CHANGE IN MEMBRANE ELASTIC MODULUS ON ACTIVATION OF GLUCOSE TRANSPORT SYSTEM OF BRUSH BORDER MEMBRANE VESICLES STUDIED BY OSMOTIC SWELLING AND DYNAMIC LIGHT SCATTERING

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ABSTRACT The cell membrane having a transport system is inferred to be flexible when its function is being activated. For the brush border membrane vesicles prepared from rat small intestine, which have the co-transport system of Na^+ and glucose, the membrane elasticity was measured as a function of the d-glucose concentration in the presence of Na^+ ions. The elastic modulus of the vesicle membrane was obtained by an osmotic swelling method. Osmolality was changed by diluting the extravesicular d-mannitol concentration. The change in the diameter of the membrane vesicle in response to an osmolality change was measured by the dynamic light-scattering method. The elastic modulus of the vesicle membrane decreased from 150 dyn/cm to 80 (45) dyn/cm with the increase of d-glucose, from 0 mM to 10 (30) mM in the presence of 10 mM Na^+ ions. On the other hand, in the presence of 1 mM phlorizin, a glucose-transport inhibitor, the elastic modulus remained at a constant value of 160 dyn/cm in the same range of the d-glucose concentration. This indicates that the vesicle membrane becomes flexible when its transport function is activated. In a broad osmolality range, the brush border membrane vesicle showed cycles of "swell-burst-reseal." The vesicle membrane became flexible after every cycle, namely, the modulus was 150, 120, and 55 in units of dyn/cm in the presence of 1 mM d-glucose and 50 mM Na^+ ions.

INTRODUCTION

The living cell is always changing its shape flexibly to activate its biological function and, in some cases, to adapt itself to its surroundings. A red blood cell, as a most interesting example, moves smoothly even in the thin vascular tubes, fulfilling its function effectively by changing its shape flexibly (Evans and Parsegian, 1983). Inside the cell, granules are moving smoothly in the cytoskeletal network from organella to organella and transporting materials (Allen, 1987). On the process of secretion in a secretory cell, a secretory granule exhibits a size change in a step of exocytosis (Finkelstein et al., 1986; Holz, 1986). Secretory granules, after fusion to the cell membrane, swell and release the contents to the luminal space (Zimmerberg et al., 1987; Breckenridge and Almers, 1987). The membrane elasticity can account for the size change of the vesicle in these processes. Of these membrane functions, the active transport of ions and low molecular weight materials plays an important role in the size change. Accordingly, it is important to make clear the correlation between membrane transport and its elasticity.

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The elastic modulus of the lipid bilayer vesicles was measured by an osmotic swelling method (Li et al., 1986; Sun et al., 1986). The response to an osmolality change can be measured as a size change of the vesicle by a dynamic light scattering method. Within the osmolality range where the change in the vesicle size is linear, the elastic modulus can easily be obtained. The osmotic swelling method in combination with the dynamic light scattering method seems to be very powerful, especially for vesicles with submicron sizes, such as brush-border membrane vesicles, secretory granules, and synaptic vesicles in the presynaptic axon.

The epithelial cell of the small intestine of the mammal has a remarkable transport system for ions and low molecular weight materials (nutrition). The transport system of the brush border membrane has been intensively investigated biochemically and physiologically (Schultz et al., 1974; Hopfer et al., 1973; Hopfer and Groseclose, 1980; Ullrich, 1979; Will et al., 1981). Conveniently, the membrane vesicles with a relatively sharp size-distribution can be easily made from the brush border membrane of the epithelial cell (Forstner et al., 1968; Hopfer et al., 1973; Eichholz and Crane, 1974; Hopfer et al., 1983). In this system, the intravesicular osmolality increases with glucose

transport and, as a result, the vesicle shows swelling (Miyamoto et al., unpublished observation).

Here, we discuss a correlation between the membrane elastic modulus for the change in the area of the vesicle membrane on swelling and an activation of the Na^+-glucose co-transport system of brush-border membrane vesicles. Some technical problems specific to dynamic light scattering from suspensions of spherical shells (or more generally, ellipsoidal shells of revolution) are discussed in an accompanying paper (Fujime et al., 1988).

MATERIALS

Vesicle Preparation

Brush-border membrane vesicles were prepared by the method of Hopfer et al. (1983). Sprague Dawley rats (male 150 ~ 300 g body weight) were anethesized with ethyl ether vapor or injection of Na-pentobarbital (0.1 ml per 100 g body weight) after fasting for 12 h, small intestines were cut out. Crude brush borders were isolated from small intestinal scrapings of the rat by the method of Forstner et al. (1968). The scrapings were homogenized in 500 ml of a hypotonic EDTA buffer (5 mM Na_3EDTA and 1 mM Hepes/Tris at pH 7.5) by use of a blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.) with its maximum speed; first for 2 s to disperse scrapings, and then for 10 s after being left standing on ice for 5 min. The homogenate was centrifuged at 400 *g* for 15 min at 2–4°C. This centrifuge was repeated several times until the supernatant became clear. The white pellets were suspended in 15 ml of an NaCl buffer (90 mM NaCl, 0.8 mM EDTA, and 1 mM Hepes/Tris at pH 7.5), and left standing on ice for 20–25 min for precipitation to form and to settle out of the solution. The white suspension on top was brought up to 30–40 ml in the NaCl buffer. The solution was centrifuged at 500 *g* for 10 min in order to collect brush border membrane while removing EDTA. Purity of the brush borders was monitored by a phase contrast microscope. More than 90% of the particles were brush borders at this step. The brush borders were resuspended in 10 ml of a mannitol buffer (200 mM d-mannitol and 1 mM Hepes/Tris at pH 7.4; the d-mannitol concentration was changed depending on experimental aims), and gently homogenized by hand (a few strokes) with a Dounce (glass/teflon) homogenizer (Ikemoto Rika Co. Ltd., Tokyo, Japan). After the addition of 5 M NaSCN to the final concentration of 0.5 M, the suspension was homogenized in glass/teflon homogenizer with a Craftmann-type drill press (Enshu Industrial Co. Ltd., Shizuoka, Japan) at 1,000 rpm (10 slow strokes). The homogenate was filled up to 100 ml with the mannitol buffer, and swirled in order for cytoskeletal materials to aggregate. In an ionic strength lower than 50 mM, the cytoskeletal meterials aggregated completely.

The filamentous materials were removed by filtration through gauze with a large mesh size. The filtrant was centrifuged at 6,000 *g* for 10 min. The supernatant was collected, and span at 34,000 *g* for 30 min. The pellets were resuspended, on ice, in 1 ml of the mannitol buffer containing 0.2 mM $MgSO_4$ by sucking up and down through a 27-gauge needle. The suspension was centrifuged at 3,000 *g* for 5 min to minimize dusts in the light-scattering sample. The supernatant was carefully aspirated by use of a 1-ml syringe with a 27-gauge needle. The membrane vesicles were immediately used for the osmotic swelling and transport experiments.

Biochemical Assay

The protein concentration was determined by the method of Lowry et al. (1951) and modified by Bensadoun and Weinstein (1976), with bovine serum albumin (BSA) as standard. Alkaline phosphatase activity was measured by the method of Forstner et al. (1968) with p-nitrophenyl phosphate (104; Sigma Chemical Co., St. Louis, MO) as substrate. Disaccharidase (sucrase) activity was measured by the method of Dahlqvist (1964).

Reagents

Phlorizin was purchased from Sigma Chemical Co., and recrystallized several times from hot water before use. Because of low solubility of phlorizin, precautions were paid for its use in the light-scattering experiment. All other reagents used in this study were of special grade from Sigma Chemical Co.

METHODS

Measurements of Elastic Modulus by Osmotic Swelling

The elastic modulus of the membrane vesicle was determined by the swelling method. Vesicles (spherical in shape) with an initial diameter d_o are prepared in an aqueous solution containing C_o (mol/l) of solute. A dilution buffer is then added to the sample in the scattering cell to reduce the final external concentration of the solute to C_e. Assume that the vesicle is permeable to water but not to the solute. Then, water flows into the vesicle, and the vesicle expands until the final internal concentration C_i of solute is reached with the final diameter d_f, where the osmotic pressure difference, $P_1 - P_2$, across the vesicle wall just balances against the elastic force produced by the expansion. Van't Hoff's law gives $P_1 - P_2 = K_o(C_i - C_e)$, where K_o is the osmotic coefficient. On the other hand, the stress, T_s, which induces the change in the membrane area, $\Delta A = (d_f^2 - d_o^2)/d_o^2$, can be given by $T_s = \pi \times (d_f/2)^2(P_1 - P_2)/(\pi d_f) = (d_f/4)(P_1 - P_2)$. Let us define the elastic modulus, M (dyn/cm), by $T_s = M\Delta A$. By noting $C_i = (d_o/d_f)^3 C_o$ (the factor $(d_o/d_f)^3$ is a correction due to the volume change), the following relation is established;

$$d_f - d_o = \frac{d_o^2 d_f K_o}{4(d_o + d_f)} (1/M)[(d_o/d_f)^3 C_o - C_e] \tag{1}$$

$$\doteq (d_o d_f K_o/4)(1/2M)[(d_o/d_f)^3 C_o - C_e]. \tag{1a}$$

In Eq. 1a, use was made of an approximation $d_o + d_f \doteq 2d_o$ for a small expansion. The modulus M in Eq. 1 is the same as the "elastic area compressibility modulus K" of Evans et al. (1976), and k of Sun et al. (1986). If we put $M = M_e t$ (t being the thickness of the membrane bilayer), Eq. 1a becomes the same expression as that of Li et al. (1986). M_e of Li et al. is defined as the purely elastic modulus, whereas M is defined as the elastic modulus including the effect of the surface tension. In deriving Eq. 1, we ignored the changes in the thickness and curvature of the vesicle membrane on swelling. These are supported by the estimation in Li et al. (1986). If we can measure the exact diameters, d_o and d_f, of the membrane vesicle at the initial and final concentrations of the solute, C_o and C_e, respectively, the membrane modulus M can be easily evaluated by use of Eq. 1.

Measurements of Vesicle Diameter by Dynamic Light Scattering

The dynamic light-scattering method was used to obtain the exact value of the average diameter of the membrane vesicles in the solution. General background information about the dynamic light-scattering method is found in standard textbooks (Chu, 1974; Berne and Pecora, 1975). A 488.0-nm beam from an Ar^+ laser (Model 95; Lexel Corp., Palo Alto, CA) was used as the light source. Details of our spectrometer were described elsewhere (Fujime et al., 1984). The temperature of the sample was controlled at (20.0 ± 0.05)°C. An (8 × N)-bit digital correlator (K7032-CE; Malvern Instruments, Worcestershire, U.K.) was used to measure the intensity correlation function, $G^2(\tau)$, of the scattered light. $G^2(\tau)$ is related to the normalized field correlation function, $g^1(\tau)$, of the scattered light by

$$G^2(\tau) = B[1 + \beta|g^1(\tau)|^2], \tag{2}$$

where B is the baseline (known from data in the monitor channels of the correlator) and β is a machine constant. For a polydisperse system, $g^1(\tau)$ is generally expressed as

$$g^1(\tau) = \int_0^\infty G(\Gamma) \exp(-\Gamma\tau)d\Gamma \text{ with } \int_0^\infty G(\Gamma)d\Gamma = 1, \quad (3)$$

where $G(\Gamma)$ is the distribution function of the decay rate Γ of $g^1(\tau)$. To obtain the average decay rate $\bar{\Gamma}$ of $g^1(\tau)$, we routinely adopted the second-order cumulant expansion method (Koppel, 1972):

$$g^1(\tau) = \exp[-\bar{\Gamma}\tau + (\mu_2/2!)\tau^2], \quad (4)$$

where $\bar{\Gamma} = \int \Gamma G(\Gamma)d\Gamma$ and $\mu_2 = \int (\Gamma - \bar{\Gamma})^2 G(\Gamma)d\Gamma$. $(\mu_2/\bar{\Gamma}^2)$ is a measure of the dispersion in $G(\Gamma)$. The average diffusion coefficient, D, and the diameter, d, of the membrane vesicle can be obtained, respectively, by the relations

$$\bar{\Gamma} = DK^2 \quad \text{and} \quad D = k_B T/(3\pi\eta d) \quad (5)$$

where $K = (4\pi n/\lambda_o)\sin(\theta/2)$ is the length of the scattering vector (n: the index of refraction of the solvent, λ_o: wavelength of the incident light in vacuum, and θ: the scattering angle), k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity. In our experiments described below, d-mannitol was used as the solute. The viscosity and index of refraction of the mannitol solution was obtained from the CRC Handbook of Chemistry and Physics (Weast, 1976). The most probable d-value for a given condition was determined by averaging d-values from ten successively measured $G^2(\tau)$s.

RESULTS

Membrane Vesicles

Table I summarizes the results of assay of the protein concentration and enzyme activities. The activities of the marker enzymes, sucrase, and alkaline phosphatase, of the membrane vesicles were, respectively, 100 and 50 times over those of the initial homogenate. These enrichments ensured that brush border membrane vesicles were pretty purified.

From several examinations in the accompanying paper (Fujime et al., 1988), our vesicles were concluded to be ellipsoidal in the shape, and relatively narrow in the size distribution. Possible complications due to the nonspherical shape and size distribution of the vesicles have been discussed in the accompanying paper. Here, we describe only the swelling experiments, which were carried out at the scattering angle of 90°.

TABLE I
SUMMARY OF BIOCHEMICAL ASSAY*

Fraction	Total protein	Alkaline phosphatase	Sucrase
	mg	U/mg‡	
Homogenate	784	0.028	0.04
Brush border	14.3	1.09	1.34
Membrane vesicle	1.8	1.39	3.94
Protein enrichment	—	50 ± 9	98 ± 35

*Figures in the table show the results of assay for one particular preparation.
‡U: international units (in μmol/min).
§The average values of five preparations.

Osmotic Swelling

The membrane vesicles were prepared in the solution containing 200 mM d-mannitol (as the solute), 1 mM Hepes/Tris at pH 7.4, and 0.2 mM $MgSO_4$. The vesicle suspension in the scattering cell was incubated for 10 min after addition of 10 mM NaSCN and the indicated amount of d-glucose, and then the swelling experiment was initiated. The dilution buffer contained 1 mM Hepes/Tris at pH 7.4, 10 mM NaSCN, the indicated amount of d-glucose, and 0.2 mM $MgSO_4$, unless otherwise stated. Addition of the dilution buffer to the vesicle suspension in the scattering cell was made at a low speed of 10 μl/min by use of a micro-injection pump. Every after addition of the 50-μl dilution buffer, ten correlation functions, $G^2(\tau)$s, were measured successively with a data accumulation period of 120 s/run.

An example of the results of the dynamic light-scattering measurements is listed in Table II, which gives an overall impression about the sizes of the diameter, of its standard deviation (S.D.), and of $(\mu_2/\bar{\Gamma}^2)$. The dynamic light-scattering method gave quite exact values of the average vesicle diameter.

Fig. 1 shows examples of a series of the swelling experiments at various d-glucose concentrations. On lowering the external osmolality C_e, a good linear relationship between $(d_f - d_o)$ and $(C_o - C_e)$ was observed for a certain osmolality range. Eq. 1 shows that $(d_f - d_o)$ is practically linear in $(C_o - C_e)$ for the present range of $(d_f - d_o)/d_o < 0.04$. The slope of the straight line became larger with the increase of the d-glucose concentration. The membrane vesicle swelled until its elastic limit, and then burst. As indicated by arrows, the linear relationship broke at a certain osmolality, which shifted toward the higher osmolality region with the increase of the d-glucose

TABLE II
AN EXAMPLE OF THE RESULTS OF SWELLING EXPERIMENTS OF BRUSH BORDER MEMBRANE VESICLES

External osmolalily	Diameter	S.D.	$\mu_2/\bar{\Gamma}^2$
200	327.5	1.7_5	0.14_5
191	329.1	2.4	0.13
183	329.4	3.3_5	0.14_5
176	329.7	1.6	0.13
169	332.2	2.3	0.13_8
163	333.3	2.2	0.13_5
157	334.1	0.8	0.15_6
152	334.7	1.2_5	0.15
147	335.0	0.8_5	0.11_8
142	335.4	3.6	0.11_8

Each figure shows the average of ten measurements at each osmolality. For experimental conditions, see legend to Fig. 1.

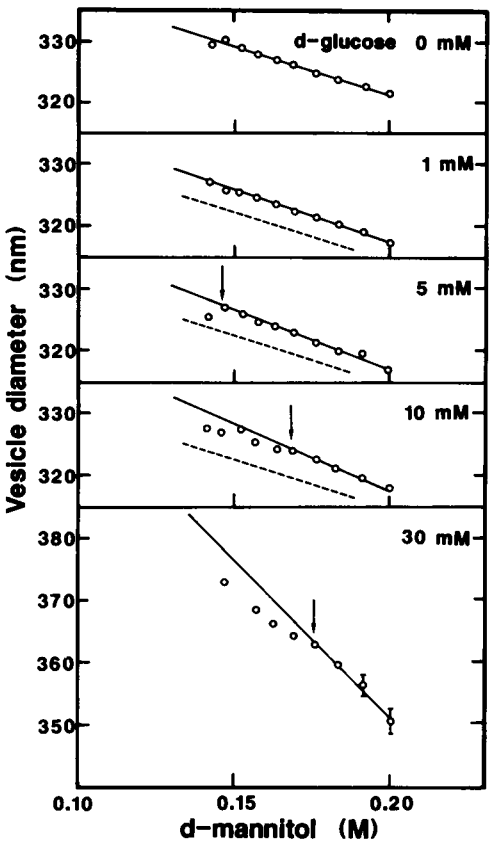

FIGURE 1 Osmotic swelling curves of brush border membrane vesicles. The vesicle suspension (100 μg/ml protein) was prepared in 200 mM d-mannitol, 1 mM Hepes/Tris at pH 7.4, and 0.2 mM $MgSO_4$, and incubated for 10 min at 20°C after the addition of 10 mM NaSCN, and indicated amounts of d-glucose. The dilution buffer containing 1 mM Hepes/Tris at pH = 7.4, 10 mM NaSCN, the indicated amount of d-glucose, and 0.2 mM $MgSO_4$ was added in steps of 50 μl at a speed of 10 μl/min. In each panel, the arrow indicates the burst point, and the solid line shows the best-fit swelling curves based on Eq. 1. The error bars represent the standard deviation (S.D.) of the vesicle diameter. The circles without error bars have S.D. of about the double-size of the symbol. The dashed lines show the slope of the straight line at 0 mM d-glucose. See text for an anomalously large initial diameter at 30 mM d-glucose.

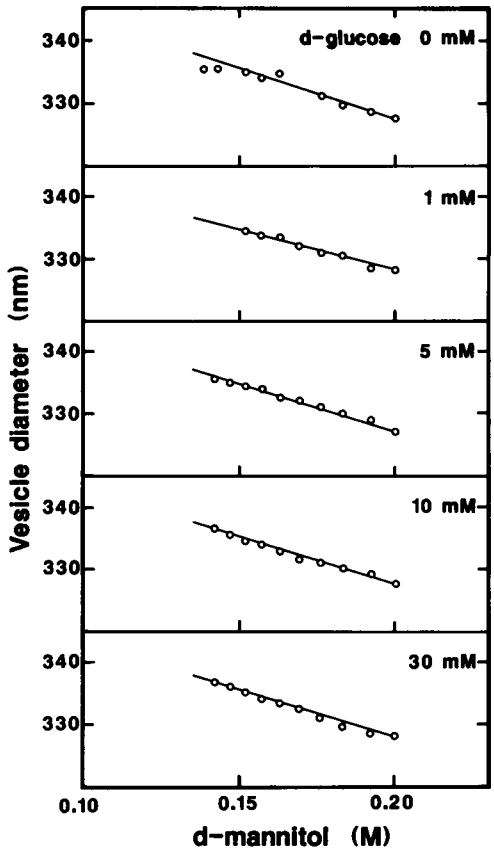

FIGURE 2 Osmotic swelling curves of brush border membrane vesicles in the presence of 1 mM phlorizin. All other conditions were the same as those in Fig. 1.

concentration. These results suggest that the membrane became flexible with the increase of the d-glucose concentration. We do not know what happened during the incubation after the addition of NaSCN and d-glucose, and we have no definite idea about a very large initial diameter of the vesicle at 30 mM d-glucose. If this was a result of swelling due to uptake of d-glucose, the vesicle burst because of very large expansion, (350–320)/320 = 0.09. Thus, the data at 30 mM d-glucose provide only a reference.

On the other hand, osmotic swelling was observed to be independent of the d-glucose concentrations in the presence of 1 mM phlorizin, which has been known as an inhibitor of d-glucose transport. As shown in Fig. 2, all the straight lines were highly parallel with each other in all conditions studied so far. This result shows that the effect of the glucose–transporter protein on the elasticity of the membrane vesicle was completely inhibited by binding of phlorizin. The binding constant of phlorizin to the glucose carrier has been estimated to be 1–10 μM (Aronson, 1978). Consequently, all glucose carriers of the membranes at a vesicle concentration of 100 μg/ml protein are blocked in the presence of 1 mM phlorizin. It should be noted that the initial diameter of the vesicle at 30 mM d-glucose had a normal size in contrast with the case in Fig. 1.

Eq. 1 was applied to the data in Figs. 1 and 2 to obtain the elastic modulus M. Fig. 3 shows the elastic modulus of the membrane vesicle as a function of the d-glucose concentration in the absence (open circles) and presence (filled circles) of 1 mM phlorizin. In the presence of phlorizin, the elastic modulus was constant, 160 dyn/cm, over the glucose concentrations studied so far. In the absence of phlorizin, on the other hand, the elastic modulus decreased from 150 dyn/cm at 0 mM glucose to 80 (45) dyn/cm at 10 (30) mM d-glucose. These results suggest that the flexibility of the vesicle membrane has a certain relation with the membrane function of d-glucose transport.

Fig. 4 shows the elastic modulus of the vesicle membrane as a function of the Na^+ ion concentration in the

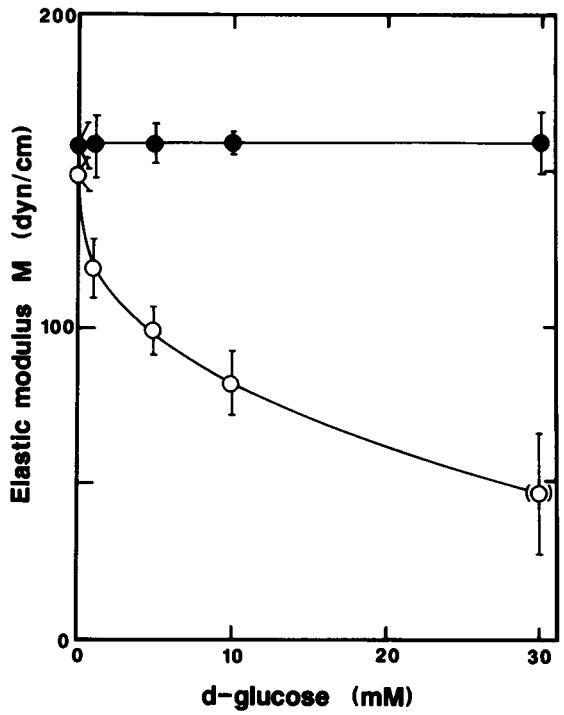

FIGURE 3 The membrane elastic modulus as a function of the d-glucose concentration. Eq. 1 was applied to the data in Figs. 1 and 2 to obtain the elastic modulus values in the absence (*open circles*) and presence (*filled circles*) of 1 mM phlorizin.

absence of d-glucose. Sodium ions rather increased the elastic modulus. This is consistent with the results for the synthetic phospholipid vesicles (Li et al., 1986). Namely, the apparent charge density on the membrane surface was reduced in the presence of neutral salt, and as a result the repulsive forces in the surface direction could be decreased.

For a comparison, we also prepared the membrane vesicles, where the cytoskeletal materials were not removed by intentionally skipping a step to treat the brush borders with high salt (0.5 M NaSCN; see Materials section). Fig. 5 shows the elastic modulus of such vesicle membranes as a function of the d-glucose concentration. Here, we did not take account of a dead volume occupied by cytoskeletal materials in the vesicle. This dead volume would decrease the size of the modulus, and the correction would become large when the membrane became flexible (the d-glucose concentration increased). Since, however, the size of the dead volume was not known, this correction was not made in Fig. 5. The sizes of the modulus were two to three times larger than those shown in Fig. 3, but they should be regarded as upper bounds of the modulus of the vesicle with cytoskeletal materials inside. The large error size was mostly due to less accuracy in the measurement of $(d_f - d_o)$ in this preparation. In spite of the large error size, the data again showed that the modulus clearly decreased with the increase of the d-glucose concentrations in the presence of 10 mM NaSCN.

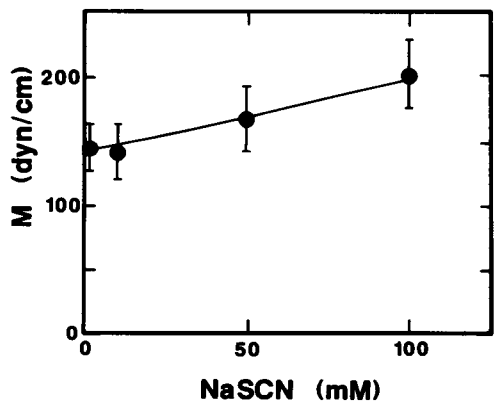

FIGURE 4 The membrane elastic modulus as a function of the Na^+ ion concentration in the absence of d-glucose. All other conditions were the same as those in Fig. 1.

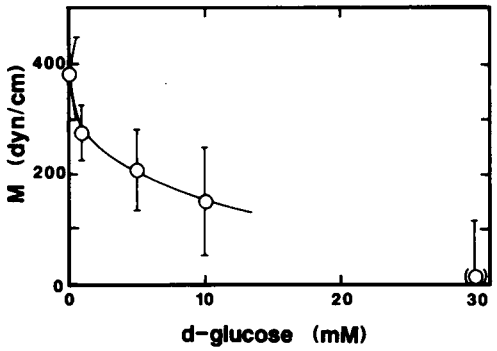

FIGURE 5 The membrane elastic modulus as a function of the d-glucose concentration. Only in this preparation of the membrane vesicles, the cytoskeletal materials were not removed by intentionally skipping a step to treat the brush borders in 0.5 M NaSCN (see Materials section). All other conditions were the same as those in Fig. 1.

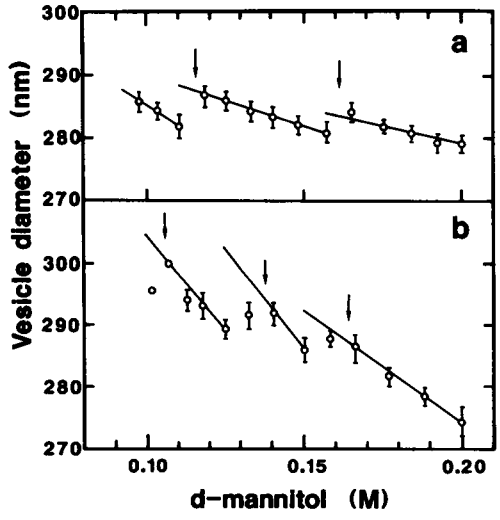

FIGURE 6 Osmotic swelling curves of brush-border membrane vesicles. All conditions were the same as those in Fig. 1, except 50 mM NaSCN and the d-glucose concentrations of 1 mM in *a* and 50 mM in *b*. Arrows indicate the burst points.

Burst and Reseal

Fig. 6 shows the diameter of the membrane vesicle as a function of the osmolality in a broad range from 200 mM to <100 mM d-mannitol. The quality of data in Fig. 6 is not so good, because the measurements were made as a gross survey at the very initial stage of this study. However, the data show some features characteristic of osmotic swelling of the membrane vesicle. The size of the vesicle changed with maxima and minima (and plateaus). These results reflect that the membrane vesicle repeated cycles of "swell-burst-reseal" along with the dilution (Li et al., 1986). For the data in Fig. 6 *a* where 50 mM NaSCN and 1 mM d-glucose were present, the elastic modulus was obtained to be ~140, 120, and 44 dyn/cm in the osmolality ranges, respectively, from 200 to 165 mM, 157 to 118 mM, and below 110 mM. This result shows that the vesicle membrane became more flexible after every bursting. In the presence of 50 mM NaSCN and 50 mM d-glucose (Fig. 6 *b*), the swelling rate of the vesicle became larger, and the burst point shifted to the higher osmolality than those in the presence of 1 mM d-glucose. For the data in Fig. 6 *b*, the elastic modulus was obtained to be ~70, (65) and 25 dyn/cm in the osmolality ranges, respectively, from 200 to 176 mM, (152 to 148 mM) and 124 to 105 mM.

Table III lists the transmembrane pressure difference (ΔP_b) and surface area increase (ΔA_b) at bursting of vesicles. In spite of the large changes in ΔP_b with d-glucose concentrations, ΔA_b changed very little.

DISCUSSION

Measurements of Membrane Elastic Modulus

For several biological membranes, the elastic modulus has been measured by various methods. A method of capilary aspiration has been extensively applied in order to obtain the membrane modulus, especially of the erythrocyte (Evans et al., 1976; Waugh and Evans, 1979; Dimitrov et al., 1978). However, this method has disadvantages such as: (*a*) A mechanical damage will occur in the membrane during aspirating. (*b*) Application of this method is limited to cells and huge vesicles whose sizes can be measured easily under a light microscope. Consequently, this method is not applicable to the granules and vesicles with submicron sizes. Katcharsky et al. (1960) were the first to study erythrocytes by the method of osmotic swelling kinetics. Recently, the osmotic swelling method in combination with the dynamic-light scattering method was used to obtain the membrane modulus of the synthetic vesicles with diameters ranging from 200 to 450 nm (Li et al., 1986), and with diameters ranging from 60 to 110 nm (Sun et al., 1986).

By the method of capilary aspiration, the elastic modulus of the biological membranes was obtained to be 250 dyn/cm for the erythrocyte (Evans et al., 1976) and 120 dyn/cm for Rye protoplast (Wolfe and Steponkus, 1983). By the method of osmotic swelling, Haines et al. (1987) gave $M_e = 9.0 \times 10^8$ dyn/cm^2 ($M = M_e t = 450$ dyn/cm for $t = 5$ nm) for *E. coli* Lac permease vesicles. Here, we obtained the values of (45–200) dyn/cm (cf., Figs. 3 and 4) for vesicles which were prepared by the method including a step to remove cytoskeletal materials. For the vesicles prepared by the method which did not include a step to remove the cytoskeletal materials, we obtained the values of (100–400) dyn/cm (cf., Fig. 5). These values for brush border membrane vesicles fall in the same range as those for other biological membranes.

Since our vesicles are believed to be prolate ellipsoidal, the absolute values of M given in this paper are conditional upon discussion in the accompanying paper (Fujime et al., 1988); the size of the modulus given above may be $(1 + \Delta)$ times larger than the true one for $d_f = (1 + \Delta)d_f^o$ and $d_o = (1 + \Delta)d_o^o$, where the superscript "o" denotes the true quantity. Although the size of expansion is very small, applications of Eq. 1 to the ellipsoidal shell produces another ambiguity. However, the relative sizes of the M values for a given preparation (Figs. 3–5) are highly meaningful.

TABLE III
TRANSMEMBRANE PRESSURE DIFFERENCE (ΔP_b) AND SURFACE AREA INCREASE (ΔA_b)* AT BURSTING OF VESICLES

run	d-glucose	Na^+	ΔP_b	ΔA_b
	mM	mM	mOsm	%
Fig. 1	0	10	39	5.5
	1	10	38	6.0
	5	10	32	6.4
	10	10	18	6.4
	30	10	(5)	(6.6)
Fig. 6 *a*	1	50	26	3.6
	1	50	28	4.3‡
Fig. 6 *b*	50	50	15	10

*$\Delta A_b = (d_b^2 - d_0^2)/d_0^2 \doteq 2(d_b - d_0)/d_0$ (assumed spherical in vesicle shape), where d_b is the diameter of the vesicle at bursting.
‡$\Delta A_b \doteq 2(d_b - d_r)/d_r$, where d_b and d_r are the diameters of the vesicle at the second bursting and the first resealing, respectively.

Membrane Elastic Modulus and Glucose Transport

As is well known, brush border membranes from the small intestine of mammals have the transport systems for various ions, amino acids, and saccharides. Especially, amino acids and saccharides are transported by the co-transport systems with Na^+ ions. In these transport systems, the electro-chemical gradient of Na^+ ions drives the transport process. For the co-transport system of d-glucose and Na^+ ions, glucose uptake increases with the increase of extravesicular Na^+ and glucose concentrations, as extensively investigated by a method of radio-isotope uptake (Hopfer and Groseclose, 1980).

The membrane elastic modulus rather increased with

the increase of the Na^+ ion concentration in the absence of glucose (Fig. 4). In the absence of Na^+ ions, the elastic modulus was independent of the d-glucose concentration in the present range (data not shown). In the presence of 1 mM phlorizin, the membrane elastic modulus did not show any change depending on concentrations of Na^+ ions and d-glucose (Fig. 3). As is well known, phlorizin is a competitive inhibitor, and its binding constant is much greater than that of glucose. In the presence of 1 mM phlorizin, all glucose carriers in the membrane vesicles bind phlorizin as schematically shown in Fig. 7 *c*. In the absence of phlorizin, on the other hand, the elastic modulus greatly decreased with the increase of the d-glucose concentration when Na^+ ions were present (Figs. 3, 5, and 6). This can be interpreted as schematically shown in Fig. 7 *b*: In the carrier-mediated transport system, a change in the conformation of the carrier protein plays an important role in the interaction between the carrier proteins and lipids. An idea is then probable that the vesicle membrane becomes flexible (or flaccid) as a result of a change in the conformation of the carrier protein due to the binding of glucose, the transport substrate. The present result suggests that the elasticity of the brush border membrane has a strong correlation with the activation of the glucose carriers. This idea is consistent with the result of Brasitus' group (Brasitus et al., 1979; Brasitus and Schachter, 1980): The Arrhenius plot of the d-glucose transport activity of the rat brush-border membrane vesicle exhibits a slope change at 27–29°C. This corresponds to a thermotropical transition of fluorescence polarization at (26 ± 2)°C, suggesting that the d-glucose transport protein has a strong interaction with annular lipids through the hydrophobic core.

The surface area increase ΔA_b at bursting (Table III) is ~6% in our brush-border membrane vesicles. This size of ΔA_b is comparable with that of synthetic phospholipid bilayer vesicles, 4–9% (Li et al., 1986). Very weak dependence of ΔA_b on glucose concentrations suggests that the size of ΔA_b is mostly determined by the lipid–lipid interaction. On the other hand, strong dependence of ΔP_b and M on glucose concentrations suggests that the sizes of ΔP_b and M are again determined by the lipid–lipid interaction, but largely modified by the protein-lipid interaction.

From the kinetic experiment on this Na^+-glucose co-transport system, the following Na^+-gating scheme is considered to be the most acceptable (Hopfer and Groseclose, 1980). (*a*) A role of Na^+ ions to the glucose carriers is to increase the binding activity of glucose to the carrier; Na^+ ions play a role of the trigger to open the gate for glucose. (*b*) The binding of glucose to the carrier protein induces a change in the conformation of the protein. Simultaneously, a significant change takes place on the interaction between membrane lipids and carrier proteins. As a result, the whole vesicle membrane becomes flexible or flaccid. Then, Na^+ ion and d-glucose move across the membrane in this order. (*c*) On the inside surface of the vesicle, Na^+ ion and glucose come off from the carrier. This scheme is consistent with our observation.

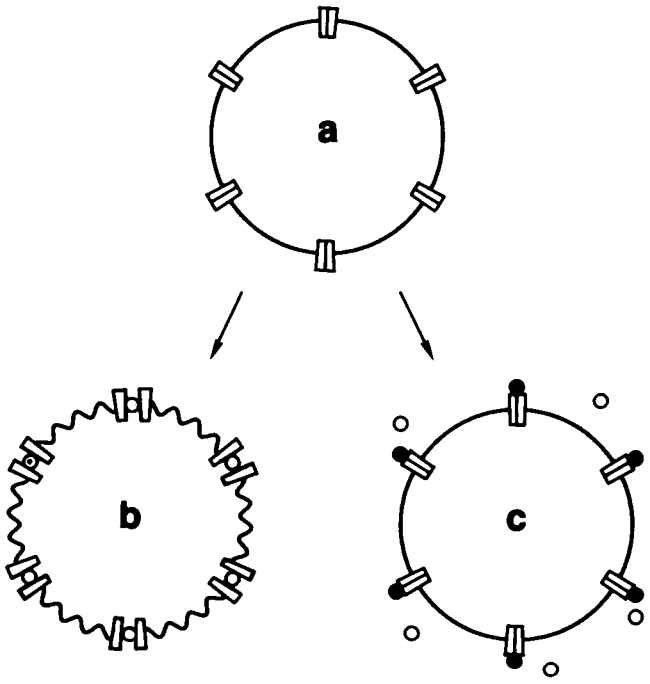

FIGURE 7 Schematic illustration of the brush border membrane vesicle. Only d-glucose carriers are explicitly shown. Open circles denote d-glucose molecules, and filled circles denote phlorizin molecules. The vesicle in the resting (*a*), in the activated (*b*), and in the inhibited (*c*) states of d-glucose carriers. The wavy surface in *b* symbolically denotes a different carrier-lipid interaction from those in *a* and *c*.

CONCLUSIONS

To the best of our knowledge, we first observed a clear correlation between the membrane elastic modulus and the activation of the Na^+-glucose co-transort system by use of the brush border membrane vesicles. As a result, it is generally speculated that the biological membrane becomes more flexible when its function is activated.

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REFERENCES

Allen, R. D. 1987. The microtubule as an intracellular engine. *Sci. Am.* 256:26–33.

Aronson, P. S. 1978. Energy-dependence of phlorizin bindings to isolated renal microvillus membranes. *J. Membr. Biol.* 42:81–98.

Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* 70:241–250.

Berne, B., and R. Pecora. 1975. Dynamic Light Scattering. John Wiley & Sons, Inc., New York. 376 pp.

Brasitus, T. A., D. Schachter, and T. G. Mamouneas. 1979. Functional interactions of lipids and proteins in rat intestinal microvillus membranes. *Biochemistry.* 18:4136–4144.

Brasitus, T. A., and D. Schachter. 1980. Lipid dynamics and lipid-protein interactions in rat enterocyte basolateral and microvillus membranes. *Biochemistry.* 19:2763–2769.

Breckenridge, L. J., and W. Almers. 1987. Final steps in exocytosis observed in a cell with giant secretory granules. *Proc. Natl. Acad. Sci. USA.* 84:1945–1949.

Chu, B. 1974. Laser Light Scattering. Academic Press, Inc., New York. 317 pp.

Dahlqvist, A. 1964. Method for assay of intestinal disaccharidases. *Anal. Biochem.* 7:18–25.

Dimitrov, D. S., I. Ponalotov, P. Richmond, and L. Ter-Minnassian-Saraga. 1978. Dynamics of insolble monolayers; dilatational or elastic modulus, friction coefficient, and Marangoni effect for dipalmitoyl lecithin monolayers. *J. Colloid Interface Sci.* 65:483–492.

Eichholz, A., and R. K. Crane. 1974. Isolation of plasma membranes from intestinal brush borders. *In* Method in Enzymology. S. Fleischer, and L. Packer, editors. 31:123–134.

Evans, E. A., R. Waugh, and L. Melnik. 1976. Elastic area compressibility modulus of red cell membrane. *Biophys. J.* 16:585–595.

Evans, E. A., and V. A. Parsegian. 1983. Energetics of membrane deformation and adhesion in cell and vesicle aggregation. *Ann. NY Acad. Sci.* 416:13–33.

Finkelstein, A., J. Zimmerberg, and F. S. Cohen. 1986. Osmotic swelling of vesicles: its role in the fusion of vesicles with planar phospholipid bilayer membranes and its possible role in exocytosis. *Annu. Rev. Physiol.* 48:163–174.

Forstner, G. G., S. M. Sabesin, and K. J. Isselbacher. 1968. Rat intestinal microvillus membranes: purification and biochemical characterization. *Biochem. J.* 106:381–390.

Fujime, S., S. Ishiwata, and T. Maeda. 1984. Dynamic light-scattering study of muscle F-actin. *Biophys. Chem.* 20:1–21.

Fujime, S., M. Takasaki-Ohsita, and S. Miyamoto. 1988. Dynamic light scattering from polydisperse suspensions of thin ellipsoidal shells of revolution with submicron diameters. *Biophys. J.* 53:497–503.

Haines, T. H., W. Li, M. Green, and H. Z. Cummins. 1987. The elasticity of uniform, unilamellar vesicles of acidic phospholipids during osmotic swelling is dominated by the ionic strength of the media. *Biochemistry.* 26:5439–5447.

Holz, R. W. 1986. The role of osmotic forces in exocytosis from adrenal chromattin cells. *Annu. Rev. Physiol.* 48:175–189.

Hopfer, U., T. D. Crowe, and B. Tandler. 1983. Purification of brush border membrane by thiocyanate treatment. *Anal. Biochem.* 131:447–452.

Hopfer, U., and R. Groseclose. 1980. The mechanism of Na^+-dependent d-glucose transport. *J. Biol. Chem.* 255:4453–4462.

Hopfer, U., K. Nelson, J. Perroto, and K. J. Isselbacher. 1973. Glucose transport in isolated brush border membrane from rat small intestine. *J. Biol. Chem.* 248:25–32.

Kachalsky, A., O. Kedem, C. Klibansky, and A. DeVries. 1960. Rheological considerations of the haemolysing red blood cell. *In* Flow Properties of Blood and Other Biological Systems. A. L. Copley, and G. Stainsby, editors. Pergamon Press Inc., Oxford, pp 155–171.

Koppel, D. E. 1972. Analysis of macromolecular polydispersity in intensity correlation spectroscopy: the method of cumulant. *J. Chem. Phys.* 57:4814–4820.

Li, W., T. S. Aurora, T. H. Haines, and H. Z. Cummins. 1986. Elasticity of synthetic phospholipid vesicles and submitochondrial particles during osmotic swelling. *Biochemistry.* 25:8220–8229.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Schultz, S. G., R. A. Frizzell, and H. N. Nellans. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* 36:51–92.

Sun, S. T., A. Milon, T. Tanaka, G. Ourisson, and Y. Nakatani. 1986. Osmotic swelling of unilamellar vesicles by the stopped flow light scattering method: elastic properties of vesicles. *Biochim. Biophys. Acta.* 860:525–530.

Ullrich, K. J. 1979. Sugar, amino acid, and Na^+ cotransport in the proximal tubule. *Annu. Rev. Physiol.* 41:181–195.

Waugh, R., and E. A. Evans. 1979. Thermoelasticity of red blood cell membrane. *Biophys. J.* 26:115–132.

Weast, R. C. 1976. CRC Handbook of Chemistry and Physics, 57th Edition (1976–1977). R. C. Weast, editor. CRC Press, Cleveland, Ohio.

Will, P. C., R. C. DeLisle, R. N. Cortright, and U. Hopfer. 1981. Induction of amiloride-sensitive sodium transport in the intestines by adrenal steroids. *Ann. NY Acad. Sci.* 372:64–78.

Wolfe, J., and P. L. Steponkus. 1983. Mechanical properties of the plasma membrane of isolated plant protoplasts. *Plant Physiol. (Bethesda).* 71:276–285.

Zimmerberg, J., M. Curran, F. S. Cohen, and M. Brodwick. 1987. Simultaneous electrical and optical measurements show that membrane fusion precedes secretory granule swelling during exocytosis of beige mouse mast cells, *Proc. Natl. Acad. Sci. USA.* 84:1585–1589.