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Ionic Permeability of Sarcoplasmic Reticulum Vesicles Measured by Light Scattering Method

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Summary. The volume change of sarcoplasmic reticulum vesicles was followed by measuring the light scattering intensity. When the salt concentration of the suspension of sarcoplasmic reticulum vesicles was increased by using a stopped flow apparatus, the light scattering intensity rapidly increased at the beginning and then decreased. The fast increase in the light scattering intensity is caused by the decrease of the volume of sarcoplasmic reticulum vesicles due to the outflow of water. The following decrease in the light scattering intensity is caused by the increase of the volume due to the inflow of the solutes and water. From the former and the latter rates, the permeation times of water and the solutes could be calculated, respectively. According to the same method, permeation times of various salts were determined. The rate of the inflow of the salts was dependent on the movement of the slower ions, that is, ions move as a pair.

In the case of potassium salts, an increase in the permeation rate of the salts was observed when valinomycin was added to the membrane suspensions. From these experiments, as a measure of permeability, half permeation times of various ions and molecules were determined. The following are typical results: water 0.1, Li^+ 36, Na^+ 26, K^+ 20, Rb^+ 16, Cl^- 0.4, methanesulfonate 20, phosphate 10.5, oxalate 40 in seconds at room temperature. As a whole, sarcoplasmic reticulum was found to be an anion permeable membrane.

In the previous papers (Kasai & Miyamoto, 1976*a–b*), we examined calcium release from sarcoplasmic reticulum (SR) vesicles and showed that SR responds to the anion replacement from methanesulfonate (MS) to chloride by releasing Ca ions. This anion exchange was considered to cause depolarization of the SR membrane. To clarify whether such an anion replacement truly depolarizes the SR membrane, the measurement of ionic permeability was important.

The measurement of permeability of various ions across the SR membrane has been carried out by several investigators mainly with tracer methods (Kasai *et al.*, 1976*a–b*; Meissner & McKinley, 1976; Duggan & Martonosi, 1970; Jilka, Martonosi & Tillack, 1975). However, various

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important ions such as Na^+, K^+, and Cl^- permeate very fast and their permeabilities could not be determined. New methods for the determination of fast permeation were needed.

Rapid flow of solute or water can be measured by following the time course of the volume change of membrane vesicles. The volume change of the vesicles or cells was followed by measuring the light scattering or the turbidity (absorbance) of the membrane suspensions by various workers (Bangham, De Gier & Greville, 1967; Rich *et al.*, 1968; Sha'afi *et al.*, 1970; Knauf *et al.*, 1977). However, such works mainly treated the problem of the permeability of nonelectrolytes. There are very few studies on the permeability of ions (Knauf *et al.*, 1977; de Kruijff *et al.*, 1974).

Materials and Methods

Materials

SR vesicles were prepared from rabbit dorsal and hind leg muscle by the method described in the previous paper (Kasai *et al.*, 1976*a*) and were stored in 100 mM KCl and 5 mM Tris-Maleate (pH 6.5) at 0 °C. SR vesicles were used within three days of isolation.

Valinomycin was purchased from Sigma Chemical Co. Other reagents were commercial products of analytical grade.

Measurement of the the Volume Change of SR Vesicles

The volume change of SR vesicles was monitored with 90° light scattering at 400 nm. Temperature was maintained at 23 °C with a temperature controlling apparatus (Lauda-K2R, West Germany). To follow the fast volume change, a stopped flow spectrophotometer (Union RA-401 and RA-450, Japan) was used with a 2-mm round cell. To follow the slow volume change, a fluorescence spectrometer (Union FS-501, Japan) and a rapid mixing apparatus (Union MX-7, Japan) were used with a 1-cm square cell.

In most experiments, SR vesicles were preincubated in 2 mM KCl and 5 mM Tris-Maleate (pH 6.5), 0.4–0.6 mg SR protein per ml for more than 3 hr at room temperature. This incubated suspension and an equal volume of the solution containing 200 mosmol salts of molecules in addition to 5 mM Tris-Maleate buffer were mixed and the change in the light scattering intensity was followed.

When valinomycin was used, valinomycin was added to the incubated suspension about 30 min before the mixing. Valinomycin was dissolved in ethanol at 1 mg/ml.

Results

Volume Change in SR Vesicles Measured by Light Scattering

SR vesicles incubated in 5 mM Tris-Maleate (pH 6.5) and 2 mM KCl were mixed with an equal volume of the salt solution containing 5 mM Tris-

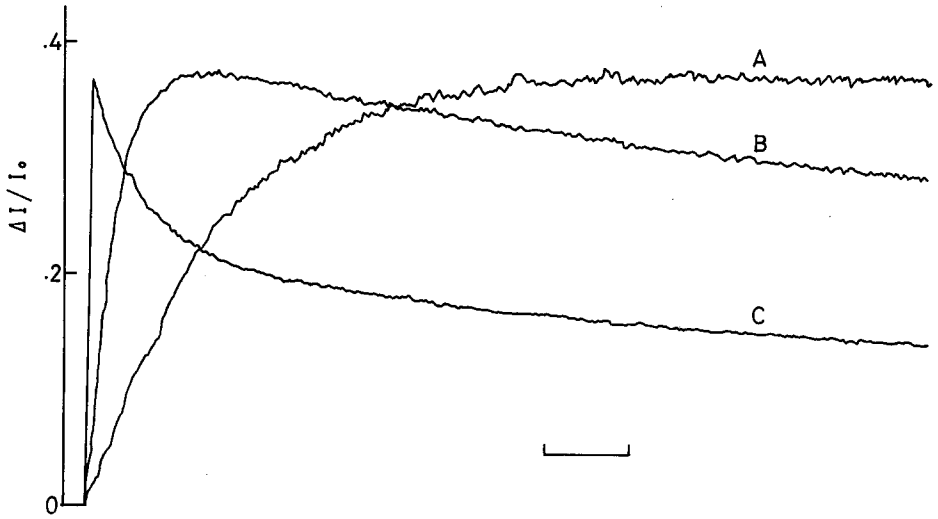

Fig. 1. Change in the light scattering intensity of SR vesicles caused by the volume change. SR vesicles incubated in 2 mM KCl, 5 mM Tris-Maleate (pH 6.5) and 0.4 mg SR protein/ml was mixed with an equal volume of a solution containing 100 mM KCl and 5 mM Tris-Maleate using a stopped flow apparatus; and the change of the light scattering intensity was followed. Time calibration: (*A*) 100 msec; (*B*) 500 msec; (*C*) 10 sec. The ordinate shows the change of the relative scattering intensity

Maleate (pH 6.5) and 100 mM KCl using a stopped flow apparatus, and the change in the light scattering intensity was followed. As shown in Fig. 1, the scattering intensity increased rapidly and then decreased. The fast increase in the scattering intensity is caused by the decrease of the volume of SR vesicles due to the outflow of water through the membrane, which is driven by the osmotic pressure difference. From this rate, the permeation time of water can be calculated. The later decrease in the light scattering intensity is caused by the increase of the volume due to the inflow of water which was accompanied by the inflow of salt ions driven by the chemical potential difference of these ions. From the later rate of change of light scattering, the permeation times of salt ions can be calculated. However, the change of the light scattering intensity did not follow the simple exponential curve. Probably the deviation from the exponential function is attributable mainly to the heterogeneity of the size and the permeability of SR vesicles, and to a lesser extent to the nonlinear relationship between the scattering intensity change and the volume change. Therefore, as a measure of the permeation time, the half permeation time, τ, was defined as the time to reach the half value of the maximal increment of the light scattering intensity, ΔI, which

was estimated by an extrapolation of the later phase to time zero. At 23 °C, the permeation times of water and KCl were 0.1 and 10 sec, respectively.

Contrary to this experiment, when SR vesicles incubated in 100 mM KCl were mixed with a solution of 2 mM KCl, only a slight decrease of the light scattering was observed. This shows only a slight increase in the volume from the incubated (resting) state. In the case of red blood cells, the symmetrical change of the light scattering intensity was reported (Rich *et al.*, 1968). Probably SR vesicles exist in the most expanded state under the incubation conditions.

Similar change of light scattering as in Fig. 1 was observed when the ionic concentration was raised from 100 to 200 mM KCl without preincubation in low ionic strength. In order to check the effect of preincubation in low ionic strength, SR vesicles preincubated in low ionic strength for 3 hr at room temperature were once transferred to 100 mM KCl, and then the change of the light scattering was followed by raising the ionic concentration. The same time course of the scattering change as above was observed. This result shows that the incubation in low ionic strength seemed not to affect the permeability properties of SR vesicles. In most experiments, therefore, we used SR vesicles preincubated in low ionic strength so as to eliminate the effect of the ions in preincubation.

Permeabilities of Various Kinds of Salts and Neutral Molecules

When different kinds of salts or neutral molecules were used in similar experiments to the previous section, their permeation times were obtained. In the case of slowly permeable ions or molecules, two solutions were mixed and the change of the scattering intensity was followed with a spectrofluorimeter.

In Table 1, permeation times, τ, and maximal increments of the scattering intensity, ΔI, are summarized. In the case of neutral molecules, the permeation time shows just the movement of the molecules. However, in the case of salt ions, there is a problem since the permeation time gives the permeability of only one ion, i.e., anion or cation. From this table, in the case of potassium salts, when the anion was changed from chloride to acetate, propionate, butyrate, etc., the permeation time did not change. On the contrary, in the case of chloride salts, when the cation was changed from K^+ to Li^+, Na^+, Rb^+, Choline, etc., the permeation time changed. This result indicates that such anions permeate faster than the cations and the

Table 1. Permeability properties of the sarcoplasmic reticulum membrane

Species	Final concentration (mM)	Permeation time (τ) sec	$\Delta I/I_0$
KCl	50	10	0.45
K-Acetate	50	10	0.45
K-Propionate	50	10	0.45
K-Butyrate	50	10	0.45
K_2-Oxalate	35	90	0.51
KMS	50	50	0.48
NaMS	50	50	0.48
NaCl	50	13	0.45
LiCl	50	18	0.48
RbCl	50	8	0.45
Choline-Cl	50	360	0.69
Tris-Cl	50	600	0.73
$MgCl_2$	33	1800	0.75
$CaCl_2$	33	6000	0.75
K_2-EDTA	20	3000	0.51
Tris-Maleate	50	900	0.71
$K_2HPO_4+KH_2PO_4$	14	25	0.26
Thiourea	100	0.6	0.69
Urea	100	1.2	0.69
Ethyleneglycol	100	0.6	0.69
Glycerol	100	1.8	0.69
Glucose	100	1500	0.69
H_2O		0.1	

Permeation time, τ, and increment of light scattering, $\Delta I/I_o$, were determined as described in the *text*.

permeation time of salts depends on the permeability of the slower ion. This conclusion is quite reasonable. During the net flow of the ions the electroneutrality must be held, and ions move as a pair. This conclusion will be confirmed by the experiment shown in the next section.

In the case of methanesulfonate salts, i.e., KMS or NaMS, the permeation times were larger than those of KCl or NaCl. This might be due to the slow permeation of methanesulfonate ions.

In the case of slowly permeable ions or molecules, such as $CaCl_2$, $MgCl_2$, choline chloride, glucose, etc., the maximal increments of the light scattering intensity, ΔI, were about 50% larger than those of NaCl or KCl. Small differences less than 10% cannot be eliminated because the osmolarity of these solutions was not necessarily the same. At first, specific effects

of such ions on the light scattering intensity can be considered. However, long after mixing, the scattering intensity reached the initial level, and there was little difference between $CaCl_2$ and KCl. The effect of binding of divalent cations to membranes seems not to contribute much to the scattering intensity. Therefore, the big change in light scattering should also be attributed to the volume change. A possible interpretation follows. As discussed in the previous section, SR vesicles are of heterogeneous size and permeability (Meissner *et al.*, 1976; Arrio *et al.*, 1974). We can assume that SR is composed of fast and slow permeating vesicles. In the case of KCl, KCl permeates through the fast vesicles as fast as the permeation of water through the slow vesicles. In the case of $CaCl_2$, however, the permeation of salts was slower than the permeation of water at both vesicles. Accordingly, in the case of KCl, the maximal increment of the light scattering intensity becomes smaller than in the case of $CaCl_2$. As a result, the permeation rate of KCl might be underestimated when compared with $CaCl_2$.

Effect of Valinomycin on the Permeability of Ions across SR Membrane

It is well known that valinomycin increases the permeability of K^+. As concluded in the previous section, if the permeation rate of potassium salts such as KCl is limited by the permeability of K^+, an increase in the permeation rate of KCl is expected in the presence of valinomycin. Experiments similar to those shown in Fig. 1 were carried out in the presence of valinomycin. As shown in Fig. 2, an increase of the permeation rate of KCl was observed. In this case the permeation rate of water seemed not to be changed.

In this experiment, valinomycin was added to the SR suspension alone before mixing. When valinomycin was added to both solutions, the permeation of KCl was almost the same as above if the final concentration of valinomycin was the same. On the contrary, when valinomycin was added to KCl solution alone, the initial rate of KCl permeation was slightly slowed. From these results, the rate of binding of valinomycin was estimated to be about 1 sec. Thus, the following experiments were carried out by adding valinomycin to the SR suspension only.

To clarify the specificity of the effect of valinomycin on SR membrane, similar experiments were carried out by using different kinds of salts. As shown in Table 2, the effect of valinomycin was specific for K^+ and Rb^+ in the case of SR membranes, too.

In the solution of valinomycin a small amount of ethanol was present.

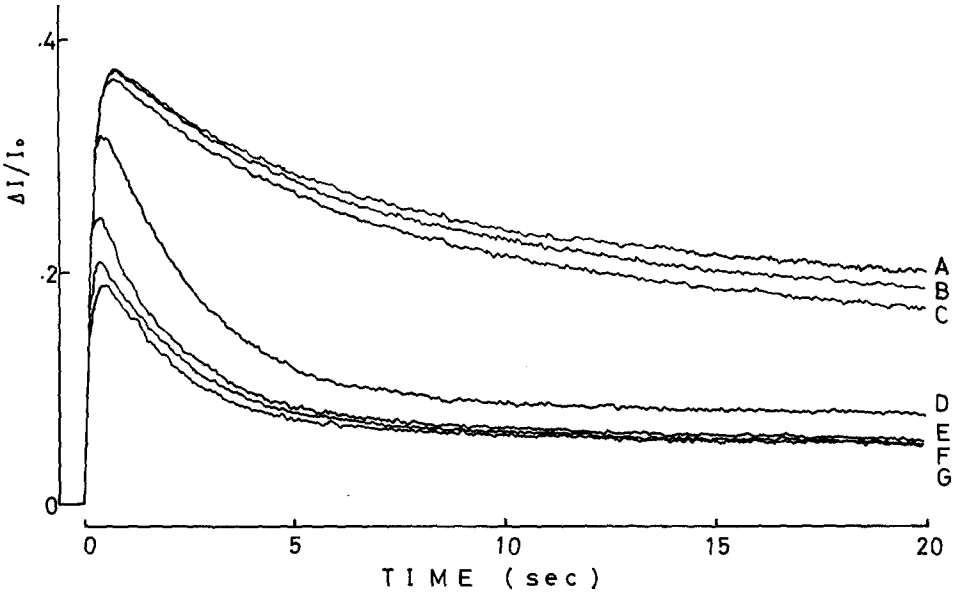

Fig. 2. Effect of valinomycin on the volume change of SR vesicles in KCl solution. An experiment similar to that in Fig. 1 was carried out in the presence of valinomycin. Valinomycin was added 30 min before mixing to the SR suspensions. Concentration of valinomycin in g/ml was following: (*A*) 0; (*B*) 1.3×10^{-9}; (*C*) 4×10^{-9}; (*D*) 5×10^{-8}; (*E*) 1.5×10^{-7}; (*F*) 5×10^{-7}; (*G*) 1.5×10^{-6}. The final concentration of SR was 0.2 mg/ml

Table 2. Effect of valinomycin on the permeability of ions and molecules

Species	Permeation time		Effect
	−Valinomycin (τ_o) (sec)	+Valinomycin (τ) (sec)	
KCl	11.0	0.5	+
KMS	51.7	10.9	+
NaCl	14.0	14.0	−
LiCl	18.7	18.7	−
RbCl	10.0	0.5	+
Choline-Cl	400	400	−
Glucose	1800	1800	−
$MgCl_2$	1800	1800	−
$CaCl_2$	6000	6000	−

Experiments similar to those in Fig. 2 were carried out using different ions and molecules. Valinomycin concentration, 5×10^{-7} g/ml.

Alcohol also increases the permeability of various kinds of salts (Hara & Kasai, 1977). However, the small amount of alcohol used in this work had no effect on the permeability of ions and molecules.

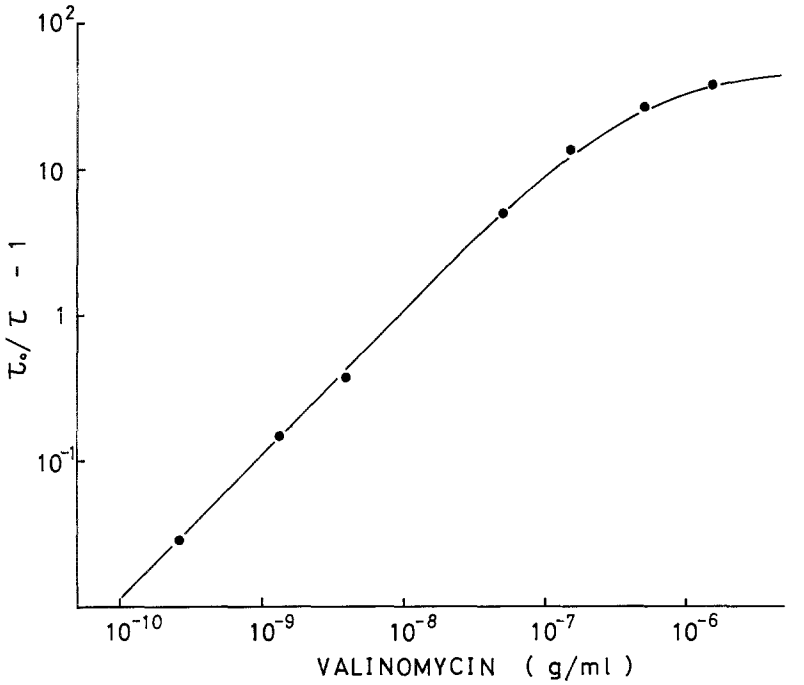

Fig. 3. Increase of the permeability of KCl as a function of valinomycin concentration. As a measure of the increase in the permeability of KCl, $(\tau_0/\tau)-1$ was used, where τ_0 is the permeation time in the absence of valinomycin, and τ is the permeation times in the presence of valinomycin. The data was taken from Fig. 2. All curves were extrapolated to the maximal increment of the control curve ($\Delta I/I_o = 0.4$) and τ were determined as times when these curves came down to the half value (0.2). Under the assumption that permeability of potassium ions, P_K, increased linearly to the concentration of valinomycin, C, we can put

$$P_K = P_{K0} + \alpha C$$

where P_{K0} is permeability of potassium ions in the absence of valinomycin and α is a constant. Since the permeability is inversely proportional to the permeation time, the following relation can be obtained using Eq. (2),

$$\frac{\tau_0}{\tau} - 1 = \frac{\alpha P_{Cl} C}{P_K (P_{Cl} + P_{K0} + \alpha C)}$$

where P_{Cl} is permeability of chloride ions. The curve was calculated using this relation.

Concentration Dependence of the Effect of Valinomycin

In Fig. 2, the effect of valinomycin on the permeation of KCl was shown when the concentration of valinomycin was increased. In the presence of a large amount of valinomycin a decrease of the maximal increment of the light scattering intensity was observed. This decrease is also explained by the fact that the permeability of KCl becomes close to the water permeability. For this reason, the time at which the increment in scattering intensity reached a half value of the maximal increment of the control sample was taken as a measure of the permeation time. In Fig. 3, the increase of the permeation rate was plotted as a function of the concentration of valinomycin. The permeation rate initially increased proportionally to the concentration of valinomycin and then reached a constant value. From our assumption, the following interpretation can be derived. In the low concentration of valinomycin, the permeability of K^+ increased but the rate is still slower than that of Cl^-. Thus, the permeation rate of KCl remains dependent on the permeation of K^+. In the higher concentration of valinomycin, the permeability of K^+ exceeds that of Cl^-,

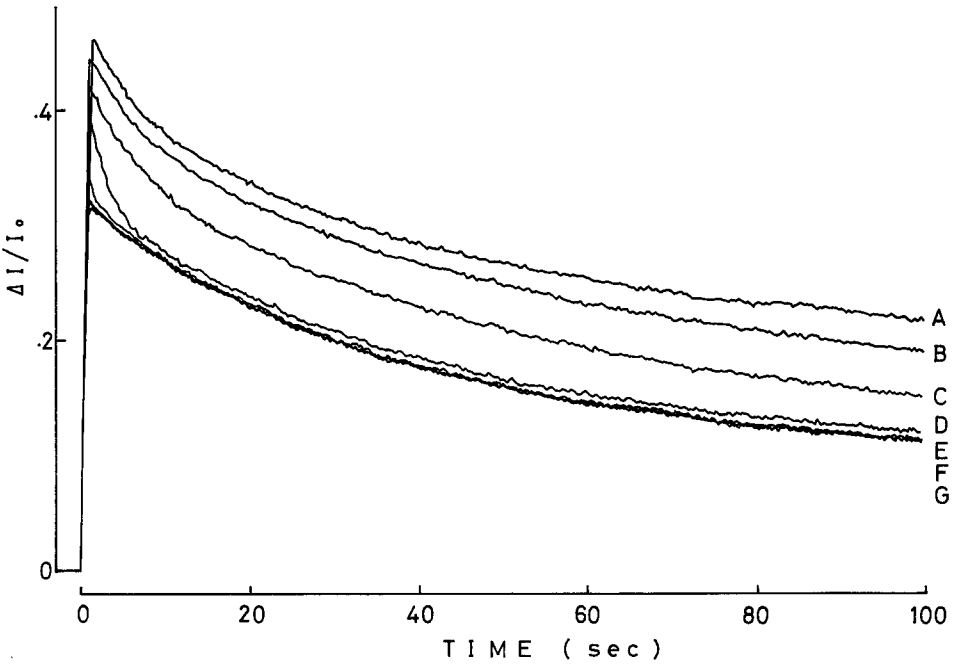

Fig. 4. Effect of valinomycin on the volume change of SR vesicles in KMS solution. An experiment similar to that in Fig. 2 was carried out in KMS. Other conditions were the same as in Fig. 2

Table 3. Permeability of ions

Ions	Permeation time (τ_{ion}) (sec)	Relative permeability
K^+	20	1
Na^+	26	0.77
Li^+	36	0.56
Rb^+	16	1.3
Cl^-	0.4	50
MS^-	20	1
Phosphate	11	1.9
Oxalate	40	0.5

Experiments similar to those in Fig. 3 were carried out using various kinds of salts and by changing valinomycin concentration. Permeation time of ions, τ_{ion}, was determined by using Eq. (2).

and the permeation of KCl becomes dependent on the permeability of Cl^-. The limit value of the permeation time gives the permeation time of Cl^-. The permeation time of Cl^- was about 0.40 sec. It was about 50 times smaller than that of K^+.

In the case of KMS, a similar experiment was carried out. The increase in the permeation rate of KMS was also observed in the presence of valinomycin as shown in Fig. 4. The increase in the permeation rate was also plotted against the concentration of valinomycin in Fig. 5. By using the same analysis as in the case of KCl, the permeation time of MS^- was about 20 sec.

A similar analysis was carried out on some interesting anions such as phosphate, oxalate. Their permeation times were determined and shown in Table 3. Some discrepancies between the data shown in Tables 1 and 3 are seen. This might be due to the heterogeneity of the SR vesicles, which will be discussed in *Discussion.*

Dependence of Change of Light Scattering on Ionic Concentration

In above experiments, SR vesicles preincubated in a low salt concentration was brought to 100 mosmol, and the change of light scattering intensity was followed. In this section, in order to know the relationship between volume change and the change of light scattering, salt concentration of preincubated medium was changed. SR vesicles preincubated in various concentrations of choline chloride was brought to 200 mM

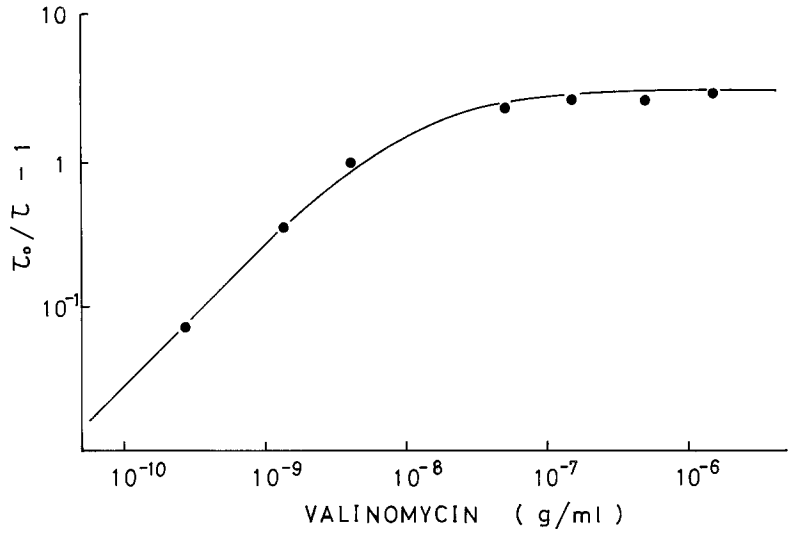

Fig. 5. Increase of the permeability of KMS as a function of valinomycin concentration. The same analysis as in Fig. 3 was made about the data in Fig. 4

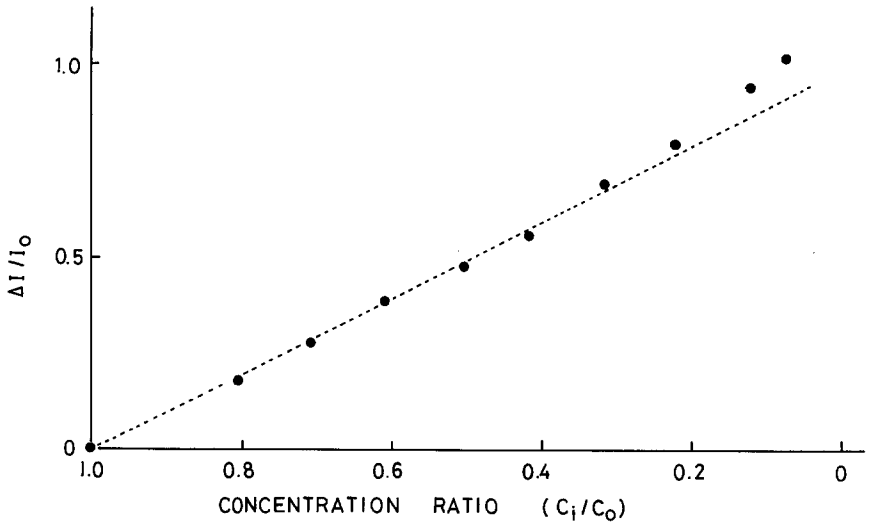

Fig. 6. Dependence of change of light scattering on salt concentration. SR vesicles preincubated in various concentrations of choline chloride, 5 mM Tris-Maleate (pH 6.5) and 0.4 mg SR Protein/ml was mixed with an equal volume of a solution containing choline chloride and 5 mM Tris-Maleate and the change of the light scattering intensity was followed. The final concentration of choline chloride was adjusted to be 200 mosmol. The relative values of increment of light scattering, $\Delta I/I_o$, were plotted against C_i/C_o, where C_i and C_o are concentrations of total salts inside and outside the vesicles, respectively. C_i and C_o are assumed to be the same as the concentration of salts in preincubated medium and after mixing, respectively

choline chloride. As shown in Fig. 6, an increment of scattering intensity was plotted against the preincubated salt concentration. Since the permeability of choline chloride is very low, the volume change is expected to be proportional to the concentration ratio C_i/C_o, where C_i and C_o are the salt concentrations of the inside and the outside of the vesicles after mixing. This figure shows the change of light scattering is almost proportional to the volume change.

Discussion

In this paper, it was demonstrated that the permeability of the ions which are too fast to be followed by the tracer method could be determined by the optical method. This should be a useful method. From results of the experiments carried out using SR vesicles, the permeability of anions such as Cl^- is about 50 times faster than that of cations such as K^+. Then the SR membrane is an anion selective one.

One of the most important points of these experiments is whether the change of the light scattering intensity is proportional to the change of the volume of the vesicles. Bangham *et al.* (1967) and Rich *et al.* (1968) showed such proportionality in the case of liposomes and red blood cells. Our result also indicates the apparent proportionality to hold. However, the time course of the scattering change did not follow the simple exponential curve; this suggested the existence of the heterogeneity of the size and the permeability of the vesicles. Therefore, the permeation time given in this paper should be considered as an apparent value. In this paper, however, such apparent values give us important information about the permeability properties of the SR membrane.

As mentioned in *Results*, the net movement of salt ions depended on the movement of the slower ion. The movement of ions across the membrane seems to follow the relation of Nernst as follows.

$$\frac{1}{D_{\text{salt}}}=\frac{1}{2}\left(\frac{1}{D_{\text{cation}}}+\frac{1}{D_{\text{anion}}}\right) \tag{1}$$

where D is a diffusion constant, and the suffix shows the corresponding species. Since the permeability is proportional to the diffusion constant, the apparent permeation time given in this paper must be proportional to the inverse of the diffusion constant. That is,

$$\tau_{\text{salt}}=\tfrac{1}{2}(\tau_{\text{cation}}+\tau_{\text{anion}}). \tag{2}$$

If we assume that the permeability of potassium ion increases linearly to the concentration of valinomycin in the case of Figs. 3 and 5, we can calculate the theoretical behaviors of τ_{KCl} and τ_{KMS}. It is clearly shown that this relation is held in Figs. 3 and 5. The curves in Figs. 3 and 5 were drawn using this relation.

In the case of KMS, some discrepancies were seen. From the data of KCl in Fig. 3, $\tau_K = 20$ sec, and from the data of KMS in Fig. 5, $\tau_{MS} = 20$ sec. Then, the calculated value of τ_{KMS} using Eq. (2) must be 20 sec. However, the observed value of τ_{KMS} was 40–60 sec. Moreover, if τ for K and MS are equal, the increase in permeation rate in the presence of valinomycin, $(\tau_0/\tau) - 1$, in Fig. 5 must be 1. However, this value was about 3. This difference might be also attributable to the heterogeneity of the vesicles. Therefore, from this experiment, we can only say that the permeabilities of K^+ and MS^- are almost the same order.

Although the time course of scattering change did not follow the simple exponential curve, we estimated the absolute value of the permeability under the assumption of the radius of SR vesicles to be 50 nm (Arrio *et al.*, 1974). Permeabilities were about 5.8×10^{-8} cm/sec for K^+ and 2.9×10^{-6} cm/sec for Cl^-.

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