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Increase of the molecular rigidity of the protein conformation in the intestinal brush-border membranes by lipid peroxidation

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The effect of lipid peroxidation on the protein conformation of the porcine intestinal brush-border membranes was studied using a fluorogenic thiol reagent, *N*-[7-dimethylamino-4-methylcoumarinyl]maleimide (DACM). By a kinetic analysis of the reaction of the membranes with DACM, it was shown that the reaction rate of the SH groups (SH_f) of the membrane proteins, whose reaction with the dye is very fast, decreases in proportion to the extent of thiobarbituric acid-reactive substance formation. The difference in the rate of the reaction of the SH_f groups for DACM between the control and peroxidized membranes completely disappeared after denaturation of the proteins by treatment with guanidine hydrochloride. The reaction of DACM with the SH_f groups of the control membranes accelerated when the temperature was increased with an apparent transition temperature between 25 °C and 30 °C. On the other hand, no transition was observed in the peroxidized membranes over the temperature range 20–43 °C. These results suggest that the conformation around the SH_f groups of the proteins in the peroxidized membranes is apparently different from that in the control membranes. A modification of the conformation around the SH groups in the membrane proteins associated with lipid peroxidation was further demonstrated by finding that the quenching efficiency of the fluorescence of the DACM-labeled membranes by Tl^+ was markedly decreased after lipid peroxidation. Based on these results, changes in the protein conformation of the porcine intestinal brush-border membranes by lipid peroxidation are discussed.

Introduction

Lipid peroxidation is a mixed chemical process often occurring in the unsaturated fatty acids of membrane lipids. It is induced by free radical production by certain enzymatic and/or non-enzymatic pathways and consequently modifies the bilayer structure of the lipids of biological membranes [1–3].

Recently, several lines of evidence showing perturbation of membrane components associated with lipid peroxidation have been reported, including inactivation [4–7] or activation [8,9] of membrane-bound enzymes as a possible consequence of a decrease of membrane lipid fluidity [10–13]. Gut et al. [14] have demonstrated a de-

Abbreviations: DACM, *N*-[7-dimethylamino-4-methylcoumarinyl]maleimide; TBARS, thiobarbituric acid-reactive substances; SDS, sodium dodecyl sulfate; DTNB, 5,5′-dithiobis(2-nitrobenzoate).

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crease in the rotational mobility of cytochrome *P*-450 in the rat liver microsomes as result of lipid peroxidation. It may be reasonably deduced that many pathological consequence of lipid peroxidation in tissues are related to the alteration in the dynamic properties of the components of various cellular membrane systems.

We previously demonstrated that lipid peroxidation of the porcine intestinal brush-border membranes is dependent on the membrane surface charge [15] and that the inhibitory effect of α-tocopherol on the lipid peroxidation is closely related to its stabilizing effect on the membrane lipids [16].

In the present study, a fluorogenic thiol reagent, *N*-[7-dimethylamino-4-methylcoumarinyl]-maleimide (DACM), has been employed to assess the effect of lipid peroxidation on the dynamic properties of the membrane proteins. Since DACM becomes fluorescent only after forming an adduct with sulfhydryl groups [17,18], this probe is very suitable for investigating the environmental changes around sulfhydryl groups of the protein molecules in biological membranes [19,20]. The experimental results in the present study suggest that lipid peroxidation causes an increase of the rigidity of the conformation around the SH groups in the membrane proteins consequence of decreased membrane lipid fluidity.

Materials and Methods

Materials. DACM, 2-thiobarbituric acid, 3(2)-*t*-butyl-4-hydroxyanisole and 1,1,3,3-tetraethoxypropane were purchased from Wako Pure Chemical Co. A stock solution of DACM (1 mM) dissolved in acetone was stored at $-20\,^{\circ}C$ until use. All other chemicals were of the purest grade commercially available.

Preparation of membrane vesicles. The brush-border membrane vesicles were prepared from porcine small intestine according to the calcium-precipitation method described in a previous paper [19] and suspended in 10 mM Tris-maleate buffer (pH 6.85). Protein concentration was assayed by the procedure of Lowry et al. [21] using bovine serum albumin as standard.

Membrane lipid peroxidation. Lipid peroxidation of the membrane vesicles was performed by the incubation of the vesicles (2.5 mg protein/ml) with 100 μM ascorbic acid and 10 μM $FeSO_4$ in 30 mM Tris-maleate buffer (pH 6.85) at 37 °C for 30 min unless otherwise specified. The membrane vesicles with different levels of lipid peroxidation were prepared by incubating the vesicles with varying concentrations of ascorbic acid (5–100 μM) in the presence of 10 μM $FeSO_4$ for 30 min at 37 °C. The reaction was terminated by dilution of the reaction mixture with a large volume of ice-cold 10 mM Tris-maleate buffer (pH 6.85) and centrifugation at $25\,000 \times g$ for 20 min. The pellets were washed twice with the same buffer and resuspended in it. The control membranes were prepared in the same manner without ascorbic acid and Fe^{2+}. In the experiment for determination of thiobarbituric acid-reactive substances (TBARS), the reaction was terminated by addition of 3 mM 3(2)-*t*-butyl-4-hydroxyanisole (as a final concentration). And the amount of TBARS formed during the reaction was determined by the fluorometric detection after complexes with 2-thiobarbituric acid as described previously [15] and expressed as the amount of malondialdehyde.

Labeling of the membrane proteins with DACM. A suspension of the membrane vesicles (2.5 mg protein/ml) was incubated with 1 μM DACM in 10 mM Tris-maleate buffer (pH 6.85) at 0 °C for 30 min. The final concentration of acetone in the reaction mixture was 0.33%. The reaction was terminated by dilution with a large volume of the same buffer and centrifugation at $25\,000 \times g$ for 20 min. The pellets were washed twice with 10 mM Tris-maleate buffer (pH 6.85) and then resuspended in it. Lipid peroxidation of the DACM-labeled membranes was performed under the same conditions described above.

Determination of the amount of bound DACM to the membrane proteins was performed as follows. The DACM-labeled membranes (0.1 mg protein/ml) were incubated in 50 mM Tris-HCl buffer (pH 9.0) containing 2% sodium dodecyl sulfate (SDS) for 18 h at 37 °C. Then the amount of DACM bound to the membrane proteins was determined using a molar absorption coefficient of 19 800 at 380 nm [17].

Preparation of N-ethylmaleimide-labeled membranes. The membrane vesicles (1 mg protein/ml) were incubated with 1 mM N-ethylmaleimide (as a

final concentration) in 30 mM Tris-HCl buffer (pH 7.4) at 25 °C for 30 min. After termination of the reaction by addition of 5-fold excess of β-mercaptoethanol with respect to the *N*-ethylmaleimide added, the reaction mixture was diluted with a large volume of 10 mM Tris-maleate buffer (pH 6.85) and centrifuged at $25\,000 \times g$ for 20 min. The pellets were washed three times with and resuspended in the same buffer.

Fluorescence measurements. Fluorescence measurements were carried out using an Hitachi spectrofluorometer MPF-4 equipped with a rhodamine B quantum counter. The sample compartment was maintained at 25 °C by circulating water through the cell holder unless otherwise specified. The excitation and emission wavelengths used in the DACM fluorescence measurements were 397 and 460 nm, respectively. The error due to light scattering of the sample emission could be entirely prevented using a 430 nm cut-off filter.

Kinetic analysis of the reaction of the membrane proteins with DACM. The reaction of the SH groups of the membrane proteins with DACM was determined at 25 °C by recording the fluorescence development at 460 nm after the addition of 0.5 μM DACM (as a final concentration) to the membrane suspension (0.1 mg protein/ml) in 10 mM Tris-maleate buffer (pH 6.85) unless otherwise specified. The pseudo-first-order rate constant (k') of DACM incorporation in the SH groups of the membrane proteins was determined from the slope of the semilogarithmic plot of $(I_{max} - I_t)/I_{max}$ against the reaction time of DACM with the membranes, where I_{max} and I_t are the observed intensities at the end of reaction and a given time t, respectively. In the present study, the development of DACM fluorescence reached a constant level after 30 min of the addition of DACM.

Quenching studies. Quenching experiments were carried out by adding small amounts of 0.2 M thallium (I) acetate (CH_3COOTl) solution in 10 mM Tris-maleate buffer (pH 6.85) at 25 °C. The stock solution of the quencher was used within a few days after preparation. Analysis of the quenching data was performed by using the Stern-Volmer (Eqn. 1) [22] and modified Stern-Volmer (Eqn. 2) [23] equations following:

$$I_o/I = K_Q[Q] + 1 \quad (1)$$

$$I_o/(I_o - I) = \frac{1}{f_a K_Q[Q]} + \frac{1}{f_a} \quad (2)$$

where I_o, I, K_Q, [Q] and f_a denote the fluorescence intensities in the absence and presence of quencher, the quenching constant, the quencher's concentration and the effective fraction of the fluorescence that is quenchable, respectively.

Measurement of SH content in the membrane proteins. The content of SH groups in the membrane proteins was determined according to the procedure of Ellman [24] as follows. The membrane vesicles (0.5 mg protein/ml) was incubated with 100 μM 5,5-dithiobis(2-nitrobenzoate) (DTNB) in 10 mM Tris-HCl buffer (pH 8.0) in the presence or absence of 1% SDS at 25 °C for 30 min.

Results

Reaction of the membranes with DACM

Fig. 1 shows the time course of DACM incorporation in the intestinal brush-border membranes at 25 °C. Incubation of the membrane vesicles

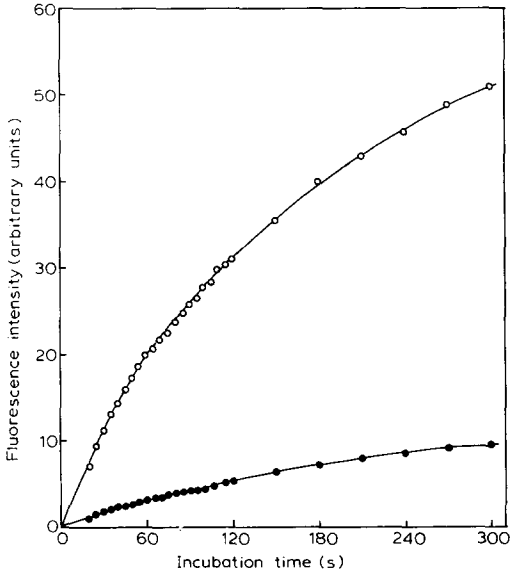

Fig. 1. Time course of the fluorescence development of DACM in 10 mM Tris-maleate buffer (pH 6.85) at 25 °C. At time zero, 0.5 μM DACM (as a final concentration) was added to the system. The membrane protein concentration was 0.1 mg/ml. ○, control membranes; ●, *N*-ethylmaleimide-labeled membranes.

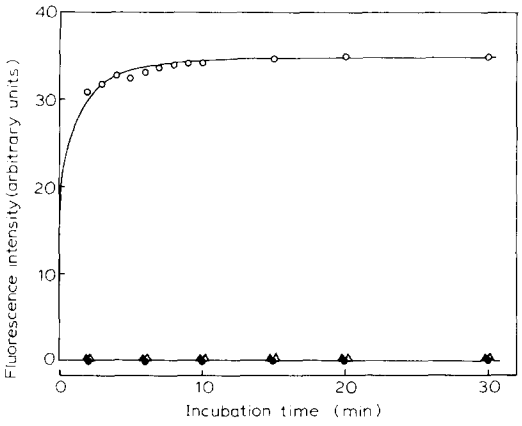

Fig. 2. Reaction of DACM with amino acids or liposomes. The concentrations of amino acids and liposomes were 1 mM and 1 mg/ml, respectively. ○, *N*-acetylcysteine; ●, glycine; △, phosphatidylcholine liposome; ▲, phosphatidylethanolamine liposome. Other experimental conditions were the same as those described in the legend to Fig. 1.

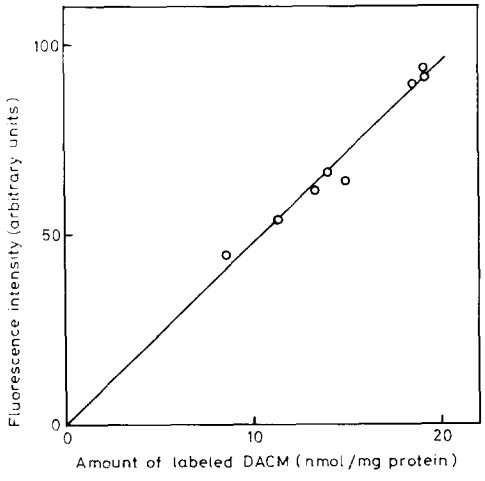

Fig. 3. Relationship between the fluorescence intensity and the amount of labeled DACM. The membrane vesicles (1.5 mg protein/ml) were incubated with 5 μM DACM (as a final concentration) in 10 mM Tris-maleate buffer (pH 6.85) at 25 °C for various periods of time (30 s to 20 min). The reaction was terminated by adding a 10-fold excess of β-mercaptoethanol with respect to DACM and then the reaction mixture was diluted with the same buffer. After centrifugation at 25000 × *g* for 20 min, the pellets obtained were washed twice with 10 mM Tris-maleate buffer (pH 6.85) and the final pellets were suspended in the same buffer. The amount of DACM labeled to the membrane proteins was determined as described in Materials and Methods.

with the dye resulted in a progressive development of DACM fluorescence at 490 nm over 30 min after the addition of the dye. On the other hand, the fluorescence development of the dye was markedly reduced by treatment of the membranes with *N*-ethylmaleimide. To determine whether the fluorescence development of DACM is due to the specific labeling of SH groups in the membrane proteins, the reaction of the dye with amino acids and phosphatidylcholine or phosphatidylethanolamine liposomes was further examined.

As shown in Fig. 2, the DACM fluorescence was also markedly increased by the addition of *N*-acetylcysteine but no fluorescence development was observed on addition of glycine or the liposomes, indicating that DACM does not react with

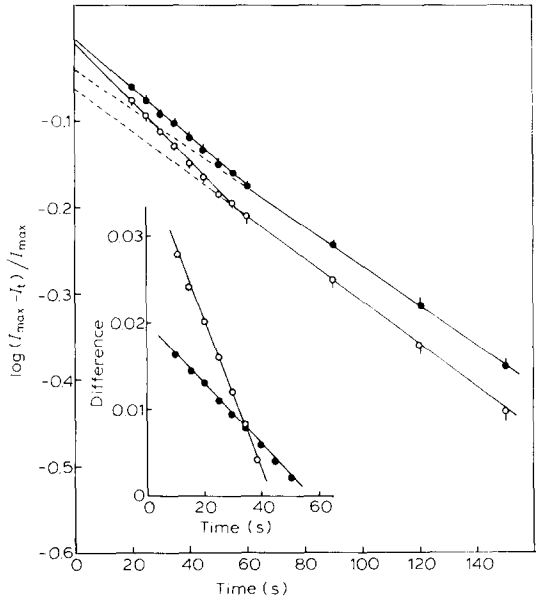

Fig. 4. The analysis of the rate of reaction of DACM with the membranes. The I_{max} and I_t represent the fluorescence intensities at the end point of the reaction (30 min after the addition of DACM) and at given time, *t*, respectively. Solid lines, fitting of observed fluorescence changes; dotted lines, extrapolation to time zero of the slow component of the observations. Values are expressed as mean ± S.D. for triplicate determinations. The procedure and conditions of lipid peroxidation are described in Materials and Methods. Other experiment conditions were the same as those described in the legend to Fig. 1. The inset figure shows the plots of the difference between respective full and dotted lines of the fast component, and values are expressed as mean of triplicate determinations. ○, control membranes; ●, peroxidized membranes.

the amino groups in these compounds. In addition, as shown in Fig. 3, the degree of the fluorescence intensity of DACM-labeled membranes was proportionate to the amount of DACM incorporated in the membrane proteins. From these results, it could be considered that an increase of the DACM fluorescence after addition of the dye to the membrane suspension is due to binding to the SH groups in the membrane proteins.

Fig. 4 shows the semilogarithmic plots of the fluorescence change versus the time of reaction with DACM in the control and peroxidized membranes.

The plots showed curves with different slopes against the incubation time and replotting of the data in the fast component of the reaction of the SH groups with DACM with correction for the contribution of the slow component is shown in the inset of Fig. 4. From the slopes of the fitted lines, the pseudo-first-order rate constants, k', of the reaction of the SH groups belonging to the fast phase, designated as SH_f, of the control and peroxidized membranes were evaluated to be $1.80 \cdot 10^{-3}$ and $0.81 \cdot 10^{-3}$ s^{-1}, respectively (Table I). On the other hand, no noticeable difference of the k' values between the control and peroxidized membranes in the late phase of the reaction were found.

In addition, the k' value of the reaction of the SH_f groups with DACM decreased proportionately to the amount of TBARS formed with varying concentrations of ascorbic acid in the presence of 10 μM Fe^{2+}, as shown in Fig. 5.

TABLE I

EFFECT OF GUANIDINE HYDROCHLORIDE ON THE RATE OF THE REACTION OF DACM WITH THE MEMBRANES

The experimental conditions were the same as those described in the legend to Fig. 4. Values are expressed as mean ± S.D. for triplicate determinations.

Guanidine HCl concn. (M)	k' (10^{-3} s^{-1})	
	control membranes	peroxidized membranes
0	1.80 ± 0.18	0.81 ± 0.06
0.3	2.54 ± 0.07	2.52 ± 0.06
1.0	4.57 ± 0.28	4.78 ± 0.28

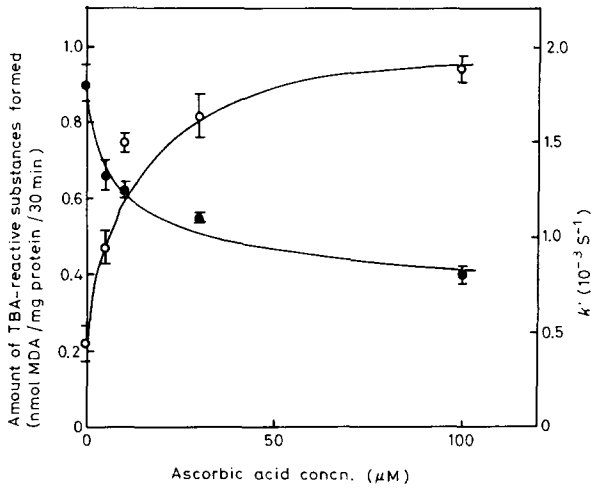

Fig. 5. Relationship between the rate of the reaction of DACM and the amount of TBARS formed. The ascorbic acid concentration was varied from 5 to 100 μM. The membrane protein concentrations used in the determinations of k' and TBARS were 0.10 and 0.50 mg/ml, respectively. Other experimental conditions were the same as those described in the legend to Fig. 4. Values are expressed as mean ± S.D. for triplicate determinations. ○, TBARS formation; ●, DACM incorporation rate.

Effect of guanidine hydrochloride on the DACM reaction.

The effect of concentrations of guanidine hydrochloride on the reaction rate of the SH_f groups with DACM is presented in Table I. The k' value of the SH_f groups in the control and peroxidized membranes markedly increased with increasing concentrations of guanidine hydrochloride and the difference of the k' values between the two membrane systems observed in the absence of the denaturant completely disappeared.

Temperature dependence of the reaction with DACM

Since we have previously reported [19] that the conformation around the SH groups in the membrane proteins is sensitive to the temperature variation, the effects of temperature on the reaction of the SH_f groups with DACM in the control and peroxidized membranes were examined (Fig. 6).

With increasing temperature, the k' value of the control membranes for DACM progressively increased with a transition point between 25 and 30 °C. This temperature is very comparable to the thermal transition temperature of the lipid layers

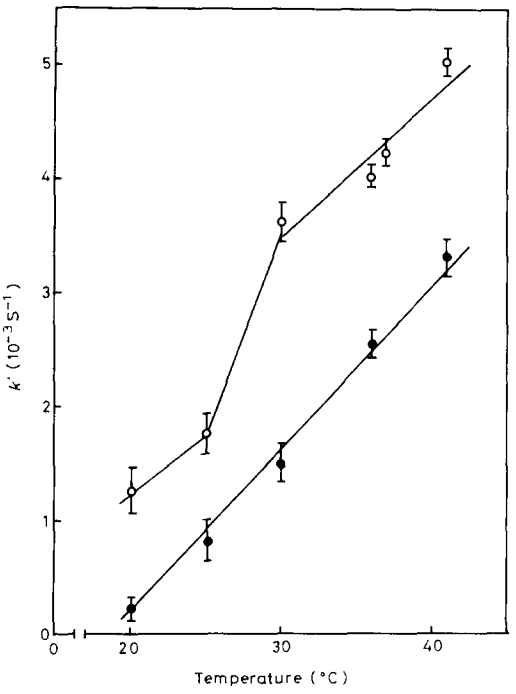

Fig. 6. Temperature dependence of the reaction of DACM with the membranes. Temperature was varied from 20 to 43° C. Other experimental conditions were the same as those described in the legend to Fig. 4. Values are expressed as mean ± S.D. for triplicate determinations. ○, control membranes; ●, peroxidized membranes.

of the membranes [19,25]. On the other hand, the k' values of the peroxidized membranes for the dye were smaller than those of the control membranes over all temperatures tested and the transition phenomenon of the DACM reaction observed in the control membranes completely disappeared on lipid peroxidation of the membranes.

Fluorescence quenching studies

In order to obtain further information about modification of the conformation around SH groups in the membrane proteins by lipid peroxidation, quenching of fluorescence of the DACM-labeled membranes by CH_3COOTl was examined.

The CH_3COOTl concentration dependence of the fluorescence intensity of the control and peroxidized membranes are presented in Fig. 7. The plots of $(I_o/I) - 1$ versus [Q] were linear in the two membrane systems over the concentration range of CH_3COOTl tested (Fig. 7A). However, the intercepts on the ordinate derived from zero indicate that quenching of the DACM fluorescence by CH_3COOTl is partial in the membranes [26]. Fig. 7B shows modified Stern-Volmer plots of the DACM-labeled membranes. From the slopes and the intercepts on the ordinate of the fitted lines, the quenching constants (K_Q) of the control and peroxidized membranes were calculated to be 207 and 133 M^{-1}, respectively (Table II). On the other hand, the f_a values of the two membrane systems were almost the same, indicating that the quenchable fraction of the probe is not altered by lipid peroxidation of the membranes.

TABLE II

THE PARAMETERS OF QUENCHING OF THE FLUORESCENCE OF THE DACM-LABELED MEMBRANES WITH Tl^+

Data were obtained from Fig. 7B and values are expressed as mean ± S.D. for triplicate determinations.

System	K_Q (M^{-1})	f_a
Control membranes	207 ± 39	0.246 ± 0.04
Peroxidized membranes	133 ± 27	0.266 ± 0.05

Effect of lipid peroxidation on SH content

As shown in Table III, the amount of DACM labeled to the SH groups in the membrane proteins was decreased from 25.6 to 18.3 nmol/mg protein by lipid peroxidation. However, the amounts of DTNB-detectable SH groups in the

TABLE III

EFFECT OF LIPID PEROXIDATION ON THE SH CONTENT OF THE MEMBRANES

The procedure and conditions of lipid peroxidation were the same as those described in the legend to Fig. 4. The concentration of SDS was 1%. A, amount of labeled DACM; B, amount of SH groups detected by the DTNB method. Values are expressed as mean ± S.D. for triplicate determinations.

System	SDS	A	B
Control membranes	+	–	44.1 ± 0.21
	–	25.6 ± 1.41	27.4 ± 0.30
Peroxidized membranes	+	–	45.9 ± 0.30
	–	18.3 ± 0.87	28.8 ± 0.10

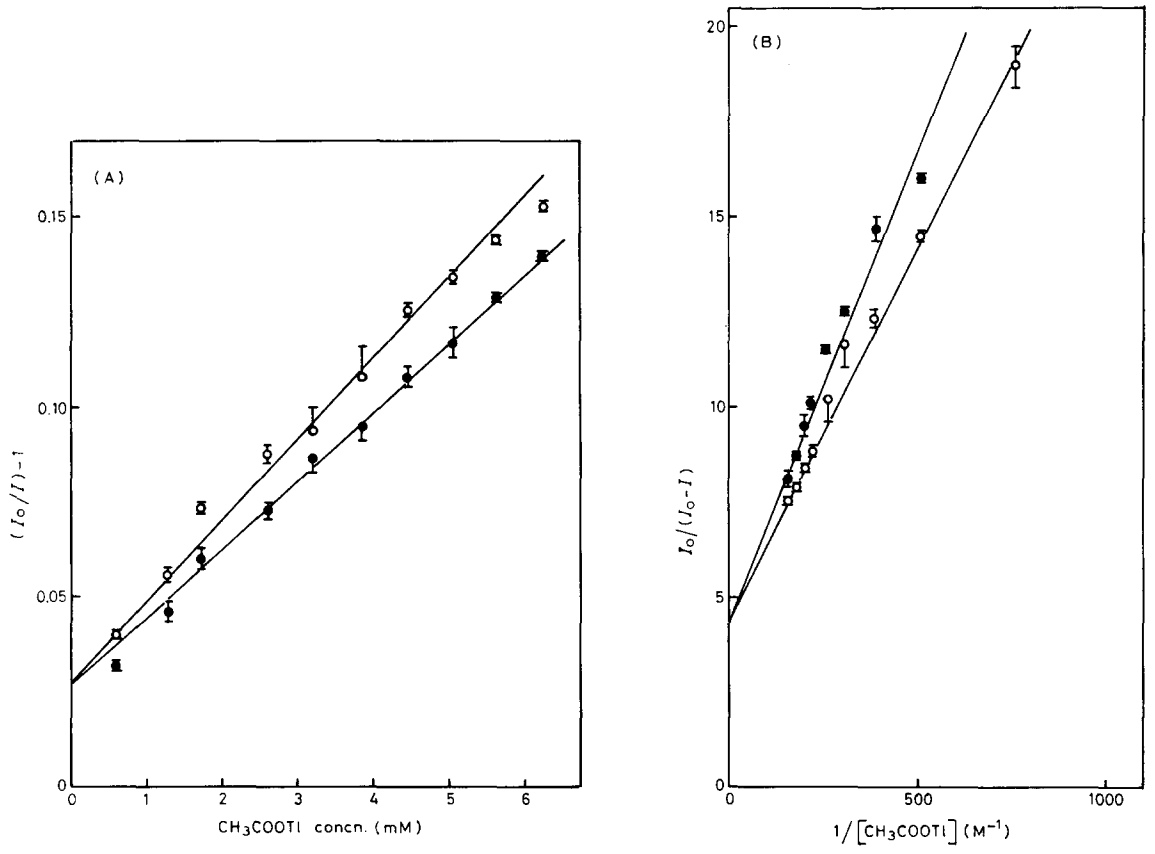

Fig. 7. (A) Stern-Volmer plots of the fluorescence intensities of the DACM-labeled control and peroxidized membranes. The concentration of membrane protein was 0.12 mg/ml. The concentration of CH_3COOTl was varied from 0.662 to 6.25 mM. ○, control membranes; ●, peroxidized membranes. Other experimental conditions were the same as those described in the legend to Fig. 4. Values are expressed as mean ± S.D. for triplicate determinations. (B) Modified Stern-Volmer plots of the data from (A).

membrane proteins in the presence and absence of 1% SDS did not show any appreciable change by lipid peroxidation. From these results, it seems that decreases in the k' value of the SH_f groups for DACM and in the amount of labeled dye by lipid peroxidation are attributed to changes in their reactivities for the dye and not due to a decrease in the amount of reactive SH groups in the membrane proteins.

Relationship between the protein conformation and lipid fluidity

We have previously reported [13] that the efficiency of fluorescence quenching of 2-(9-anthroyloxy)stearic acid incorporated into the intestinal brush-border membranes decreases in proportion

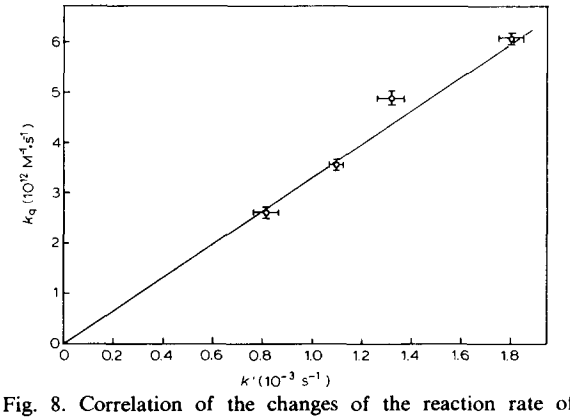

Fig. 8. Correlation of the changes of the reaction rate of DACM and the rate constant of quenching of the fluorescence of 2-(9-anthroyloxy)stearic acid by Cu^{2+} in varying the extent of lipid peroxidation of the membranes. The data were obtained from Fig. 5 and our previous work [13] for the DACM reaction and fluorescence quenching, respectively.

to the degree of lipid peroxidation of the membranes.

Fig. 8 shows the relationship between the changes of the k' value of the SH_f groups for DACM and the efficiency of the fluorescence quenching of the fatty acid embedded in the membrane lipids by Cu^{2+} with progressing lipid peroxidation. A good correlation was found between the rate constants of the DACM reaction (k') and the quenching reactions (k_q).

Effect of malondialdehyde on the reaction with DACM

Next we examined the effect of malondialdehyde, one of decomposition products of lipid peroxides, on the reaction of the SH_f groups with DACM. Malondialdehyde was prepared by acidic hydrolysis of 1,1,3,3-tetraethoxypropane as described previously [27].

As shown in Table IV, the k' value of the DACM reaction at 37 °C decreased depending on the concentration of malondialdehyde, suggesting that interaction of malondialdehyde with the membrane components also influences the reaction between the SH_f group and DACM.

To check whether or not malondialdehyde is able to react with SH groups, we examined the formation of fluorescent products. Incubation of malondialdehyde with glycine, histidine or lysine resulted in a marked fluorescence development at 500 nm, as shown in Fig. 9. However, *N*-acetylcysteine did not form fluorescent products with malondialdehyde.

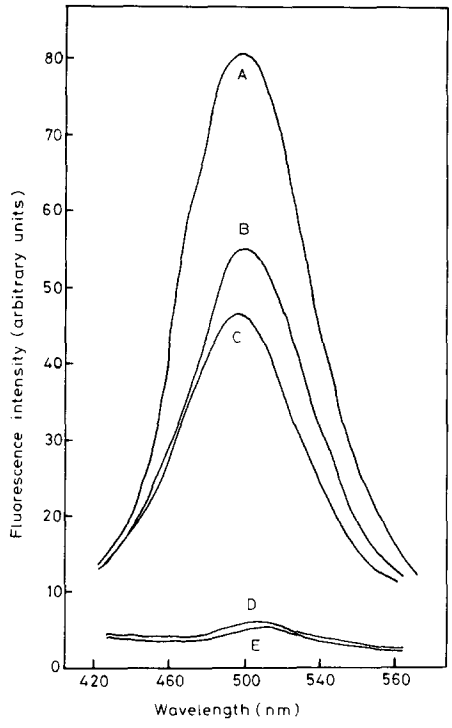

Fig. 9. Formation of fluorescent products of malondialdehyde with amino acids. The fluorescence spectra were traced with the excitation of 355 nm after 30 min of incubation of malondialdehyde (0.5 mM) and amino acids (5 mM) in 30 mM phosphate buffer (pH 7.1) at 37 °C. (A) Lysine; (B) glycine; (C) histidine; (D) *N*-acetylcysteine; (E) malondialdehyde alone.

TABLE IV

EFFECT OF TREATMENT OF THE MEMBRANES WITH MALONDIALDEHYDE ON THE DACM REACTION

Prior to determination of the rate of the DACM reaction, the membrane vesicles (0.1 mg protein/ml) were incubated with various concentrations of malondialdehyde in 10 mM Tris-maleate buffer (pH 6.85) for 30 min at 37 °C. The k' values were determined as described in the legend to Fig. 4. Values are expressed as mean ± S.D. for triplicate determinations.

Malondialdehyde concn. (μM)	k' (10^{-3} s^{-1})
0	4.27 ± 0.09
40	3.44 ± 0.07
100	2.76 ± 0.03
500	2.10 ± 0.14

Discussion

The present study has demonstrated that lipid peroxidation of the intestinal brush-border membranes induces conformational changes in the membrane proteins.

The addition of DACM to the membrane suspension resulted in a marked fluorescence development of the dye (Fig. 1) with two phases against the reaction time (Fig. 4). Judging from a decrease of the fluorescence development by treatment of the membranes with *N*-ethylmaleimide (Fig. 1) and the existence of a good correlation between the fluorescence intensity of the DACM-labeled membranes and the amount of labeled DACM (Fig. 3), it seems that DACM specifically interacts

with the SH groups in the membrane proteins. In addition, the aqueous buffer/*n*-hexane partition coefficient of DACM was found to be about 20 : 1, suggesting that the dye is able to react with the SH groups which are located in the polar and non-polar regions in the membrane proteins.

The kinetic studies of the DACM reaction revealed that the rate constant, k', of DACM to the SH groups belonging to fast reactive groups, SH_f, was decreased depending on TBARS formation in the membranes (Fig. 5). Although it is not clear at present whether the appearance of discontinuity in the DACM reaction is attributable to changes in the reactivities of the SH groups in the same or different protein moieties in the membranes, this result suggests that the conformation of protein molecules in the membranes is modified by lipid peroxidation.

The conformational changes in the membrane proteins by lipid peroxidation were also reflected in a disappearance of the thermal transition phenomenon in the DACM reaction (Fig. 6) and a decreased quenching efficiency of Tl^+ of the DACM-labeled membranes (Table II). In general, the extent of fluorescence quenching is accepted as an indication of the proximity of quencher molecules to fluorophore [22], but the quenching by an ionic quencher can also be influenced by the charge around fluorophore and by configuration of its binding sites [23,26,28]. Therefore, the decreased quenching efficiency with Tl^+ of the DACM-labeled membranes would imply a decreased accessibility of the quencher molecules to DACM-labeled SH groups and/or a decreased susceptibility of the conformation around the dye-labeled SH groups in the membrane proteins for Tl^+-binding to negatively charged groups in the membrane components, i.e., an increase of rigidity of the conformation around the labeled SH groups. The complete disappearance of a difference in the SH_f reactivities of DACM after treatment of the membranes with guanidine hydrochloride (Table I) also supports this interpretation.

In is of interest that there is a good correlation between the k' value of the SH_f groups with DACM and the k_q value of quenching of the fluorescence of 2-(9-anthroyloxy)stearic acid embedded in the membrane lipids by Cu^{2+} (Fig. 8). We have previously demonstrated [19] that the nature of the conformation around the SH groups in the membrane proteins is closely related to the dynamic properties of the membrane lipids. Therefore, it seems that alteration in the conformation around the SH_f groups in the membrane proteins associated with lipid peroxidation is partly related to decreased lipid fluidity [13] through modification of lipid–protein interactions in the membranes.

The reaction of the SH_f groups with DACM was also markedly reduced by treatment of the membranes with malondialdehyde (Table IV). In addition, malondialdehyde did not form fluorescent products with *N*-acetylcysteine (Fig. 9). These results suggest the possibility that conformation of the membrane proteins is also influenced by interaction with malondialdehyde, probably through formation of intra- and/or intermolecular Schiff bases between the amino groups in the membrane components [29,30]. However, it is well known [26,29–31] that lipid peroxidation of biological membrane systems results in the formation of many types of aldehyde including 4-hydroxyaldehydes. Furthermore, an appreciable formation of TBARS in the membranes was not found in the present study (Fig. 5). Therefore, further detailed experiments are needed in order to clarify whether or not the malondialdehyde formed during the peroxidation reaction of the membranes is directly related to the conformational changes in the membrane proteins observed in the present study.

In any case, all of the results obtained in the present study suggest that the protein conformation of the porcine intestinal brush-border membranes is sensitively modified by lipid peroxidation and that there is a good correlation between the changes in the protein conformation and lipid fluidity induced by lipid peroxidation.

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