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A study on peroxidative damage of the porcine intestinal brush-border membranes using a fluorogenic thiol reagent, *N*-(1-pyrene)maleimide

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To examine the effects of lipid peroxidation on the protein conformation in the porcine intestinal brush-border membranes, a fluorogenic thiol reagent, *N*-(1-pyrene)maleimide (NPM) was employed. By treatment of NPM-labeled membranes with 100 μM ascorbic acid / 10 μM Fe^{2+} in the presence of various concentrations of *tert*-butyl hydroperoxide (*t*-BuOOH), the fluorescence intensity of the complex decreased with the formation of conjugated diene, depending on the hydroperoxide concentration. The temperature dependence profile of the fluorescence intensity of NPM-labeled control membranes showed a thermal transition of the NPM fluorescence at 27–28 °C. The transition phenomenon of the NPM fluorescence in the membranes around this temperature disappeared by treatment of the labeled membranes with 100 μM ascorbic acid / 10 μM Fe^{2+} / 0.6 mM *t*-BuOOH. The difference in response of the fluorescence characteristics of the bound NPM for temperature variation between the control and peroxidized membranes was also observed in the quenching efficiency with acrylamide. Measurement of the fluorescence polarization revealed that the harmonic mean of the rotational relaxation times of the bound NPM molecules to the membrane proteins increased from 1.96 to 4.93 μs by lipid peroxidation of the membranes. This indicates that the movement of the region containing NPM-labeled SH groups in the membrane proteins is restricted by lipid peroxidation. Treatment of NPM-labeled peroxidized membranes with sodium dodecyl sulfate (SDS) resulted in a restoration of the intensity of the NPM fluorescence to the level of the control ones. In addition, the temperature dependence profile of the fluorescence intensity of NPM-labeled peroxidized membranes in the presence of SDS also showed an appearance of a transition phenomenon around 30 °C. The result of SDS-polyacrylamide gel electrophoresis of the peroxidized membranes revealed that high-molecular-weight aggregates of the membrane proteins were not formed by lipid peroxidation. On the basis of these results, changes in the environmental properties around NPM-labeled SH groups in the membrane proteins by lipid peroxidation are discussed.

Introduction

Lipid peroxidation is a mixed chemical process of oxidative degradation of polyunsaturated fatty acids in the phospholipids of biological membranes, resulting in a change in the chemical composition of membrane phospholipids [1–3].

It has been reported [4–6] that lipid–lipid interactions and/or molecular packing of lipid bilayers in biological membranes are strengthened as a result of lipid peroxidation, i.e. decrease of membrane lipid fluidity. We have previously reported that lipid peroxidation of the porcine intestinal brush-border membranes causes decrease of the membrane lipid fluidity [7] and membrane-bound Ca^{2+}-ATPase activity [8], depending on the degree of lipid peroxidation.

As is well known [9,10], lipid–lipid interactions in biological membranes play an important role in their functions, probably through modification of the protein

Abbreviations: NPM, *N*-(1-pyrene)maleimide; *t*-BuOOH, *tert*-butyl hydroperoxide; BHA, 3(2)-*tert*-butyl-4-hydroxyanisole; SDS, sodium dodecyl sulfate.

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conformation. A number of evidence supporting the importance of lipid fluidity on a variety of physiological functions of biological membranes have recently reported [11]. Therefore, it is of interest to study the effects of lipid peroxidation on the dynamic properties of membrane proteins in order to understand the molecular mechanisms of modification of membrane functions by lipid peroxidation. However, reports concerning the effects of lipid peroxidation on the dynamic features of the proteins in biological membranes are very few.

In previous papers [12,13], we have reported that the reactivities of SH groups in the proteins of the porcine intestinal brush-border membranes for fluorogenic thiol reagents, *N*-(7-dimethylamino-4-methylcoumarinyl)maleimide and *N*-(1-pyrene)maleimide, decrease by lipid peroxidation of the membranes.

In the present study, we examined the effects of ascorbic acid/Fe^{2+}/*t*-BuOOH-induced lipid peroxidation on susceptibility for temperature variation of the environmental properties around NPM-labeled SH groups in the membrane proteins to obtain further information about peroxidation-induced damage of the procine intestinal brush-border membranes.

Materials and Methods

Materials

NPM, 2-thiobarbituric acid, *t*-BuOOH and 3(2)-*tert*-butyl-4-hydroxyanisole (BHA) were purchased from Wako Pure Chemical Co. NPM was dissolved in acetone to make a stock solution (1 mM) and stored at −20 °C until use. EGSSX (20%)-Chromosorb WAW was obtained from Gasukuro Kogyo Co. Other chemicals were of the purest grade commercially obtainable.

Preparation of membrane vesicles

The brush-border membranes were prepared from the porcine small intestine according to the calcium-precipitation method as described in our previous paper [14] and the final pellets were suspended in 10 mM Tris-maleate buffer (pH 6.85). Protein concentration was assayed by the procedure of Lowry et al. [15] using bovine serum albumin as the standard.

Preparation of NPM-labeled membranes

A suspension of the membranes (2 mg protein/ml) was incubated with 10 μM NPM in 10 mM Tris-maleate buffer (pH 6.85) for 30 min at 0 °C. The final concentration of acetone in the reaction medium was 0.33%. The reaction was terminated by dilution with a large volume of 10 mM Tris-maleate buffer (pH 6.85) and centrifugation at 25000 × *g* for 20 min. The pellets obtained were washed twice with the same buffer and resuspended in 10 mM Tris-maleate buffer (pH 6.85). Under these conditions, NPM is mainly incorporated into 36 and 80 kDa proteins in the membranes [13].

Lipid peroxidation of membranes

The NPM-labeled membranes (1 mg protein/ml) were incubated with 100 μM ascorbic acid, 10 μM $FeSO_4$ and 0.6 mM *t*-BuOOH in 10 mM Tris-maleate buffer (pH 6.85) for 30 min at 37 °C, unless otherwise specified. The reaction was terminated by dilution with a large volume of 10 mM Tris-maleate buffer (pH 6.85) and centrifugation at 25000 × *g* for 20 min. The pellets were washed twice with and resuspended in the same buffer. The control membranes were prepared by treatment of NPM-labeled membranes in the same manner but without oxidizing agents. The amount of conjugated diene was measured by monitoring the absorbance at 233 nm as described in our previous paper [8].

Fluorescence measurements

Fluorescence measurements were performed using a Hitachi spectrofluorometer MPF-4 equipped with a rhodamine B quantum counter. The excitation and emission wavelengths used were 340 and 392 nm, respectively. The error due to light scattering of the sample could be entirely prevented using a 350 nm cutoff filter. The degree of steady-state fluorescence polarization, P, was calculated as the value of $(I_V - I_H)/(I_V + I_H)$, where I_V and I_H are the intensities of vertically and horizontally polarized emission with vertically polarized light, respectively. The harmonic mean of the rotational relaxation times, ρ_h, of labeled NPM was calculated from the slope of a $1/P$ versus T/η plot using the following equation [16] which was obtained from Perrin [17] and Weber [18] equation,

$$\rho_h = \left(\frac{1}{P_0} - \frac{1}{3}\right)\frac{3\tau}{(\text{the slope})} \cdot \frac{\eta}{T}$$

where P_0 is the limiting polarization at $T/\eta = 0$, η the viscosity of the solvent, τ the fluorescence lifetime and T the absolute temperature. The fluorescence lifetime was measured by the single photon counting method using an Ortec PRA-3000 nanosecond spectrofluorometer (Photochemical Research Associates Inc., Ontario, Canada) as described in our previous paper [19].

Quenching study

Quenching study was performed by adding small amount of 5 M acrylamide in 10 mM Tris-maleate buffer (pH 6.85). The stock solution of the quencher was used within a few days after preparation. The quenching data were analyzed using the following equation given by Lehrer [20], because quenching of the fluorescence intensity of NPM-labeled membranes

with acrylamide does not follow the simple Stern-Volmer law [13],

$$I_0/(I_0 - I) = \frac{1}{f_a K_Q[Q]} + \frac{1}{f_a}$$

where I_0, I, K_Q, [Q] and f_a denote the fluorescence intensities in the absence and presence of quencher, the quenching constant, the quencher concentration and the effective fraction of fluorophore that is quenchable, respectively. The quenching rate constant, k_q, was calculated using the equation of $k_q = K_Q/\tau_0$, where τ_0 is the fluorescence lifetime in the absence of quencher.

Lipid analysis

Change in the content of unsaturated fatty acids during lipid peroxidation was determined by gas-liquid chromatography (Shimadzu Gas Chromatograph GC-6AM) according to the procedure described in Ref. 21.

Polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was conducted in a 7.5% separating and a 5% stacking gel system in the presence of 0.1% SDS. Staining of the protein fractions in a gel was carried out using Coomassie brilliant blue.

Results

Change in fluorescence intensity of NPM-labled membranes

Fig. 1 shows relationship between the degree of change in the fluorescence intensity of NPM-labeled membranes and the amount of conjugated diene formed after incubation of the labeled membranes with 100 μM ascorbic acid/10 μM Fe^{2+} in the presence of various concentrations of *t*-BuOOH.

With increasing concentrations of *t*-BuOOH, the fluorescence intensity of the complex progressively decreased and reached almost a constant level as about 200 nmol of conjugated diene per mg protein were formed. On the other hand, the degree of decrease in the fluorescence intensity of NPM-labeled membranes by treatment with ascorbic acid/Fe^{2+} was only 3% of the control membranes (without ascorbic acid/Fe^{2+}/*t*-BuOOH), indicating that ascorbic acid/Fe^{2+} treatment of the membranes does not induce an appreciable change in the NPM fluorescence.

Table I shows change in the content of unsaturated fatty acids in the membrane phospholipids after treatment of the membranes with 100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH.

After incubation for 30 min at 37 °C, the content of arachidonic acid markedly decreased and fell to about 40% of the control membranes. On the other hand, the contents of oleic, linoleic and linolenic acids did not show appreciable change during the incubation periods.

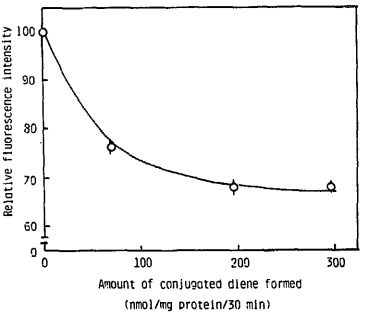

Fig. 1. Relationship between conjugated diene formation and NPM fluorescence change. The procedure and conditons of lipid peroxidation of NPM-labeled membranes were described in Materials and Methods, except variation of the *t*-BuOOH concentration (0, 0.1, 0.3 and 0.6 mM). The protein concentration of NPM-labeled membranes employed in the fluorescence measurement was 0.1 mg/ml. The fluorescence intensity was measured in 10 mM Tris-maleate buffer (pH 6.85) at 25 °C and represented as relative to that of the system without *t*-BuOOH. Values are expressed as means ± S.D. for triplicate determinations.

From these results, it is clear that change in the fluorescence intensity of the bound NPM to the membrane proteins by treatment with ascorbic acid/Fe^{2+}/*t*-BuOOH are related to alteration in the environmental properties around NPM-labeled SH groups by lipid peroxidation.

Temperature dependence of fluorescence intensity of NPM-labeled membranes

Fig. 2 shows the effects of temperature variation on the fluorescence intensity of NPM-labled membranes.

TABLE I

Change in content of unsaturated fatty acids in the membrane phospholipids during lipid peroxidation

The procedure and conditions of measurement of the content of fatty acids in the membrane phospholipids are described in Materials and Methods. The change in the content of fatty acids was expressed as a percentage of the original value in the control membranes for each fatty acid. Values are expressed as means of triplicate determinations.

Fatty acids	Content (%)
18:1	95.1
18:2	90.8
18:3	95.0
20:4	66.2

The fluorescence intensity of the control membranes decreased with a bending point at 27–28 °C by increasing temperature. However, this temperature-induced bending phenomenon in the fluorescence intensity of the complex was not detected as the labeled membranes were treated with 100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH. This result suggests that the susceptibility for temperature variation of the environmental properties around NPM-labeled SH groups in the peroxidized membranes is different from that of the control ones. This interpretation was further supported by the result of the fluorescence quenching experiment with acrylamide.

Quenching study

As previously reported [13], the quenching rate constant (k_q) of acrylamide at 25 °C for NPM-labeled membranes markedly decreases by ascorbic acid/Fe^{2+}/*t*-BuOOH treatment of the membranes. This finding suggests that the rate of penetration of the quencher molecules into the vicinity of the region containing NPM-labeled SH groups in the membrane proteins is suppressed by lipid peroxidation. In the present study, we examined the effects of temperature variation on acrylamide quenching of the NPM fluorescence to further ascertain this consideration.

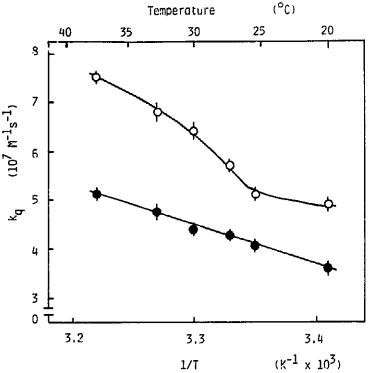

Fig. 3. Temperature dependence of quenching rate constant (k_q) of acrylamide for NPM fluorescence in the membranes. The concentration of acrylamide was varied from 65.8 to 400 mM. Other experimental conditions were the same as those described in the legend to Fig. 1, except the temperature variation. Symbols: ○, control membranes; ●, peroxidized membranes (100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH). Values are expressed as means ± S.D. for triplicate determinations.

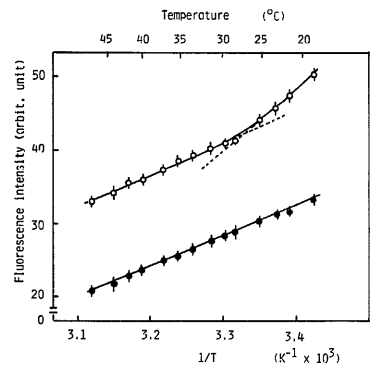

Fig. 2. Temperature dependence of fluorescence intensity of NPM-labeled membranes. Temperature was varied from 19 to 47.5 °C. Other experimental conditions were the same as those described in the legend to Fig. 1. Symbols: ○, control membranes; ●, peroxidized membranes (100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH). Values are expressed as means ± S.D. for triplicate determinations.

As can be seen in Fig. 3, the temperature dependence profile of the quenching reaction with acrylamide of NPM-labeled membranes also showed a disappearance of temperature-induced bending phenomenon of the k_q value in the temperature range from 20 to 37.5 °C by lipid peroxidation of the membranes.

Fluorescence polarization measurement

To elucidate the interanal freedom of the region containing NPM-labeled SH groups in the membrane proteins, the temperature dependence of the fluorescence polarization of NPM-labeled membranes was examined.

As shown in Fig. 4, the degrees of the fluorescence polarization of the control and peroxidized membranes decreased with increasing temperature of the medium. However, the slope in a $1/P - T/\eta$ of the peroxidized membranes was definitely smaller than that of the control ones. This result suggests that the rotational freedom of the region containing labeled NPM in the membrane proteins turned to become small by lipid peroxidation. In fact, the harmonic means of the rotational relaxation tiems of the bound NPM molecules in the control and peroxidized membranes at 25 °C were estimated to be 1.96 and 4.93 μs, respectively.

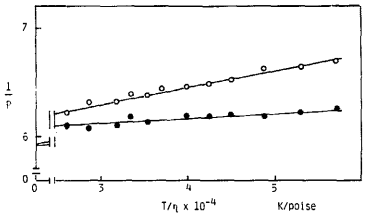

Fig. 4. Temperature dependence of fluorescence polarization of NPM-labeled membranes with 10 mM Tris-maleate buffer (pH 6.85). The membrane protein concentration was 0.08 mg/ml. The temperature was varied from 20 to 48 °C. Symbols: ○, control membranes; ●, peroxidized membranes (100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH). The fluorescence lifetimes of NPM-labeled control and peroxidized membranes at 25 °C are 54.7 and 49.2 ns, respectively [13].

Effects of SDS treatment of fluorescence intensity of NPM-labeled membranes

Fig. 5 shows the effects of SDS concentrations on the fluorescence intensity of NPM-labeled membranes.

The fluorescence intensities of NPM-labeled control and peroxidized membranes were increased depending on the SDS concentration in the medium. In addition, the fluorescence intensity of the peroxidized membranes approached that of the control ones when the SDS concentration was increased up to 0.05%. In this case, the emission maxima of NPM-labeled control and peroxidized membranes did not change by addition of SDS.

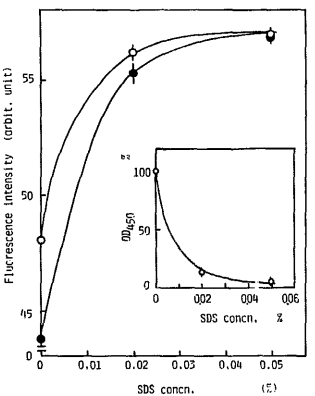

Fig. 5. SDS concentration dependence of fluorescence intensity of NPM-labeled membranes. The membrane protein concentration was 0.2 mg/ml. Symbols: ○, control membranes; ●, peroxidized membranes (100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH). Other experimental conditions were the same as those described in the legend to Fig. 1. The inset figure shows change in the absorbance at 450 nm of the membrane suspension (0.2 mg protein/ml). Values are expressed as means ± S.D. for triplicate determinations.

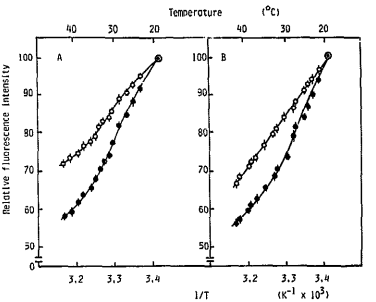

Fig. 6. Temperature dependence of fluorescence intensity of NPM-labeled membranes in the presence of SDS. Temperature was varied from 19 to 43 °C. Other experimental conditions were the same as those described in the legend to Fig. 1. (A) control membranes; (B) peroxidized membranes (100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH). Symbols: ○, in the absence of SDS; ●, in the presence of 0.05% SDS. The fluorescence intensity was expressed as relative to that at 19 °C in each system. Values are expressed as means ± S.D. for triplicate determinations.

On the other hand, the extent of the absorbance at 450 nm of the membrane suspension decreased depending on the concentrations of SDS (inset in Fig. 5). At 0.05% of SDS, the membrane suspension became clear, indicating that the membranes were almost dissolved at this concentration of SDS. Next, we examined the effects of temperature variation on the fluorescence intensity of NPM-labeled membranes in the presence of 0.05% SDS (Fig. 6, A and B). In this figure, the results from the control and peroxidized membranes without SDS are also described as a reference in each system.

In the absence of SDS, the temperature dependence profile of the fluorescence intensity of NPM-labeled peroxidized membranes against $1/T$ showed a linear curve in the temperature range tested as described in Fig. 2. On the other hand, in the presence of 0.05% SDS, the plot of the fluorescence intensity against $1/T$ of the complex showed an appearance of a bending phenomenon of the NPM fluorescence in the membranes around 30 °C (Fig. 6B). This temperature was very close to that observed in the control membranes (Figs. 2 and 6A).

Discussion

The susceptibility of the fluorescence characteristics of bound NPM to the proteins in the porcine intestinal brush-border membranes were examined in relation to lipid peroxidation of the membranes.

The temperature dependence profile of the NPM fluorescence in the control membranes indicated the existence of a bending point at 27–28 °C (Fig. 2). Such a bending phenomenon in the fluorescence intensity may be interpreted as indicative of a temperature-induced transition of the environmental properties around NPM-labeled SH groups in the membrane proteins, because NPM is specifically binds to SH groups in macromolecules [22]. This critical temperature was very close to that reported previously using *N*-(7-dimethylamino-4-methylcoumarinyl)maleimide [14]. The temperature-induced bending phenomenon of the NPM fluorescence in the membranes detected around 30 °C completely disappeared by lipid peroxidation of the membranes. This result suggests that the susceptibility for temperature variation of the NPM fluorescence in the membranes is suppressed by peroxidation-induced changes in the environmental properties around NPM-labeled SH groups. A disappearance of discontinuity in the temperature dependence profile of acrylamide quenching of NPM-labeled membranes by treatment with ascorbic acid/Fe^{2+}/*t*-BuOOH (Fig. 3) also supported this interpretation, because the rate constant of the quenching reaction with acrylamide is mainly dependent on the degree of penetration of the quencher molecules into the region where the fluorophore is located [23–25]. In addition, the result of the fluorescence polarization measurement (Fig. 4) also suggested that the movement of the region containing NPM-labeled SH groups in the membrane proteins is restricted by lipid peroxidation of the membranes.

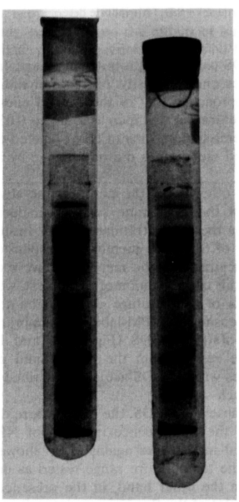

Fig. 7. SDS-polyacrylamide gel electrophoresis of the membrane proteins. The procedure and conditions of lipid peroxidation are described in Materials and Methods. A, Control membranes; B, peroxidized membranes.

As is well known [4–7], lipid peroxidation of biological membranes results in a decrease of their lipid fluidity. Such a modulation of the lipid organization may give an influence for the conformation and/or mobility of protein molecules in biological membranes [26,27]. Benga and Holmes [28] and Shinitzky [11] have reported that the mobility of membrane-bound proteins is directly modulated by lipid fluidity. In addition, recently, several investigators have reported [29–31] that membrane lipid composition is also an important factor in the regulation of certain protein-mediated activities in biological membranes.

As shown in Table I, the content of arachidonic acid in the membrane phospholipids markedly decreased by lipid peroxidation. This result suggests that the lipid environment surrounding the proteins in the peroxidized membranes is different from that of the control ones. A decrease of the content of unsaturated phospholipids in the membranes is expected to induce a decrease in the lipid fluidity, leading to modified conformation or quaternary structure of the proteins embedded in the membranes [32,33]. Therefore, it is of interest to investigate whether changes in the NPM fluorescence in the membranes by lipid peroxidation are related to alteration in the lipid organization.

To address this issue, the contol and peroxidized membranes were exposed to a detergent, SDS. As shown in Fig. 5, the fluorescence intensity of NPM-labeled peroxidized membranes approached to that of the control membranes as the labeled membranes were dissolved in SDS. In addition, the temperature dependence profile of the fluorescence intensity of the peroxidized membranes with SDS also showed an appearance of temperature-induced bending phenomenon around 30 °C (Fig. 6B). These results suggest that the environmental properties around NPM-labeled SH groups in the membrane proteins approached to the level of the control membranes by SDS treatment of the peoxidized membranes, although the exact mechanism is unclear at present.

It has been known [3] that the secondary products of lipid peroxidation, dialdehydes, are capable of interacting with the protein components in biological membranes, forming intermolecular protein cross-linking. Recently, Richter et al. [27] have demonstrated that decrease of the mobility of cytochrome *P*-450 in rat liver mircosomes induced by lipid peroxidation is due to the formation of protein aggregates. In the present study, the result of SDS-polyacrylamide gel electrophoresis analysis did not show the formation of high-molecular-weight aggreagtes of the membrane proteins by treatment of the membranes with 100 μM ascorbic acid/10 μM Fe^{2+}/0.6 mM *t*-BuOOH for 30 min at 37 °C (Fig. 7). Therefore, it seems that changes in the fluorescence characteristics of NPM-labeled membranes by lipid peroxidation may be not related to the formation of membrane protein aggregates by interaction of the membrane proteins with the peroxidation products.

On the basis of these results, it is suggested that differences in the response for temperature variation of the fluorescence characteristics of NPM-labeled membranes observed in the control and peroxidized membranes may be due to differences in the environmental properties around NPM-labeled SH groups in the membrane proteins induced by changes in the lipid organization, e.g., restriction of the mobility of the labeled amino acid side chains and/or rigidification of the conformation around the labeled SH groups in the membrane proteins. To know the exact mechanisms of the contribution of the lipid organization to the physical properties of the membrane proteins, further experiments including reconstitution study are need afterhere.

It has been recently reported the possibility that an oxidative damage of microvillus membranes of enterocytes induced by the administration of high oral doses of iron is related to lipid peroxidation of the membranes [35]. However, there have been few lines of research concerning lipid peroxidation-induced membrane damage in the intestinal brush-border membranes. Therefore, it seems that the present observations may give us an important clue in the analysis of mechanisms of peroxidation-induced membrane damage of enterocytes.

References

1 Plaa, G.L. and Witschi, H. (1976) Annu. Rev. Pharmacol. Toxicol. 16, 125–141.
2 Buege, J.A. and Aust, S.D. (1978) in Methods in Enzymology (Fleisher, S. and Packer, L., eds.), Vol. 52, pp. 302–351, Academic Press, New York.
3 Kagan, V.E. (1988) in Lipid Peroxidation of Biomembranes, pp. 55–117, CRC Press, Boca Raton, FL.
4 Rice-Evans, C. and Hochstein, P. (1981) Biochem. Biophys. Res. Commun. 100, 1537–1542.
5 Eichenberger, K., Bohni, P., Winterhalter, K.H., Kawato, S. and Richter, C. (1982) FEBS Lett. 142, 59–62.
6 Yamaguchi, T., Fujita, Y., Kuroki, O., Ohtsuka, K. and Kimoto, E. (1983) J. Biochem. 94, 379–386.
7 Ohyashiki, T., Ohtsuka, T. and Mohri, T. (1986) Biochim. Biophys. Acta 861, 311–318.
8 Ohta, A., Mohri, T. and Ohyashiki, T. (1989) Biochim. Biophys. Acta 984, 151–157.
9 Fox, C.F. (1975) in Biochemistry of Cell Walls and Membranes (Fox, C.F. ed.), Vol. 2, pp. 279–306, University Park Press, Baltimore, MD.
10 Tada, M., Yamamoto, T. and Tonomura, Y. (1978) Physiol. Rev. 58, 1–79.
11 Shinitzky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. 1, pp. 1–51, CRC Press, Boca Raton, FL.
12 Ohyashiki, T., Ohtsuka, T. and Mohri, T. (1988) Biochim. Biophys. Acta 939, 383–392.
13 Ohyashiki, T., Yamamoto, T. and Mohri, T. (1989) Biochim. Biophys. Acta 981, 235–242.
14 Ohyashiki, T., Takeuchi, M., Kodera, M. and Mohri, T. (1982) Biochim. Biophys. Acta 688, 16–22.
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
16 Ohyashiki, T., Sekine, T. and Kanaoka, Y. (1974) Biochim. Biophys. Acta 351, 214–223.
17 Perrin, F. (1926) J. Phys. Radium 7, 390–401.
18 Weber, G. (1952) Biochem. J. 51, 145–155.
19 Ohyashiki, T., Taka, M. and Mohri, T. (1985) J. Biol. Chem. 260, 6857–6861.
20 Lehrer, S.S. (1971) Biochemistry 10, 3254–3263.
21 Mowri, H., Nojima, S. and Inoue, K. (1984) J. Biochem. 95, 551–558.
22 Weltman, J.K., Bunting, R.P., Frackelton, Jr., A.R., Dowben, R.M., Bunting, J.R. and Cathou, R.E. (1973) J. Biol. Chem. 248, 3173–3177.
23 Eftink, M.R. and Ghiron, C.A. (1981) Anal. Biochem. 114, 199–227.
24 Ando, T. and Asai, H. (1980) J. Biochem. 88, 255–264.
25 Ando, T., Fujisaki, H. and Asai, H. (1980) J. Biochem. 88, 265–276.
26 Borochov, H. and Shinitzky, M. (1976) Proc. Natl. Acad. Sci. USA 73, 4526–4530.
27 Gut, J., Kawato, S., Cherry, R.J., Winterhalter, K.H. and Richter, C. (1985) Biochim. Biophys. Acta 817, 217–228.
28 Benga, G. and Holmes, R.P. (1984) Prog. Biophys. Mol. Biol. 43, 195–252.
29 East, J.M., Jones, O.T., Simmonds, A.C. and Lee, A.G. (1984) J. Biol. Chem. 259, 8070–8071.
30 Connolly, T.J., Carruthers, A. and Melchior, D.L. (1986) J. Biol. Chem. 260, 2617–2620.
31 Brasitus, T.A., Dehiya, R., Dudeja, P.K. and Bissonnette, B.M. (1988) J. Biol. Chem. 263, 8592–8597.
32 Salesse, R., Garnier, J., Leterrier, F., Daveloose, D. and Viret, J. (1982) Biochemistry 21, 1581–1586.
33 Spector, A. and Yorek, M.A. (1985) Lipid Res. 26, 1015–1035.
34 Hochstein, P. and Jain, S.K. (1981) Fed. Proc. 40, 183–188.
35 Fodor, I. and Marx, J.J.M. (1988) Biochim. Biophys. Acta 961, 96–102.