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Effect of neuraminidase treatment on the lipid fluidity of the intestinal brush-border membranes

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The effect of neuraminidase treatment on the lipid fluidity of the porcine intestinal brush-border membranes was studied using two fluorescence dyes, pyrene and 1,6-diphenyl-1,3,5-hexatriene. By treatment of the membranes with neuraminidase, the fluorescence parameters of pyrene-labeled membranes changed; i.e., a shift of thermal transition temperature, an increase in the fluorescence quenching rate for Tl^+ and a decrease in the fluorescence lifetime. These results suggest that the environmental properties around the dye molecules in the membranes change sensitively upon neuraminidase treatment. Perturbation of the lipid domain in the membranes associated with neuraminidase treatment is also demonstrated by a stimulated solubilization of diphenylhexatriene molecules in the membrane lipids, an increased quenching efficiency with Tl^+ and a decreased rotational correlation time of diphenylhexatriene-labeled membranes. Based on these results, we conclude that the lipid organization of the membranes is susceptible to neuraminidase treatment and that the membrane lipid fluidity increases by desialylation by the enzyme treatment.

Introduction

Sialic acids are distributed on the external surface of plasma membranes of animal cells as constituents of carbohydrate moieties associated with glycoproteins and glycolipids. It has been argued that sialic acids in biological membranes play an important role in various physiological functions of cells, such as cell adhesion, recognition and growth regulation and as receptors for many hormonal peptides [1–3].

Several investigators have demonstrated by chemical modification techniques that the carboxy groups of sialic acid are vital for various biological and physiological reactivities of the acid [4–7].

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It has been recognized that the lipid–lipid and/or lipid–protein interactions in biological membranes play a major role in their functions [8,9]. Therefore, it will be very important to study the role of sialic acids in these dynamic features of membrane components in order to understand the physiological function of the acids in biological membranes. However, there have been few lines of research reported concerning the contribution of sialic acids to the physical properties of biological membrane systems. Recently, Uchida et al. [10] have reported that the number and linkage portion of the sialic acid residues in a DPPC-ganglioside mixed dispersion influences the molecular packing of hydrocarbon cores in the dispersion, suggesting a contribution of sialic acid in controlling the physical properties of lipid organization.

In the present study, therefore, we have examined, using two fluorescence dyes, pyrene and

diphenylhexatriene, the effect of neuraminidase treatment on the lipid fluidity of the porcine intestinal brush-border membranes and have hypothesized that sialic acids in the membranes are related to regulation of lipid–lipid interactions therein.

Materials and Methods

Materials

Pyrene and 1,6-diphenyl-1,3,5-hexatriene (diphenylhexatriene) were purchased from Wako Pure Chemical and dissolved in ethanol and tetrahydrofuran, respectively, to make their stock solutions (1 mM of each). Neuraminidase (*Clostridium perfringens*, Type IV) was obtained from Sigma and dissolved in 0.1 M Tris-acetate buffer (pH 5.5) containing bovine serum albumin (0.3 mg/ml).

Preparation of membrane vesicles

Brush-border membrane vesicles were prepared from the porcine small intestine by the calcium-precipitation method described in our previous paper [11] 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. [12] using bovine serum albumin as standard.

Treatment of the membrane vesicles with neuraminidase

The membrane vesicles (1 mg protein/ml) were incubated with neuraminidase (10 μg/ml) in 30 mM phosphate buffer (pH 6.5) for 30 min at 37 °C unless otherwise specified. The reaction was terminated by dilution 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 and resuspended in the same buffer. The control membranes were treated in a similar manner but without neuraminidase. The content of sialic acids in the membrane vesicles was assayed according to the thiobarbituric acid method of Warren [13].

Labeling of the membrane vesicles with fluorescence dyes

After the treatment of the membrane vesicles with neuraminidase, the vesicles (3 mg protein/ml) were incubated with 1 μM pyrene of diphenylhexatriene in 10 mM Tris-maleate buffer (pH 6.85) for 30 min at 0 °C. The final concentration of ethanol or tetrahydrofuran in the reaction mixture was 0.33%. The reaction was terminated by dilution 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 obtained were washed three times with, and resuspended in, the same buffer.

Fluorescence measurements

Fluorescence measurements were carried out using a Hitachi MPF-4 spectrofluorometer 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 for pyrene and diphenylhexatriene fluorescence measurements were 340 and 375 nm, and 330 and 420 nm, respectively. Measurements of the fluorescence anisotropy and fluorescence lifetime were performed using an Ortec PRA-3000 nanosecond fluorometer (Photochemical Research Associates Inc., Ontario, Canada) and the rotational correlation time of diphenylhexatriene molecules in the membranes was calculated directly by analysing data obtained from measurement of the time-resolved anisotropy of the fluorescence emission [14] with a Digital MINC-11 computer system (Digital Equipment Co., Maynard, U.S.A.).

Quenching studies

Quenching studies were carried out at 25 °C by adding small amounts of 5 M acrylamide or 0.1 M thallium(I) acetate (CH_3COOTl) in 10 mM Tris-maleate buffer (pH 6.85). The stock solutions of these quenchers were used within a few days after preparation. Quenching parameters were calculated according to the Stern–Volmer equation [15]:

$$(I_0/I) - 1 = K_Q[Q]$$

where I_0, I, K_Q and [Q] denote the fluorescence intensities in the absence and presence of quencher, the quenching constant and the quencher concentration, respectively.

If the quenching reaction did not follow a

simple Stern–Volmer law, the quenching parameters were determined by the modified Stern–Volmer equation [16] as follows:

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

where f_a represents the fractional maximum accessible probe fluorescence, which is associated with the accessibility of the probe to the quencher. The quenching rate constant, k_q, was estimated by $k_q = K_Q/\tau_0$, where τ_0 is the fluorescence lifetime in the absence of quencher.

Results

Sialic acid content of the membranes

The content of sialic acids in the porcine intestinal brush-border membranes was determined in the presence and absence of 1% Triton X-100.

As shown in Table I, the total amount of sialic acids in the membranes was estimated at 21.5 ± 1.3 nmol/mg protein in the presence of 1% Triton X-100, a little more than that reported for rat intestinal brush-border membranes (15.0 nmol/mg protein) [17].

On the other hand, total sialic acids were 20.9 ± 0.9 nmol/mg protein in the absence of the detergent, indicating that about 97% of the membrane vesicles are of right-side-out orientation. The amounts of sialic acids liberated from the membranes by incubating for 30 min with different concentrations of neuraminidase are also presented in Table I.

TABLE I

AMOUNT OF SIALIC ACID LIBERATED BY NEURAMINIDASE TREATMENT

The incubation of the membrane vesicles (1 mg protein/ml) with neuraminidase was carried out in 30 mM phosphate buffer (pH 6.5) for 30 min at 37 °C. Each value was expressed as mean ± S.D. for triplicate determinations.

Neuraminidase concn. (μg/mg protein)	Amount of sialic acid liberated (nmol/mg protein)
100 [a]	21.5 ± 1.3 [a]
100	20.9 ± 0.7
20	14.7 ± 0.3
10	6.8 ± 1.5
2	5.6 ± 0.4
1	4.6 ± 0.7

[a] In the presence of 1% Triton X-100.

Changes in fluorescence parameters of pyrene-labeled membranes

The effect of temperature variation on the fluo-

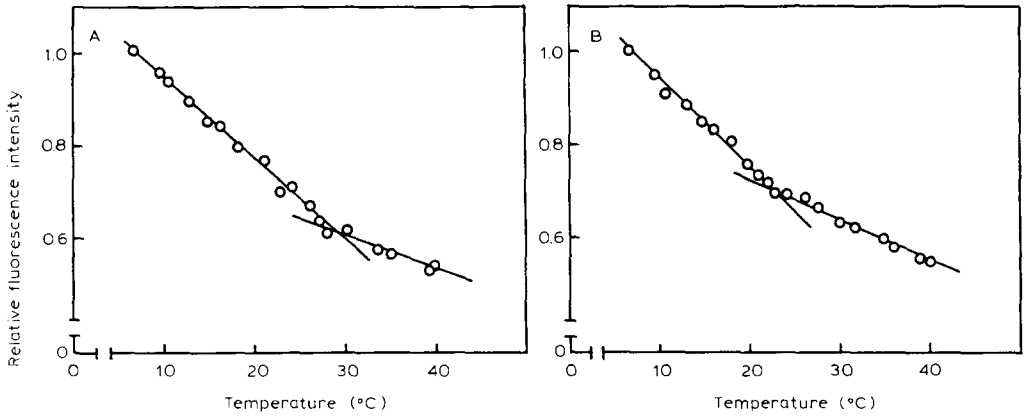

Fig. 1. Temperature-dependence of fluorescence intensity of pyrene-labeled membranes in 10 mM Tris-maleate buffer (pH 6.85). Membrane protein concentration was 110 μg/ml. Temperature was varied from 7 to 40 °C. The fluorescence intensities were represented as relative to that at 7 °C in each system. (A) Control membranes; (B) neuraminidase-treated membranes. Neuraminidase-treated membranes were prepared by incubating the membrane vesicles (1 mg protein/ml) with the enzyme (10 μg/ml) in 30 mM phosphate buffer (pH 6.5) for 30 min at 37 °C. The bending points in these plots were determined from the intersection of the two curves calculated by a least-squares procedure.

TABLE II

FLUORESCENCE LIFETIME OF PYRENE-LABELED MEMBRANES

Fluorescence lifetime measurements were carried out in 10 mM Tris-maleate buffer (pH 6.85) at 25°C. Membrane protein concentrations were 120 and 119 μg/ml for control and neuraminidase-treated membranes, respectively. Neuraminidase treatment conditions were the same as described in the legend to Fig. 1. The data were shown as mean ± S.D. for triplicate determinations.

Membrane systems	Lifetime (ns)	
	τ_1	τ_2
Control membranes	6.93 ± 0.10	180.0 ± 0.25
Neuraminidase-treated membranes	7.06 ± 0.09	165.2 ± 0.15

rescence intensity of pyrene-labeled membranes was examined.

As shown in Fig. 1A, the fluorescence intensity of the control membranes progressively decreased by increasing temperature with a transition temperature of about 29°C. On the other hand, the temperature-dependence profile of the neuraminidase-treated membranes revealed a transition point at 23°C (Fig. 1B). This result suggests that the environmental properties around pyrene molecules embedded in the membrane lipids are modified by neuraminidase treatment. This interpretation was further supported by a decrease in the fluorescence lifetime of pyrene-labeled membranes by treatment with neuraminidase.

As reported previously [18,19], the fluorescence decay curve of pyrene in the membranes does not follow an single exponential function. We assumed, therefore, that there are two components in the decaying process of the fluorescence to analyze its lifetime. By a computer fitting, the lifetimes of pyrene-labeled control membranes were determined to be 6.93 ns (τ_1), accounting for 12% of the total fluorescence, and 180 ns (τ_2), accounting for 88%, respectively. Treatment of the membranes with neuraminidase resulted in a slight but definite decrease in the τ_2 value (Table II).

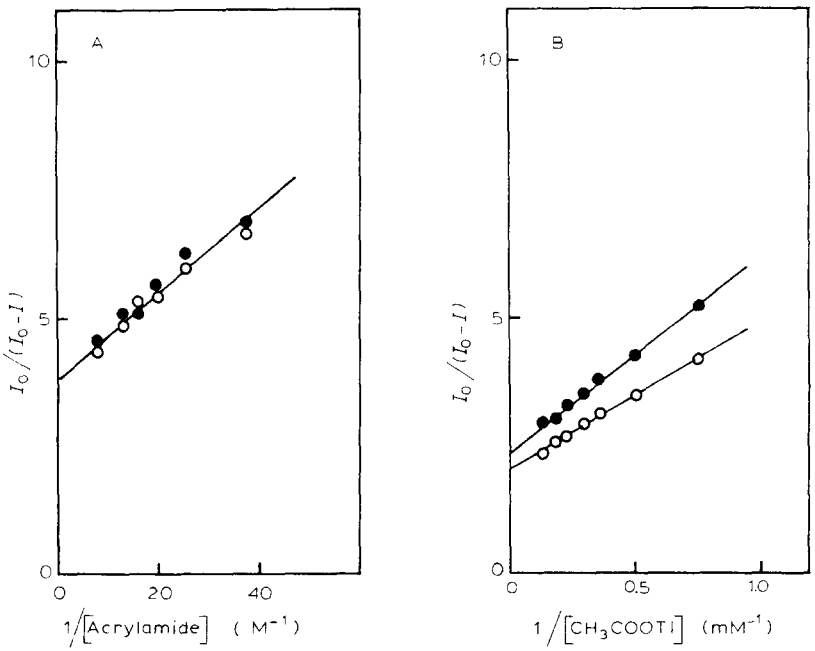

Fig. 2. The modified Stern–Volmer plots of pyrene-labeled membranes at 25°C. Membrane protein concentration was 80 μg/ml. The concentrations of acrylamide and CH_3COOTl were varied from 26.6 to 125 mM and from 0.66 to 6.25 mM, respectively. (A) Acrylamide; (B) CH_3COOTl. Symbols: ○, Control membranes; ●, Neuraminidase-treated membranes. Neuraminidase treatment conditions were the same as described in the legend to Fig. 1.

Quenching studies of pyrene fluorescence in the membranes

The effects of acrylamide and CH_3COOTl on the fluorescence intensities of pyrene-labeled control and neuraminidase-treated membranes were examined to obtain further information about changes in the lipid organization by neuraminidase treatment. Since quenching of the fluorescence intensity of the pyrene-labeled membranes with acrylamide or Tl^+ does not follow a simple Stern–Volmer law [19,20], the determination of fluorescence quenching parameters was performed using a modified Stern–Volmer equation proposed by Lehrer [16].

As shown in Fig. 2, the plots of $I_0/(I_0 - I)$ vs. $1/[Q]$ were linear over the concentration ranges of these quenchers tested, suggesting that each of the pyrene molecules in the membranes is subjected to a similar degree of fluorescence quenching. The quenching rate constants, k_q, for acrylamide and Tl^+ of the control membranes were estimated to be $34 \cdot 10^7$ and $495 \cdot 10^7$ $M^{-1} \cdot s^{-1}$, respectively (Table III). In addition, the f_a values for acrylamide and Tl^+ determined from the intercepts on the ordinates in these plots were 0.27 and 0.48 in the control membranes, respectively. These results indicate that about 27 and 48% of pyrene molecules in the membranes are accessible for acrylamide and Tl^+, respectively.

The k_q value for Tl^+ was markedly increased by the treatment with neuraminidase, but the quenching efficiency for acrylamide did not change. On the other hand, the f_a values for these quenchers were almost the same in both membrane systems, indicating that the amount of pyrene molecules in the membranes which are accessible to acrylamide and Tl^+ did not change by neuraminidase treatment.

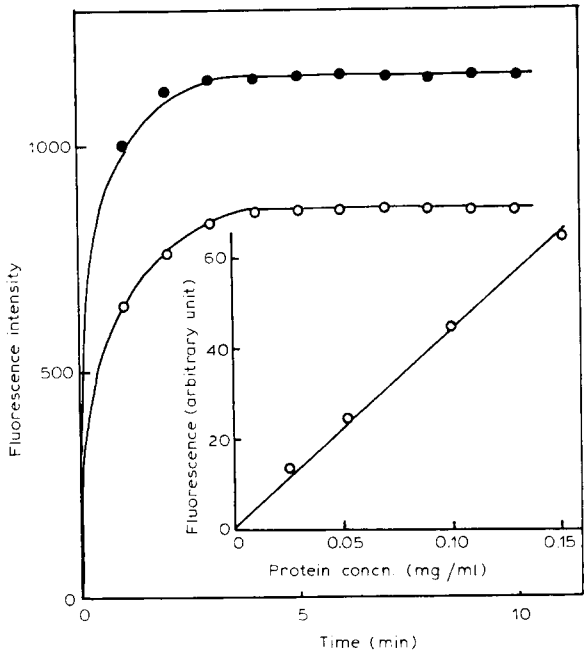

Fig. 3. Time-course of incorporation of diphenylhexatriene in the membranes at 25 °C. 30 mM Tris-maleate buffer (pH 6.85). Membrane protein concentrations were 47 and 25 μg/ml for control (○) and neuraminidase-treated (●) membranes, respectively. At time zero, 1.66 μM diphenylhexatriene (as a final concentration) was added to the membrane suspension. The fluorescence intensities were represented as the value per mg protein. Neuraminidase treatment conditions were the same as described in the legend to Fig. 1. The inset shows the concentration dependence of the membrane protein on the fluorescence development of diphenylhexatriene. Membrane protein concentration was varied from 25.4 to 152.4 μg/ml. The fluorescence intensities were measured after 5 min of addition of the dye to the membrane suspension. Other experimental conditions were the same as those described above.

Incorporation of diphenylhexatriene in the membranes

The time-course of development of diphenylhexatriene fluorescence after the addition of the

TABLE III

QUENCHING PARAMETERS OF PYRENE-LABELED MEMBRANES WITH ACRYLAMIDE AND CH_3COOTl

The data were obtained from Fig. 2 and expressed as mean ± S.D. for triplicate determinations.

Quencher	Control membranes			Neuraminidase-treated membranes		
	K_Q (M^{-1})	k_q (10^7 $M^{-1} \cdot s^{-1}$)	f_a	K_Q (M^{-1})	k_q (10^7 $M^{-1} \cdot s^{-1}$)	f_a
Acrylamide	40.1 ± 1.5	34.0 ± 1.3	0.270 ± 0.002	34.0 ± 1.4	27.6 ± 1.1	0.283 ± 0.003
CH_3COOTl	583.6 ± 3.5	495.4 ± 3.0	0.485 ± 0.029	727.7 ± 49.7	591.6 ± 40.4	0.415 ± 0.002

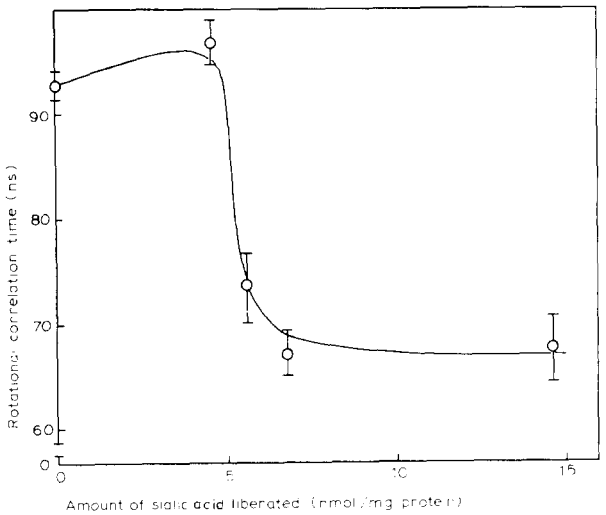

Fig. 4. Relationship between the rotational correlation time of diphenylhexatriene in the membranes and the amount of sialic acid liberated. The fluorescence anisotropy was measured under the following conditions: 10 mM Tris-maleate buffer (pH 6.85); membrane protein concentration, 150 μg/ml; 25°C. Values are expressed as mean ± S.D. for triplicate determinations.

dye to the membrane suspensions at 25°C was explored. Since diphenylhexatriene lacks any polar groups in the molecule, similarly to pyrene, an increased fluorescence intensity indicates an increase in solubilization of the dye molecules in the lipid domain of the membranes [21–23].

As can be seen in Fig. 3, the fluorescence development, which is represented per mg membrane protein, was dependent on the incubation time and reached an almost constant level within 5 min after the addition of the dye. The development of diphenylhexatriene fluorescence was marked enhanced by neuraminidase treatment of the membranes. In addition, the degree of fluorescence development of the dye after addition to the membranes was in proportion to the membrane protein concentration in the range from 25.4 to 152.4 μg/ml (inset, Fig. 3). Therefore, an increased fluorescence intensity of diphenylhexatriene in the neuraminidase-treated membranes suggests that solubilization of the dye molecules in the membrane lipids was enhanced.

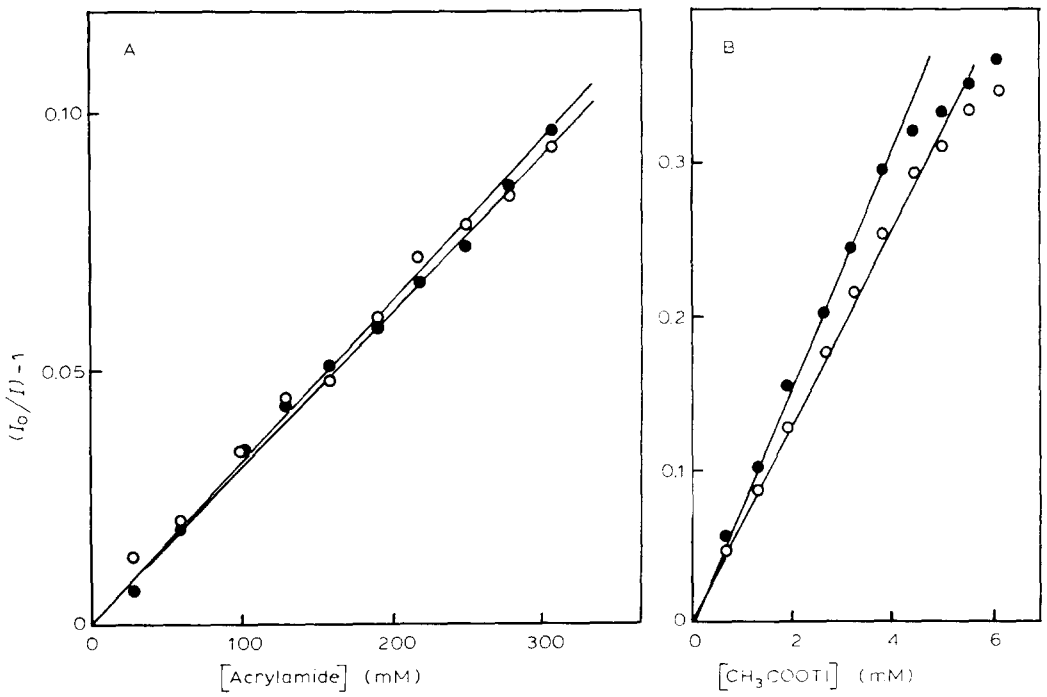

Fig. 5. Stern–Volmer plots of diphenylhexatriene-labeled membranes in 30 mM Tris-HCl buffer (pH 7.4) at 25°C. Membrane protein concentrations were 28.6 and 30.0 μg/ml for control (○) and neuraminidase-treated (●) membranes, respectively. The concentration of acrylamide and CH_3COOTl were varied from 33.1 to 312.5 mM and from 0.66 to 6.25 mM, respectively. (A) Acrylamide; (B) CH_3COOTl. Neuraminidase treatment conditions were the same as described in the legend to Fig. 1.

Rotational correlation time of diphenylhexatriene in the membranes

Fig. 4 shows the relationship between the rotational correlation time (θ) of diphenylhexatriene molecules embedded in the membrane lipids and the amount of sialic acid liberated by treatment with neuraminidase. In this experiment, the membrane vesicles with different levels of sialic acid content were prepared by incubating the membrane vesicles (1 mg protein/ml) with various concentrations of neuraminidase (1–20 μg/ml) for 30 min at 37° C.

when about 25% of the total sialic acids had been digested by neuraminidase treatment, the θ value of diphenylhexatriene in the membranes markedly decreased and then maintained a constant level, despite further digestion by the enzyme.

Quenching studies of diphenylhexatriene-labeled membranes

The plots of $(I_0/I) - 1$ vs. [Q] of diphenylhexatriene-labeled membranes for acrylamide and Tl^+ are shown in Fig. 5. In contrast to the case of pyrene-labeled membranes, the quenching of diphenylhexatriene fluorescence in the membranes by these quenchers followed a simple Stern–Volmer law. This difference may be due to highly ordered orientation of diphenylhexatriene molecules in the membrane lipids. The fluorescence lifetimes of diphenylhexatriene-labeled control and neuraminidase-treated membranes were 12.0 and 11.1 ns, respectively. Therefore, the quenching rate constants of the control membranes for acrylamide and Tl^+ were estimated to be $0.0267 \cdot 10^9$ and $5.56 \cdot 10^9$ $M^{-1} \cdot s^{-1}$, respectively. By treatment of the membranes with neuraminidase, the quenching rate constant for Tl^+ was slightly but definitely increased ($k_q = 6.76 \cdot 10^9$ $M^{-1} \cdot s^{-1}$). On the other hand, the quenching efficiency with acrylamide was hardly changed by the treatment ($k_q = 0.0279 \cdot 10^9$ $M^{-1} \cdot s^{-1}$), similar to the case of pyrene-labeled membranes.

Discussion

In the present study, it has been demonstrated that sialic acids in the porcine intestinal brush-border membranes are related to the dynamic properties of the lipid layers of the membranes.

A study of the temperature dependence of the fluorescence intensity of pyrene-labeled control membranes revealed a discontinuity at about 29° C (Fig. 1A). This result indicates that the ordering of lipid layers of the membranes transitionally alters at this temperature [11]. Upon treatment of the membranes with neuraminidase, the thermal transition temperature shifted to 23° C (Fig. 1B), suggesting that the environmental properties around pyrene molecules located in the membranes become more sensitive to temperature variation. The perturbation of the lipid layers by neuraminidase treatment was also reflected in a decrease in the fluorescence lifetime (Table II) and an increase in quenching efficiency for Tl^+ (Table III) of pyrene-labeled membranes. As is well known [14], the rate constant of quenching reaction between the quencher and a fluorophore bound to macromolecules is dependent on a diffusion-controlled process. Therefore an increased quenching rate generally suggests an increase in the proximity of quencher molecules to the fluorophore. When the quencher carries an electric charge, however, the quenching rate is also influenced by other facts, such as electrostatic forces and configuration of the binding sites [16,24–26]. Ando and Asai have recently reported [26] that Tl^+ interacts with phosphate groups of $1,N^6$-ethanoadenosine oligophosphate. In the present study, the quenching rate for acrylamide of pyrene- or diphenylhexatriene-labeled membranes was not influenced by neuraminidase treatment, although the quenching rates for Tl^+ were increased in both the membrane systems (Table III and Fig. 5). Therefore, it seems that an increased quenching rate for Tl^+ can be attributed mainly to the effect of the binding of Tl^+ ions to negatively charged groups of the membrane components rather than to an increase in the proximity of the quencher molecules to fluorophores in the membrane lipids. This indicates, therefore, that the environment of these fluorescence dyes embedded in the membrane lipids becomes more sensitive for to the binding of Tl^+ to membrane components.

In addition, an increase in solubilization of diphenylhexatriene in the membranes (Fig. 3) and a decrease in the rotational correlation time of diphenylhexatriene molecules in the membranes

(Fig. 4) also support the hypothesis that the lipid fluidity of the membranes increases by desialylation with neuraminidase treatment. From these results, it could be considered that sialic acids partially contribute to the maintenance of the lipid organization in the membranes.

The rotational correlation time of diphenylhexatriene molecules in the membrane lipids sharply decreased when only 25% of the total sialic acids in the membranes were cleaved by the enzyme. It is of interest that the lipid fluidity of the membranes is sensitively modified by elimination of very small amounts of sialic acids, because the lipid fluidity of biological membranes is known to be an important parameter in controlling their functional processes, including ion permeability [27,28] and glucose transport [29,30].

Sialic acid contributes as the charge-bearing group in biological membrane systems. Recently, Walter et al. [31] have reported that treatment of neuraminidase of rat erythrocytes results in a decrease in the surface charge. We have also found that the apparent dissociation constant of 1-anilino-8-naphthalene sulfonate (ANS) to the intestinal brush-border membranes decreases from 36.2 ± 1.44 to 27.0 ± 0.75 (S.D.) μM by neuraminidase treatment of the membranes. This increase in binding affinity of ANS anions to the membranes may be due to a decrease in negatively charged groups on the membrane components by desialylation. In addition, we have previously reported [20] that the lipid fluidity of the membranes increases with the shielding of negatively charged groups of the membranes with increasing ionic strength of medium. From these findings, therefore, it could be considered that changes in the membrane surface charge associated with desialylation by the enzyme treatment is partly related to an increase in the membrane lipid fluidity. Although it is not clear at present that changes in the fluorescence parameters of dye-labeled membranes by treatment with neuraminidase is due to elimination of sialic acids from the glycoproteins and/or glycolipids in the membranes, it seems likely that the sialic acids are one of the important elements regulating the lipid–lipid interactions in biological membranes.

References

1 Winzler, R.J. (1969) in Red Cell Membrane Structure and Function (Jamieson, G.A. and Greenwalt, P.J., eds.), pp. 157–171, Lippincott, Philadelphia, PA
2 Martinez-Polomo, A. (1970) Int. Rev. Cytol. 29, 29–75
3 Hakomori, S. (1983) Annu. Rev. Biochem. 50, 733–764
4 Sattler, J., Schwartmann, G., Staerk, J., Ziegler, W. and Wiegandt, H. (1977) Hopp-Seylers Z. Physiol. Chem. 358, 159–163
5 Momoi, T., Tokunaga, T. and Nagai, Y. (1982) FEBS Lett. 141, 6–10
6 Handa, S. and Nakamura, K. (1984) J. Biochem. 95, 1323–1329
7 Nakamura, K. and Handa, S. (1986) J. Biochem. 99, 219–226
8 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
9 Tada, M., Yamamoto, T. and Tonomura, Y. (1978) Physiol. Rev. 58, 1–79
10 Uchida, T., Nagai, Y., Kawasaki, Y. and Wakayama, N. (1981) Biochemistry 20, 162–169
11 Ohyashiki, T., Takeuchi, M., Kodera, M. and Mohri, T. (1982) Biochim. Biophys. Acta 688, 16–22
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
13 Warren, L. (1959) J. Biol. Chem. 234, 1971–1975
14 Kinoshita, K., Jr., Kataoka, R., Kimura, Y., Gotoh, O. and Ikegami, A. (1981) Biochemistry 20, 4270–4277
15 Stern, O. and Volmer, M. (1919) Z. Phys. 20, 183–188
16 Lehrer, S.S. (1971) Biochemistry 10, 3254–3263
17 Kim, Y.S. and Perdomo, J.M. (1974) Biochim. Biophys. Acta 342, 111–124
18 Ohyashiki, T., Ushiro, H. and Mohri, T. (1986) Biochim. Biophys. Acta 858, 294–300
19 Ohyashiki, T., Ohtsuka, T. and Mohri, T. (1986) Biochim. Biophys. Acta 861, 311–318
20 Ohyashiki, T., Taka, M. and Mohri, T. (1985) J. Biol. Chem. 260, 6857–6861
21 Fulford, A.J.C. and Peel, W.E. (1980) Biochim. Biophys. Acta 598, 237–246
22 King, G.P., Turner, J.S. and Thompson, G.A., Jr. (1980) Biochim. Biophys. Acta 598, 217–236
23 Ohyashiki, T., Kodera, M. and Mohri, T. (1985) J. Biochem. 98, 1441–1446
24 Pownall, H.J. and Smith, L.C. (1974) Biochemistry 13, 2594–2597
25 Eftink, M.R. and Ghiron, C.A. (1976) Biochemistry 15, 672–680
26 Ando, T. and Asai, H. (1980) J. Biochem. 88, 255–264
27 Lee, A.G. (1975) Prog. Biophys. Mol. 29, 5–56
28 Jain, M.K. and White, H.B. (1977) in Advances in Lipid Research (Paoletti, R. and Kritchevsky, D., eds.), Vol. 15, pp. 1–60, Academic Press, New York
29 Amatruda, J.M. and Finch, E.D. (1979) J. Biol. Chem. 254, 2619–2625
30 Czech, M.P. (1980) Diabetes 28, 399–409
31 Walter, H., Tamblyn, C.H., Krob, E.J. and Seaman, G.V.F. (1983) Biochim. Biophys. Acta 734, 368–372