The Specificity of Ionophore A 23187 in Cation Transport across Lipid Membranes
Studies with Lecithin Vesicles

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Lecithin vesicles containing different cations were prepared by sonication and characterized. When the concentration of the divalent cations in the buffer was increased from 1 mM to about 100 mM the cation-concentration inside of the vesicles did not increase proportional to the outside concentration, but showed a saturation behaviour. The efflux of various cations mediated by ionophore A 23187 was measured and relative transport-rates were determined. The following sequence was obtained:

$$Zn^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+} > Sr^{2+} \gg Ba^{2+} \approx Li^{+} > Na^{+}.$$

The efflux of Ca^{2+} increased proportional to the square of the A 23187-concentration. To lecithin-vesicles containing ethylenediamine tetraacetate (EDTA) inside different cations were added on the outside. In the presence of A 23187 divalent cations are transported into the vesicles and bound there by EDTA. During the influx of Me^{2+}-ions into vesicles a H^{+}-efflux was observed. The resulting pH-decrease was measured. The rate of the pH-change depended on the Me^{2+}-ion used. The sequence was:

$$Cd^{2+} > Zn^{2+} > Ca^{2+} > Mn^{2+} > Mg^{2+} \gg Sr^{2+},\ Ba^{2+}.$$

Introduction

The divalent cations Ca^{2+} and Mg^{2+} play an important role in several functions of living organisms. The transport of these cations across membranes is often critical for their regulatory role, but the transport mechanisms are not yet known in detail. The antibiotic A 23187 is the most specific ionophore for divalent cations known at present and has been studied in biological and artificial systems. A selected list of references is given in an earlier paper [1] where Ca^{2+}-transport across Müller-Rudin membranes has been investigated. Since the smallest Ca^{2+}-flux detectable by that experimental technique was about 10^{-12} mol cm^{-2} s^{-1} we wanted to overcome this difficulty by increasing the membrane area. This can easily be done by using single bilayer vesicles prepared by sonication. In this system the membrane area is increased by several orders of magnitude compared to Müller-Rudin bilayer membranes. Therefore much smaller Ca^{2+}-fluxes than with the Müller-Rudin-system can be followed continuously when the same optical indicator Arsenazo III is used as in the earlier study [1]. This has the following advantages:

1. The concentration dependence of the ion flux at low ionophore concentrations (10^{-7} M to 10^{-9} M) is accessible.
2. The flux of other ions which are less effectively transported than Ca^{2+} becomes measurable. We have followed the efflux of Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Li^{+} and Na^{+} from vesicles by methods described later.
3. An antiport of H^{+} can be detected under certain conditions. Its relation to the influx of Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+} into vesicles was investigated.

Materials and Methods

Chemicals

Egg-phosphatidylcholine (Egg-PC) was prepared by K. Janko in our laboratory. Other lipids, HEPES and Arsenazo III were products from Fluka, Buchs (Switzerland). Inorganic salts of analytical grade or better were products from Merck, Darmstadt (GFR) A 23187 was a gift from Dr. R. L. Hamill, Eli-Lilly and Co. Ind., USA and used without purification.

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Vesicle preparations

20 mg Egg-PC was dried from ethanolic solution by a stream of N_2 in a Corning borosilicate culture tube 13 × 100 mm. 2 ml of the appropriate aqueous solution was added, the tube covered with parafilm and sonicated in a bath type sonifier Bransonic 12 at 50–60 °C for 1 h. Several types of vesicles were prepared in this manner and gave faintly opalescent, stable solutions.

a) Ca^{2+}-vesicles: Vesicles were prepared from solutions containing between 1 and 65 mM $CaCl_2$, 10 mM HEPES, pH adjusted to 8, 7 and 6. The osmolarity was adjusted by KCl to that of 100 mM KCl, 10 mM HEPES. This Ca^{2+}-free buffer solution was also used for elution of the vesicles from a Sephadex G-50 (0.9 × 60 cm) or Sepharose 4B (2.6 × 25 cm) column. From the Sephadex-column the vesicles were eluted in the void volume. The eluate from the Sepharose 4B column was monitored at 254 nm (Unicord or similar instrument) [2]. The first peak representing large particles was usually small. The amount of Ca^{2+} enclosed in the vesicles was determined both by the Arsenazo-method after dissolving the vesicles by detergent (see below) and by atomic absorption. The Beckmann 1272 Digital model was used with the instrumental settings given in the manual for Ca^{2+}. Ca^{2+}-contents of unfractionated vesicles (separated from outside Ca^{2+} by the Sephadex G-50 column) and of the descending part of the second peak fractionated on the Sepharose 4B-column [2] were measured. The lipid concentration was determined by the method of Bartlett [3]. [^{14}C]sucrose was used to determine the inner volume of the vesicles in some cases. The results given in Fig. 1 will be discussed below.

b) Sr^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}-vesicles: These types of vesicles were prepared in analogy to Ca^{2+}-vesicles. Zn^{2+}-vesicles could only be prepared at pH 6, Mn^{2+}-vesicles at pH 6 and 7, due to the low solubility-product of the hydroxides.

c) Li^+ and Na^+-vesicles: Vesicles containing 100 mM LiCl, 10 mM HEPES pH 8 were prepared, eluted with 10 mM NH_4Cl, 10 mM HEPES from a Sephadex column and the Li^+-content and Li^+-efflux determined by atomic absorption. Vesicles containing 100 mM NaCl, 10 mM HEPES pH 8 were prepared and eluted with 100 mM NH_4Cl, 10 mM HEPES from a Sephadex-column. Na^+ was determined by atomic absorption.

d) EDTA-vesicles containing 100 mM EDTA were prepared and eluted with 195 mM NaCl, 5 mM NaN_3 from a Sephadex-column. The vesicles were used to measure the efflux of H^+ during the influx of divalent cations (These experiments were initiated and first done together with Dr. Anthony Durham, Institute de Biologie Moleculaire et Cellulaire, CNRS, 15 Rue Descartes, 67000 Strasbourg).

Cation efflux from Me^{2+}-vesicles

The effects of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+} was followed by the Arsenazo-method. After elution of the vesicles from the column Arsenazo III was added to make the solution about 5×10^{-5} M. The solution was kept in a 1 cm standard cuvette, thermostated at 25 °C in a Zeiss DMR 10 Spectrophotometer. For the different cations the extinction was measured at the following wavelengths: Mg 630 nm, Ca 652 nm, Sr and Ba 645 nm, Mn 616 nm, Zn 612 nm (Extinction range 0.1; band width 0.6 nm). The extinction was plotted with a chart speed of 1 cm per minute. A 23187 solutions in ethanol were added usually in 5 µl steps to make the solutions between 3×10^{-9} M and 10^{-6} M. The extinction increased linearly for about 10 to 20 minutes. Finally detergent was added to dissolve the vesicles. Before and (or) after the experiment known amounts of Me^{2+} were added to the solution to obtain a calibration curve from which the Me^{2+}-efflux could be calculated in moles per minute. Fig. 2 of the results section shows an example.

H^+ efflux from EDTA-vesicles

The vesicles were pipetted into a Metrohm titration vessel, kept at 25 °C under N_2 with continuous magnetic stirring. A combined Metrohm glass electrode EA 147 and a Metrohm Ion-Activity-Meter E 850 were used to measure the pH which was plotted on a Hewlett-Packard strip chart recorder at speeds between 1.25 and 5 cm per minute. Solutions of Mg, Ca, Sr, Ba, Mn, Zn and Cd-chloride were added to make the solutions between 1 and 65 mM in Me^{2+}. Solutions of A 23187 were added to a final concentration between 10^{-8} M and 10^{-6} M. Except for the case of $BaCl_2$ a pH-decrease was observed which continued for several minutes. (Usually a decrease between 0.5 and 1.5 pH-units occurred.) The order of addition of A 23187 and $MeCl_2$ did not significantly change the results. Fig. 3 of the results section shows an example.

Results and Discussion

Me^{2+}-content inside of the vesicles

When Ca^{2+}-vesicles were prepared with buffer solutions of increasing Ca^{2+}-concentrations (c^0_{Ca}) the Ca^{2+}-concentration inside the vesicles did not increase linearly but showed a saturation behaviour (Fig. 1). The concentration (c^{Ves}_{Ca}) is defined as number of moles Ca^{2+} inside the vesicles (n^i_{Ca}) per liter of vesicle suspension. Since the total volume of the vesicle suspension (V_{tot}) can be measured directly c^{Ves}_{Ca} is readily obtained as n^i_{Ca}/V_{tot}. The concentration inside the vesicles (c^i_{Ca}) on the other hand is n^i_{Ca}/V_i were V_i is the internal volume of all vesicles in the suspension. Both concentrations are connected by the expression:

$$c^{Ves}_{Ca} = c^i_{Ca}\,(V_i/V_{tot}).$$

Since V_i may vary with c^0_{Ca} as discussed later we prefer to use c^{Ves}_{Ca}. In our case V_i/V_{tot} was about 5.85×10^{-3}. V_i was determined with $K_3\,Fe(CN)_6$ by the method of Newman and Huang [4]. The saturation curve of Fig. 1 may be fitted under the assumption that Ca^{2+} is adsorbed to the inner surface of the vesicle membrane. With a limited number of binding places for Ca^{2+} a maximal concentration of adsorbed Ca^{2+} would be $(c^{Ves}_{Ca})^{max}_{ads}$. Then we can express the measured curve by the following equation:

$$c^{Ves}_{Ca} = \frac{(c^{Ves}_{Ca})^{max}_{ads}\, c^0_{Ca}}{K_M + c^0_{Ca}} + K \cdot c^0_{Ca}. \qquad (1)$$

With $K_M = 6.88$ mM, $(c^{Ves}_{Ca})^{max}_{ads} = 0.112$ mM and $K = 1.59 \cdot 10^{-3}$ the solid curve of Fig. 1 is calculated. The calculated values are also given in Table I. When c^{Ves}_{Ca} is calculated under the assumption of constant vesicle size and shape (with $V_i = 450$ ml per mol Egg-PC) without adsorption a rather poor fit with the measured values is obtained. A large change of V_i with varying c^0_{Ca} was excluded by determining the amount of included [^{14}C]sucrose at the same time. This amount varied only by about ± 10% and increased with increasing c^0_{Ca}. (Between c^0_{Ca} 3 mM and 30 mM.) A change in the shape of the vesicles which might explain the saturation of c^{Ves}_{Ca} without a change of V_i cannot be excluded.

The ratio of included Ca^{2+} to Egg-PC molecules was determined and is given in Table I for the unfractionated vesicles. The same was done with the fractionated vesicles (descending part of the second peak) and a ratio $c^{Ves}_{Ca}: c_{PC}$ about 10% smaller than in the unfractionated vesicles was found.

For Sr^{2+}, Ba^{2+}, Mg^{2+}, Zn^{2+} vesicles c^{Ves}_{Me} was determined in an analogous manner as for Ca^{2+}-vesicles. The results are given in Table II. At $c^0_{Me} = 3$ mM the data for all Me^{2+}-ions are in good agreement. At $c^0_{Me} = 30$ mM the experimental data (from at least 4 determinations) show a large standard error. The transport-rates for different Me^{2+}-ions, which are given in the next section were therefore only calculated for vesicles prepared with $c^0_{Me} = 3$ mM.

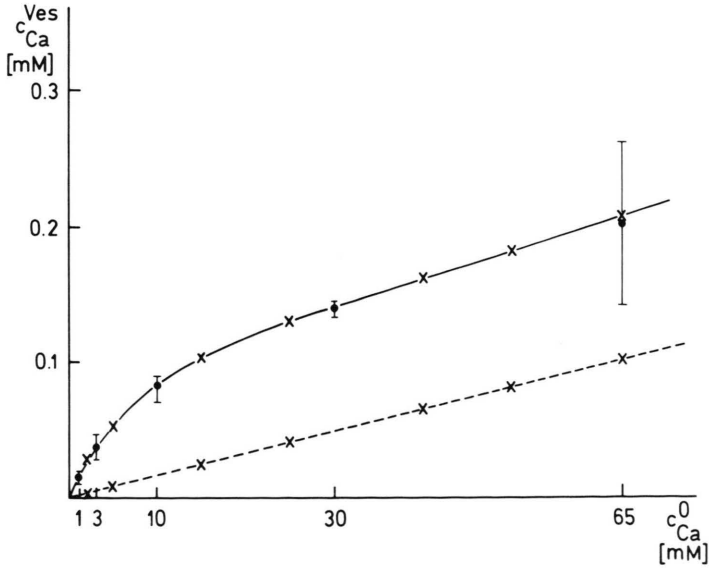

Fig. 1. Ca^{2+}-concentration inside lecithin-vesicles (c^{Ves}_{Ca} . . . for definition see text) versus Ca^{2+}-concentration in the buffer used for preparation of vesicles (c^0_{Ca}); ● measured values of c^{Ves}_{Ca} with S. E.; —×— c^{Ves}_{Ca} calculated using equation (1); - - × - - calculated "free" Ca^{2+}-concentration inside of lecithin-vesicles (see text and disscussion).

Table I. Ca^{2+}-concentration inside vesicles in dependence on the Ca^{2+}-concentration in the buffer solution (outside) (c_{Ca}^{Ves} is defined in the text)

c_{Ca}^{0} [mM]	c_{Ca}^{Ves} [mM] measured		c_{Ca}^{Ves} [mM] calculated with adsorption formula (1)	c_{Ca}^{Ves} [mM] calculated * without adsorption	$\frac{c_{Ca}^{Ves}}{c_{PC}}$ measured
	pH 6	pH 8			
1	–	0.0162 ± 0.005	0.0158	0.0058	0.14×10^{-2}
3	0.045 ± 0.010	0.0380 ± 0.010	0.0388	0.0173	0.35×10^{-2}
10	0.079 ± 0.003	0.0820 ± 0.001	0.0823	0.0577	0.77×10^{-2}
30	0.135 ± 0.004	0.1390 ± 0.005	0.1390	0.1730	1.35×10^{-2}
65	0.180 ± 0.020	0.2050 ± 0.060	0.2050	0.3750	1.65×10^{-2}

* under the assumption, that the vesicle size stays constant and the internal volume of the vesicles is $V_i = 450$ ml per mol PC.

Table II. Me^{2+}-concentration inside vesicles in dependence on the Me^{2+}-concentration in the buffer solution (outside)

c_{Me}^{0} [mM]	c_{Me}^{Ves} [mM]				
	Ca^{2+}	Sr^{2+}	Ba^{2+}	Mg^{2+}	Zn^{2+}
3	0.038 ± 0.01	0.038 ± 0.006	0.034 ± 0.014	0.041 ± 0.005	0.036 ± 0.009
30	0.139 ± 0.005	0.134 ± 0.017	0.161 ± 0.060	0.117 ± 0.050	0.186 ± 0.090

For Li^+-vesicles prepared at $c_{Li}^{0} = 100$ mM we determined $c_{Li}^{Ves} = 0.20 \pm 0.06$ mM. This is within the range of saturation values obtained for Me^{2+}-vesicles.

Efflux of Me^{2+}-ions mediated by A 23187

Fig. 2 shows a typical efflux-experiment with Ca^{2+}-vesicles. The increase in extinction is plotted versus time as described under methods. From the slope of the straight lines together with a calibration curve (not shown) the Ca^{2+}-efflux v (change of Ca^{2+}-concentration outside of the vesicles per unit time) is calculated. The inset of Fig. 2 shows $\log v$ *versus* $\log C_{A23}$ which gives a slope 2. This quadratic dependence of the Ca^{2+}-efflux on c_{A23} was verified between $c_{A23} = 3 \times 10^{-9}$ M and 3×10^{-7} M, when c_{Ca}^{0} was between 3 to 30 mM. The Egg-PC-concentration was between 2.5×10^{-3} M and 2.4×10^{-4} M in these experiments, the pH = 8. A similar result has been reported before with multilayered liposomes [5]. In experiments at pH 7 and pH 6 a gradual change from the quadratic to a linear dependence of the Ca^{2+}-efflux on c_{A23} was observed but not studied in detail. The Ca^{2+}-efflux decreased by a factor 4 to 5 when the pH decreased from 8 to 6. This is in agreement with results found with Müller-Rudin membranes [1]. The Ca^{2+}-flux in the vesicle experiments was in the range of 10^{-16} mol cm^{-2} s^{-1} to 2.5×10^{-14} mol cm^{-2} s^{-1}. This is much smaller than the flux in the Müller-Rudin system (between 10^{-12} and 2.5×10^{-10} mol cm^{-2} s^{-1}) due to the smaller ratio c_{A23}/c_{PC} in the vesicle experiments. The flux was calculated under the assumption of a surface of 1.2×10^{-11} cm^2 per vesicle (corresponding to the outer radius 12 nm, the inner radius 8 nm and the mean radius 10 nm). This gave a value of 3.2×10^3 cm^2 membrane area per mg PC.

The temperature-dependence of the Ca^{2+}-efflux between 25 °C and 45 °C was measured. An Arrhenius-plot yielded an activation energy of 58.8 kJ/mol (14 kcal/mol). This is a value intermediate between the activation energy of K^+-transport by valinomycin and Na^+-transport by gramicidin.

In vesicles prepared form L-dipalmitoyl-phosphatidylcholine (DPPC) the Ca^{2+}-efflux was strongly inhibited in the gel state at 25 °C and increased significantly around 30 °C. In the liquid crystalline state at 45 °C DPPC-vesicles showed a comparable Ca^{2+}-efflux as Egg-PC-vesicles at 25 °C, when c_{Ca}^{Ves} and c_{A23} were equal in both systems. It is interesting to note that the Ca^{2+}-transport by ionophore A23187 starts well below the gel to liquid crystalline phase transition. The specificity of A23187 for different divalent cations was compared by preparing Me^{2+}-vesicles with $c_{Me}^{0} = 3$ mM and measuring the Me^{2+}-efflux at $c_{A23} = 1.5 \times 10^{-7}$ M. From v as used in Fig. 2 relative transport rates were calculated and summarized in Table III. Relative binding constants determined in a macroscopic two-phase system by Pfeiffer and Lardy [6] are included for comparison.

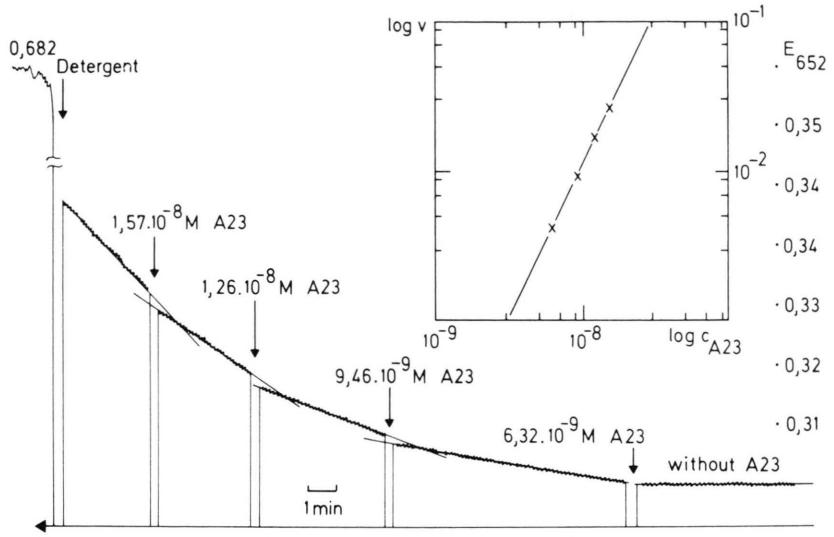

Fig. 2. Ca^{2+}-efflux from vesicles mediated by A 23187 at 25 °C. Vesicles were prepared with $c^{in}_{Ca} = 30$ mM, pH = 8. The solution contained 2 mg/ml Egg-PC and 4.8×10^{-5} M Arsenazo III. The extinction at 652 nm was plotted *versus* time. At the points marked by arrows ethanolic solutions of A23187 were added in 5 µl steps to give the indicated concentrations. The Ca^{2+}-efflux v was calculated from the extinction as described in the text. Inset: double logarithmic plot of Ca^{2+}-efflux *versus* concentration of A23187.

Table III. Relative transport rates for Me^{2+}. Mean values ± 20% (Vesicles prepared with $c^{0}_{Me} = 3$ mM, $c_{A23} = 1.5 \times 10^{-7}$ M, $T = 25^{6}$ C).

pH	Ba^{2+}	Sr^{2+}	Ca^{2+}	Mg^{2+}	Mn^{2+}	Zn^{2+}
8	< 0.005	0.070	1	0.25	–	–
7	< 0.001	0.040	0.4	0.15	0.4	–
6	< 0.001	0.002	0.2	–	0.4	0.6
Relative Binding Constants [6]	4×10^{-6}	0.004	1.0	0.35	360	3600

It is clear that the differences between transport rates are much smaller than between binding constants. This seems quite reasonable because the diffusion of the ionophore-cation-complex across the membrane will be a rate-determining step.

The overall structure of the A_2 Me-complexes is probably very similar (for different Me^{2+}). Only Ba^{2+} forms such an unstable complex that it is not significantly transported under our experimental conditions.

Efflux of Me^{+}-ions mediated by A 23187

According to its known properties [1, 6] A 23187 should not mediate the efflux of monovalent cations. Since only Li^+ is transported into a bulk organic phase [7] by the antibiotic we compared Li^+ and Ca^{2+}-efflux under similar conditions. The efflux of Ca^{2+} from vesicles with $c^{Ves}_{Ca} = 0.14$ mM after addition of 3×10^{-7} M A23187 was $(4 \pm 0.4) \times 10^{-10}$ mol Ca^{2+}/s. The efflux of Li^+ from vesicles with $c^{Ves}_{Li} = 0.20$ mM after addition of 3×10^{-7} M A23187 was $(1.2 \pm 0.7) \times 10^{-12}$ mol Li^+/s.

The efflux of Na^+ from vesicles was not mediated under comparable conditions. K^+ was not studied since it is known to bind A23187 less [7, 6] than Na^+ or Li^+.

H^{+}-Me^{2+} antiport mediated by A 23187

Several authors [1, 8] have suggested an electroneutral exchange of Me^{2+} for $2H^+$ via formation of a charge neutral complex $Me A_2$.

$$Me^{2+}_{aq} + 2AH_{membr} \rightleftharpoons (Me\,A_2)_{membr} + 2H^{+}_{aq}. \quad (2)$$

During the transport of Me^{2+} from the high concentration side to the low concentration side protons should be transported in the other direction. Most experimental evidence supports this view, although the stochiometry was found closer to $H^+/Ca^{2+} = 1$ and not 2 [9]. In our study with Müller-Rudin-membranes we could not detect a pH-change during Ca^{2+}-transport. We suspected that the initially formed pH-gradient was rapidly dissipated by back diffusion of H^+ via an unknown mechanism different from (2). When we prepared Ca^{2+}-vesicles without HEPES-buffer and measured the pH during Ca^{2+}-efflux, again we could not find a pH-change. When the Ca^{2+}-vesicles were prepared with 100 mM HEPES and eluted from the Sephadex column with 100 mM KCl without buffer, the expected pH increase was observed during ionophore-mediated

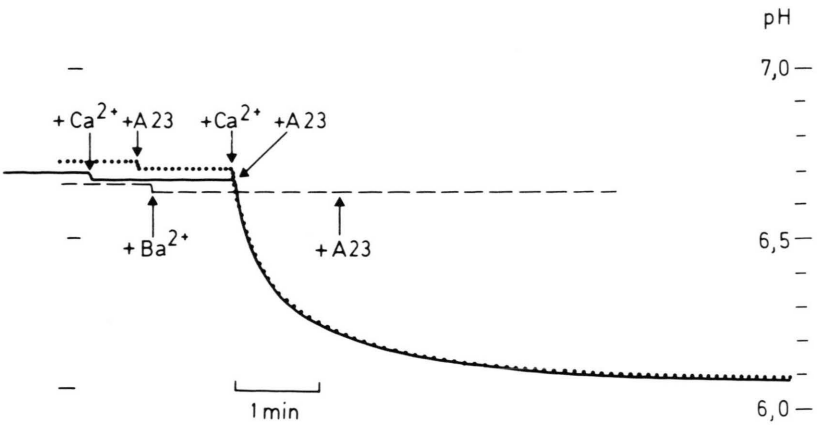

Fig. 3. H^+-efflux from EDTA-vesicles (0.1 M EDTA) after addition of 1 mM $CaCl_2$ and 5×10^{-7} M A23187 at 25 °C. The pH was plotted versus time. No pH-change occurred in the presence of 1 mM $BaCl_2$ —— $CaCl_2$ added first, A23187 later; ····· A23187 added first, $CaCl_2$ later; - - - - $BaCl_2$ and A23187.

Ca^{2+}-efflux. It was of transient nature and disappeared after several minutes. Since we wanted to compare different cations a more convenient way was to prepare EDTA-vesicles. Since EDTA readily complexes most divalent cations, externaly added Me^{2+} will be transported into the vesicles and complexed there. At the same time H^+ would be transported out of the vesicles. Indeed these experiments showed a large pH-decrease under conditions of Me^{2+} influx (Fig. 3). It can be seen in Fig. 3 that the pH-change only occurred when a transported cation (like Ca^{2+}) and A 23 are present. The order of addition of cation and ionophore makes no difference. When the cation is not transported (like Ba^{2+}) no pH-change was noted after addition of ionophore. This fact and the time course of the pH-change in the presence of transported cations show that EDTA is indeed inside of the vesicles. The total ΔpH dependend only slightly on the concentration and type of cations added outside but became more and more negative with increasing EDTA-concentrations: $-(0.4 \pm 0.1)$ at 0.05 M EDTA $-(0.7 \pm 0.1)$ at 0.1 M EDTA and $-(1.1 \pm 0.1)$ at 0.2 M EDTA inside of the vesicles. The pH plotted versus log time gave a straight line for the first few minutes (3 to 10 min depending on concentration of Me^{2+}, A 23 and EDTA). Comparing the initial rate of pH-decrease at equal concentrations of Me^{2+} (1 or 3 mM) A 23187 (5×10^{-7} M) added to the same vesicles filled with 0.1 M EDTA gave the following relative transport rates (at an initial pH 6.7) Ca^{2+} 1; Mg^{2+} 0.3; Mn^{2+} 0.8; Zn^{2+} 1.2; Cd^{2+} 3. Ba^{2+} and Sr^{2+} gave no pH-change under these conditions. The values are similar to those given in Table III as obtained from efflux measurements. Cd^{2+} induced the fastest pH-decrease of the cation tested.

Comparison of the H^+-efflux with the Me^{2+}-efflux measured under similar conditions gave no clear answer concerning the stochiometry of the Me^{2+}-H^+ antiport. Usually the initial H^+-efflux (corresponding to a Me^{2+}-influx) was faster than the Me^{2+}-efflux (measured under similar conditions but in a different experiment) by a factor of 2 to 10. At the moment we cannot draw conclusions from this result because the H^+-efflux was measured with vesicles containing EDTA and Me^{2+}-efflux was measured in the absence of EDTA. If the dissociation of the A_2Me complex at the membrane-water interface is a rate-determining step of the Me^{2+}-transport, the presence of EDTA might influence the Me^{2+}-flux substantially. It would be necessary to measure Me^{2+}- and H^+-transport at the same time with the same vesicles to get a conclusive answer concerning the stochiometry of the Me^{2+}-H^+-antiport. This was experimentally not possible at the moment, but should be done in the future.

Conclusion

We have shown that the Ca^{2+}-content of vesicles can be calculated by assuming adsorption of Ca^{2+} (maximal 1 Ca^{2+} for 60 lecithins) to the vesicle membrane. This is only taken as a phenomenological description. It would be difficult to understand the physical basis of Ca^{2+}-adsorption because we used neutral lipids throughout the studies. We have also shown that the Ca^{2+}-transport across vesicle-membranes depends on the square of the ionophore con-

centration as one would expect if the transported species was a complex of 1 Ca^{2+} and 2 ionophores. Due to the higher ionophore to lipid ratio necessary in the Müller-Rudin system the ionophore is completely bound in the complex which explains the linear dependence on the ionophore-concentration found there [1]. This may also be the case for Pr^{3+}-transport studied recently by NMR-techniques [10]. The specificity of A 23187 for different ions was determined by measuring the transport rates of these ions during the efflux from vesicles. These rates run qualitatively parallel to the equilibrium constants for the cation-ionophore complexes as determined (by two-phase extraction experiments [6]. Quantitatively the differences between these kinetic parameters are much smaller than between the equilibrium constants both for low affinity ions (Sr^{2+}, Ba^{2+}) and high affinity ions (Mn^{2+}, Zn^{2+}). In the latter case the existence of polynuclear hydroxy complexes may reduce the formation rate of the cation-ionophore complex. It is interesting to note that ionophoric substances which are analogues of 1,2-ethylene dioxydiacetic acids [11] did not show any measurable transport of Ca^{2+} or Zn^{2+} in our vesicle system even at concentrations 100 times higher than that of A 23187. (N,N′-Di[(11-ethoxy carbonyl) undecyl]N,N′-4,5-tetramethyl-3,6-dioxaoctanediamide a very potent Ca^{2+}-ionophore in macroscopic two phase systems was a gift of Prof. Dr. W. Simon from the ETH-Zürich.)

A very similar result has been reported by Wun and Bittman [12]. One reason for this reduced transport potency compared with A 23187 is the positive charge of the complex formed by neutral ligands and the cations. The specificity of A 23187 for divalent ions is high. We found that only Li^+ is transported but about 500 times less effective than Ca^{2+}.

The specificity for divalent ions (especially Ca^{2+}) is much higher than in the case of "calciphorin" isolated recently from inner mitochondrial membranes [13].

We have also detected the proton antiport predicted by equation (2) but only under certain experimental conditions. Although it was found that probably more than one H^+ is transported per Me^{2+} the H^+/Me^{2+}-stochiometry could not be established. This question should be solved since, as shown above, A 23187 remains the most specific and potent ionophore available for divalent cations.

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