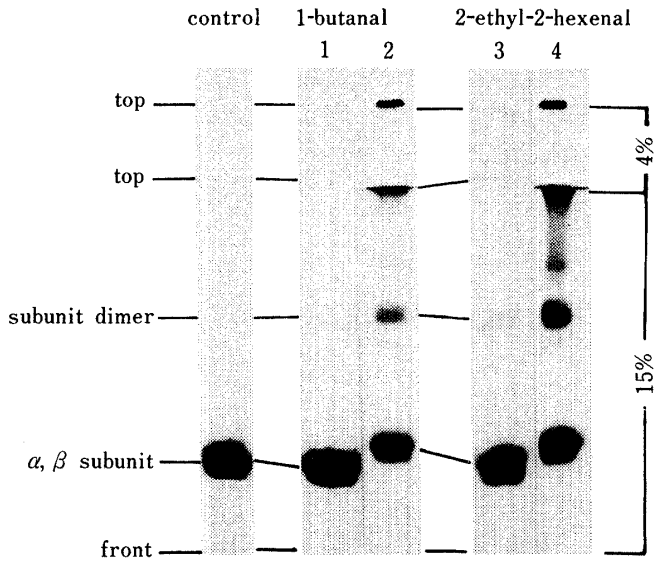

Fig. 3. SDS–PAGE of Hemoglobin Modified with 1-Butanal and 2-Ethyl-2-hexenal

A mixture of CO-hemoglobin (10 mg) and 1-butanal (400 μmol) or 2-ethyl-2-hexenal (200 μmol) in 2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated at 37 °C for 24 h. The precipitate was collected by centrifugation at 3000 rpm and washed with the buffer, and 50 μl of the solution was electrophoresed. The supernatant was dialyzed against water. An equal volume of the sample buffer with 2-fold concentration was added to the dialysate and 100 μl of the solution was electrophoresed. The reference standard of subunit dimer was the hemoglobin cross-linked between β-subunits (ref. 21).

1-Butanal; 1, supernatant; 2, precipitate. 2-Ethyl-2-hexenal; 3, supernatant; 4, precipitate.

extensively cross-linked to form subunit dimer at the position of the reference subunit dimer and higher-molecular-weight polymers at the tops of the stacking and separating gels (lanes 2 and 4). The precipitates obtained from the reaction mixtures with borohydride treatment showed similar cross-linked subunits. The results indicated that both 1-butanal and 2-ethyl-2-hexenal produced cross-links in hemoglobin subunits.

Erythrocyte ghosts were treated with 1-butanal or 2-ethyl-2-hexenal. Fluorescence was produced in the protein fraction of ghosts, and the intensities increased with the incubation time (Fig. 1C). The rate of the increase in fluorescence intensities of the 1-butanal reaction was higher than that of the 2-ethyl-2-hexenal reaction. The fluorescence was partially decreased on treatment with borohydride. Ghosts modified with 1-butanal and 2-ethyl-2-hexenal at two different concentrations for 1 h were subjected to SDS–PAGE analysis (Fig. 4). While ghosts treated with 100 μmol of 1-butanal showed intact protein bands 1, 2 (spectrin), 3, 4.1, 4.2 and 5 (lanes 1 and 2), ghosts treated with 400 μmol of 1-butanal showed large amounts of cross-linked proteins: protein bands 3, 4.1 and 4.2 completely disappeared and protein bands 1 and 2 were markedly diminished, and instead high-molecular-weight protein bands appeared at the top of the stacking and the separating gels (lane 3). 2-Ethyl-2-hexenal showed the higher cross-linking ability. Ghosts treated with 25 or 100 μmol of 2-ethyl-2-hexenal showed large amounts of cross-linked proteins (lanes 4 and 5). When the modified ghosts were treated with borohydride, no significant changes in the electrophoretic patterns were observed. The reaction of erythrocyte membrane proteins with 1-butanal and 2-ethyl-2-hexenal produced cross-links along with fluorophores.

Isolated erythrocyte spectrin, composed of α- and β-subunits,[25] was treated with 1-butanal and 2-ethyl-2-hexenal. SDS–PAGE of spectrin modified with these aldehydes showed that the original protein bands 1 and 2 completely disappeared and intense cross-linked protein bands were observed at the tops of the stacking and separating gels (Fig. 5). The

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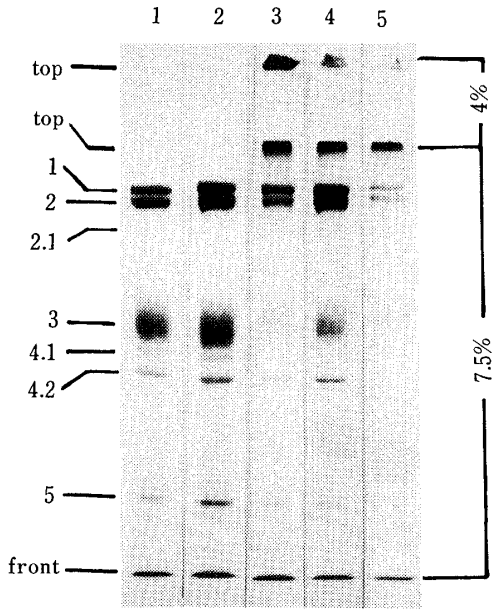

Fig. 4. SDS–PAGE of Ghosts Modified with 1-Butanal and 2-Ethyl-2-hexenal

A mixture of ghosts (10 mg protein) and 1-butanal or 2-ethyl-2-hexenal in 2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated at 37 °C for 1 h. Ghosts were recovered by centrifugation at 4 °C and 10000 rpm for 15 min, and washed with the buffer five times. Modified ghosts were resuspended in 1 ml of the buffer, and ghosts recovered were resuspended in 1 ml of the buffer. A 30 μg sample of the ghost proteins was loaded per lane.

1, control ghosts; 2, ghosts treated with 1-butanal (100 μmol); 3, ghosts treated with 1-butanal (400 μmol); 4, ghosts treated with 2-ethyl-2-hexenal (50 μmol); 5, ghosts treated with 2-ethyl-2-hexenal (200 μmol).

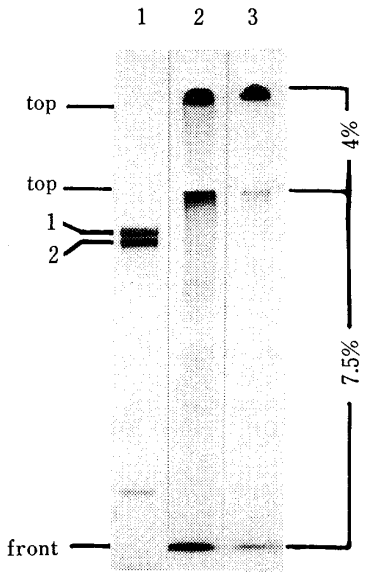

Fig. 5. SDS–PAGE of Spectrin Modified with 1-Butanal and 2-Ethyl-2-hexenal

A mixture of spectrin (0.5 mg) and 1-butanal (400 μmol) or 2-ethyl-2-hexenal (200 μmol) in 2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated at 37 °C for 24 h. The reaction mixture was dialyzed against 0.1 M phosphate buffer (pH 7.0)–0.03 mM PMSF and concentrated under vacuum in a Collodion bag. A 30 μg sample of the spectrin was loaded per lane.

1, control spectrin; 2, spectrin modified with 1-butanal; and 3, spectrin modified with 2-ethyl-2-hexenal. Incubation of spectrin without aldehydes produced no cross-linked proteins.

formation of the cross-links in spectrin was not influenced in the absence of oxygen. Thus, spectrin was readily cross-linkable by the aldehydes regardless of whether it existed in the erythrocyte membrane or in the purified form.

Discussion

Membrane lipid oxidation results in various kinds of damage to membrane proteins. Cross-linking of membrane proteins is one such event.[1-7] Monofunctional aldehydes such as alkanals, 2-alkenals, 2,4-alkadienals and 4-hydroxyalkenals generated during lipid oxidation[8,9] can form fluorophores in erythrocyte membrane proteins,[6] polylysine[14] and membrane proteins of microsomes,[16] the fluorescence spectra of which are similar to those of the proteins treated with oxidized lipids. These aldehydes also form cross-links in erythrocyte membrane proteins,[6] polylysine[14] and apoprotein of low density lipoprotein.[15]

In the present investigation, formation of fluorophores and cross-links in soluble and membrane proteins and polylysine by 1-butanal was studied. It has been previously shown that 1-butanal is converted into 2-ethyl-2-hexenal in the presence of methylamine, and both the aldehydes produce fluorophores on reaction with methylamine.[19] It was found that 2-ethyl-2-hexenal was formed in the presence of polylysine. 1-Butanal and 2-ethyl-2-hexenal produced fluorophores with similar fluorescence spectra in polylysine, bovine serum albumin and erythrocyte membrane proteins. The development of fluorescence intensities was

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different depending on the kinds of aldehydes and proteins. While the fluorescence intensities of polylysine modified with 2-ethyl-2-hexenal were higher than those with 1-butanal (Fig. 1A), the fluorescence intensities of bovine serum albumin and erythrocyte membrane proteins modified with 2-ethyl-2-hexenal were lower than those with 1-butanal (Fig. 1B and C). The rate of conversion of 1-butanal into 2-ethyl-2-hexenal may be different depending on the protein, and the reactivities of 1-butanal and 2-ethyl-2-hexenal to produce fluorophores may also be different depending on the protein. Formation of fluorescence did not require oxygen. The fluorescence produced was only partially decreased on treatment with borohydride, which is in accordance with the previous observations that the fluorescence produced in polylysine by acetaldehyde and 1-hexanal resisted borohydride treatment.[14]

While the aldehydes produced cross-links in polylysine, hemoglobin, erythrocyte membrane proteins and isolated spectrin, they were unable to cross-link bovine serum albumin. Differences in the contents of functional groups in the proteins or the quaternary structures of the proteins may be responsible for different effects in the cross-linking. Polylysine is a synthetic polypeptide with a large number of ε-amino groups and non-ordered structures which may facilitate the cross-linking reaction by the aldehydes. Hemoglobin is a highly ordered tetramer of α- and β-subunits,[29] which may facilitate the intersubunit cross-linking reaction. Erythrocyte membrane proteins exist in the membrane forming a network with specific associations,[23] and isolated spectrin tends to self-assemble.[25] The readily cross-linkable nature of erythrocyte membrane proteins may be due to these associations. Bovine serum albumin, a soluble protein, could not be cross-linked, probably owing to its non-assembling nature. Polylysine and erythrocyte membrane proteins produced fluorophores and cross-links by reaction with 1-butanal and 2-ethyl-2-hexenal, but bovine serum albumin produced only fluorophores. This may imply that fluorophores and cross-links are different in structure.

Formation of cross-links by 1-butanal and 2-ethyl-2-hexenal did not require oxygen. The cross-links produced were stable throughout the subsequent treatment, and they were not altered on treatment with borohydride. Although the mechanisms of the cross-linking reaction are obscure, cross-links may be produced by the interaction with the amino groups of proteins or polypeptides. It has been shown that acetaldehyde cross-links erythrocyte membrane proteins and spectrin,[30] and reacts with bovine serum albumin to from unstable (Schiff bases) and stable adducts,[31-33] and hemoglobin to form stable adducts.[34,35] Tuma and Sorrell proposed that the formation of stable adducts is due to the reaction of sulfhydryl groups with the unstable Schiff bases (Chart 1).[33] Esterbauer[8] suggested that 2-alkenals and

$$\underset{}{CH_3CHO} + \underset{}{R_1NH_2} \longrightarrow \underset{\text{unstable adduct}}{CH_3CH{=}NR_1} \xrightarrow{+R_2SH} \underset{\text{stable adduct}}{\begin{matrix} CH_3CH\text{-}NHR_1 \\ | \\ SR_2 \end{matrix}}$$

Chart 1

4-hydroxyalkenals are reactive with the protein sulfhydryl groups. We found in the present investigation that polylysine (having no sulfhydryl functions) was cross-linked by 1-butanal, 2-ethyl-2-hexenal and other monofunctional aldehydes.[14] Thus, it can be assumed that the monofunctional aldehydes cross-link proteins by mechanisms involving amino functions alone. An investigation on the mechanisms of the cross-linking reaction by monofunctional aldehydes is in progress.

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