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Pore kinetics reflected in the dequenching of a lipid vesicle entrapped fluorescent dye

Gerhard Schwarz *, Anna Arbuzova

Department of Biophysical Chemistry, Biocenter of the University, Klingelbergstr. 70 CH-4056 Basel, Switzerland

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Abstract

Pore formation in lipid vesicle membranes can be monitored by the fluorescence signal $F(t)$ arising from the induced release of a self-quenching dye in the course of the elapsed efflux time t. We present a basic theoretical analysis of pertinent experimental data allowing the quantitative evaluation of information on the pore kinetics and mechanism. This implies an investigation of the 'dynamic' quenching factor Q_t exhibited by that fraction of dye which is still being retained inside the liposomes at t. It is shown how Q_t depends on the mode of release which could be 'all-or-none' or more gradual as expressed by a parameter $\rho \leq 1$ (related to the pore lifetime), i.e., the average dye retention factor in a vesicle after a single pore opening. A fit to measured values of Q_t at a sufficient extent of efflux may be applied in order to determine ρ. Then the pore formation rate per liposome, $v_a(t)$, can be derived from the registered $F(t)$. We give a practical demonstration of the procedures with carboxyfluorescein-loaded phosphatidylcholine liposomes of two different sizes to which the wasp venom peptide mastoparan X had been added.

Keywords: Liposome leakage; Carboxyfluorescein; Channel; Theory; Membrane-active agent; Mastoparan X

1. Introduction

Pore formation in the membrane of liposomes may be examined by observing the apparent efflux of a suitable marker substance. Using a sufficiently concentrated self-quenching dye such as carboxyfluorescein [1] has become quite popular. The increase of fluorescence emission F due to dequenching upon dilution of dye into the external solution can be readily measured in the course of time t. This may be expressed in terms of a normalized experimental efflux function

$$E(t) = (F_\infty - F)/(F_\infty - F_0) \quad (1)$$

with F_0, F_∞ being the intensity of the fluorescence emission at $t = 0$ and $t \to \infty$, referring to the start of the release experiment and the final state of no more entrapped marker, respectively. Naturally $E(t)$ will be determined by the underlying pore kinetics. An adequate quantitative analysis of $E(t)$ should accordingly provide an access to relevant rate parameters and so open up interesting insights into the molecular mechanism of activating and inactivating pores.

A comparatively simple approach becomes applicable when equivalent pores are formed independently in uniform liposomes and these are subject to dye release in an 'all-or-none' mode so that at any time liposomes do exist only in an all full or an all empty state (indicating total release of marker by the first pore having been opened). Under these circumstances we simply have

$$p(t) = -\ln E(t)$$

where $p(t)$ stands for the average number of pore openings per liposome that have been activated during t [2]. An appropriate rate of pore formation ('activation'), $v_a = dp/dt$, can thus be evaluated from the measured fluorescence change. This procedure has been employed in the case of pores induced in unilamellar lipid vesicles by small amounts of the bee venom peptide melittin [3].

Generally one must expect, however, a more or less gradual release of dye so that all kinds of partially depleted liposomes arise in the course of the efflux experiment. A theoretical analysis of this general case has recently been presented elsewhere [4]. It applies to any kind of equivalent and non-interacting pores formed in the membranes of

* Corresponding author. Fax +41 61 2672189.

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liposomes of the same size. Instead of the empirical efflux function $E(t)$ the theoretically significant retention function $R(t)$ was derived, i.e., the fraction of marker (dye) still being retained inside the liposome moiety after a time t. The calculations resulted in the relation

$$\ln R(t) = -p(t) \cdot (1 - \rho(t)) \tag{2}$$

involving a function $\rho(t)$ defined as the average decrease of marker content in those liposomes where only one pore had been active in the given time.

If the measured efflux signal F would be linearly related to the amount of released marker, one can easily see that $R(t)$ is equal to $E(t)$. However, when we deal with a self-quenching dye such a linear relationship only applies in the 'all-or-none' release mode characterized by the condition $\rho(t) = 0$. Otherwise the non-linear concentration dependence of the quenching experienced by the entrapped dye implies differences between $E(t)$ and $R(t)$. In other words, the measured percentage of fluorescence change must not be confused with the percentage of dye release.

The apparent quenching of the entrapped dye alone may be expressed by a pertinent quenching factor $Q < 1$. It has already been pointed out that one actually deals with the 'all-or-none' case if Q does not increase in the course of an efflux experiment [5,6]. Here we present a more detailed theoretical and experimental investigation of the general problem. Procedures are developed for converting the measured $E(t)$ to $R(t)$ and also for a numerical evaluation of the actually time-independent value of ρ in the case that efflux through a single pore is comparatively fast and the pore closing is subject to a basic first order kinetics. Then the pore forming intensity $p(t)$ can be determined by means of Eq. (2). Subsequently the pore formation rate, $v_a(t)$, may be examined in view of a possible molecular mechanism.

2. Theoretical

The measured fluorescence signal F is composed of two contributions, namely

$$F_{ex} = (1 - R(t)) \cdot F_\infty;\ F_{in} = Q_t \cdot R(t) \cdot F_\infty \tag{3a,b}$$

arising from the dye that was so far released into the external medium and from the remainder being as yet retained inside the liposomes, respectively. This involves a term $Q_t = F_{in}/F'_\infty \leq 1$ standing for the apparent quenching factor of the still entrapped dye after a certain duration of the efflux (where of $F'_\infty = F_\infty - F_{ex} = R(t) \cdot F_\infty$ is the final fluorescence level at total release). The Q_t can be experimentally determined after having removed the external dye (by gel chromatography for instance). Then one will observe a respective internal signal $F_{in} = F'_0$ and add a detergent to destroy the liposomes so that the pertinent value of F'_∞ is registered.

By applying the Eq. (3a,b) the normalized efflux function evaluated from the measured total fluorescence signal $F = F_{ex} + F_{in}$ can be expressed as

$$E(t) = ((1 - Q_t)/(1 - Q_0)) \cdot R(t) \tag{4}$$

where Q_0 refers to $t = 0$. Accordingly the experimental $E(t)$ may be converted to the theoretically significant $R(t)$ once the relevant quenching factors have been determined somehow.

Now we consider a monodisperse suspension of liposomes where non-interacting equivalent pores are being induced by some external action, e.g., by adding a membrane-active substance. The pores are then subject to a Poisson statistical distribution over all the individual liposomes. In addition we assume that the relaxation time τ_0 applicable to efflux through a single pore [2] is small in comparison with the time scale of the measurement (in other words, $\tau_0 \ll t$). Furthermore, an ordinary first order kinetics of pore closing is envisaged with an average pore lifetime τ_p. Then a time-independent value

$$\rho = \tau_0/(\tau_0 + \tau_p) \tag{5}$$

is derived from the general theory [4].

Under these circumstances Q_t may be calculated for given values of $R(t)$ or $p(t)$, respectively, and the constant parameter ρ. For details of the derivation we refer to the Appendix section below. In the first stage one obtains in terms of ρ and $p(t)$

$$Q_t = \left(Q_0 + \sum_{n \geq 1} (p^n/n!) \cdot \varphi_n(\rho)\right) \cdot \exp(-\rho p) \tag{6a}$$

where

$$\varphi_n(\rho) = \int_0^\infty q(y) \cdot (x^{n-1}/(n-1)!) \cdot \exp(-x/\rho) \mathrm{d}x \tag{6b}$$

The function $q(y)$ stands for the quenching factor in the case of liposomes with an entrapped dye concentration that has dropped to a lower level of $c'_0 < c_0$ if the initial level was c_0, and setting $y = c'_0/c_0$. The actual course of $q(y)$ must be determined experimentally (see Fig. 1). In Eq. (6b) one has to set $y = \exp(-(1 - \rho) \cdot x/\rho)$. For practical reasons (because the evaluation can then easily be carried out using a programmable pocket calculator) we propose a fit of the measured quenching curve by a finite power series

$$q(y) = \sum_{m \geq 0} a_m \cdot y^m \quad (a_0 = 1) \tag{7}$$

Now the integration can be carried out resulting in

$$\varphi_n(\rho) = \sum_{m \geq 0} a_m (\rho/(1 + m(1 - \rho)))^n \tag{8}$$

Thus, Eq. (6a) eventually takes the form

$$Q_t = \sum_{m \geq 0} a_m (R(t))^{z_m} \text{ with } z_m = m\rho/(1 + m(1 - \rho)) \tag{9}$$

after having expressed $p(t)$ in terms of $R(t)$ according to Eq. (2). This demonstrates that Q_t should be a function of only $R(t)$ and ρ, provided one considers data where the initially entrapped dye concentration c_0 is the same (otherwise the coefficients a_m would change). Because of Eq. (4) one may alternatively compute Q_t as a function of the measured $E(t)$ for a given value of ρ. Some examples of calculated curves are shown in Fig. 2. When they are compared with pertinent experimental results, the relevant value of ρ may be determined.

3. Materials and methods

3.1. Buffer and dye

Our standard buffer was composed of 10 mM Hepes (from Bioprobe), 107 mM NaCl, 1 mM EDTA (both supplied by Merck, Darmstadt, Germany) and approx. 5.6 mM NaOH so that a pH of 7.4 is set. The fluorescent dye 5,6-carboxyfluorescein (CF) (mixed isomers, $M_r = 376.3$, 99% pure by HPLC) has been a product of Sigma (St. Louis, MO, USA). Ordinarily we prepared an aqueous stock solution of 50 mM CF with 10 mM NaCl, 10 mM Hepes and 1 mM EDTA. In order to facilitate the dissolution by neutralizing the two acidic groups per dye molecule and to adjust the pH to 7.4 we have added approx. 135 mM NaOH. This solution is isoosmolar to the standard buffer. For measurements with higher dye concentrations another such stock solution with 95 mM CF was prepared. In this case an additional stoichiometric amount of NaCl has been used in the standard buffer to ensure equal osmolarity. Dye concentrations were checked by measuring the optical absorption at 490 nm where the absorption coefficient was taken as $\varepsilon = 72\,000\ M^{-1}\ cm^{-1}$.

3.2. Lipid vesicles

The pure lipid, palmitoyloleoylphosphatidylcholine (POPC) in chloroform, as obtained from Avanti Polar Lipids (Alabaster, AL, USA) was used without further purification. Small unilamellar vesicles (SUV) with entrapped CF were made in the conventional way described previously [2]. A phospholipid film (10 mg) was dried by rotary evaporation and then placed overnight under an oil pump vacuum. After having dispersed the dry film in 1 ml CF solution by vortex mixing, the SUV were prepared by ultrasonic irradiation under a nitrogen atmosphere using a microtip sonicator (MSE ultrasonic disintegrator) with a cooling bath of 10°C. The suspension was centrifuged at 13 000 rpm for 10 min to remove metal debris particles. We then passed it over a Sepharose CL 4B column (1 × 30 cm) so that the smaller loaded vesicles (with a radius of about 13 nm) are separated from external dye and an apparent fraction of larger vesicles having a radius around 50 nm (see below). The so obtained SUV were used for experiments on the same day (the lipid concentration being determined by phosphate analysis [7]).

In addition, also CF-loaded large unilamellar vesicles of 100 nm diameter (LUV_{100}) have been prepared by means of the extrusion technique [8,9]. A dry film of the lipid was dispersed in 2 ml CF solution with vortex mixing for 5 min. Then a freeze-thaw cycle has been repeated five times. Subsequently the suspension was ten times extruded through a 100 nm polycarbonate membrane (Nucleopore Corporation, Pleasanton, CA) under approx. 15 bar nitrogen pressure. The stock solution was stored at 4°C. External dye was separated off by gel filtration through a Sephadex G-50 column (1 × 30 cm).

Vesicles sizes have been examined by determining the hydrodynamic radius based on dynamic light scattering (DLS) [10]. We did the measurements using a commercial apparatus at 90° detection (ALV 5000 equipped with a He-Ne-laser and a correlator), made available in the Physical Chemistry Institute of this university.

3.3. Peptide

As the pore forming agent in this study we applied mastoparan X (MPX), a constituent of the *Vespa xanthoptera* wasp venom. It is a tetradecapeptide having the sequence: I-N-W-K-G-I-A-A-M-A-K-K-L-L-NH_2 [11]. The synthesized substance was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland). A stock solution was prepared either in water or in aqueous 100 mM NaCl with 10 mM Hepes. Concentrations have been measured by means of the Trp optical absorption (on the basis of $\varepsilon = 5570\ M^{-1}\ cm^{-1}$ at 280 nm).

3.4. Fluorescence quenching

The measurements were done on a Jasco FP 777 spectrofluorometer with excitation at 490 nm (slit 1.5 nm), emission at 520 nm (slit 10 nm, cut-off filter at 495 nm), maintaining 20°C and continuous stirring. In a first series of experiments we have determined the 'static' quenching factor $Q_0 = F_0/F_\infty$ (without added MPX) as a function of the entrapped dye concentration c_0 by appropriately diluting the dye stock solution at the stage of vesicle preparation. The F_0 refers to the fluorescence signal immediately after having removed the external dye, reflecting the degree of internal self-quenching. The F_∞ was measured after having added the detergent Triton X-100 (from Merck, Darmstadt, Germany) to a final concentration of 0.1%. This decomposes the liposomes so that the dye is diluted into the whole volume, thus relieving the initial self-quenching. Vesicles (final lipid concentrations in the range of 15–60 μM) were added to a 2 ml standard buffer sample in a quartz cuvette with a Teflon stirrer. When monitoring F_0 for a while it was found to remain nearly constant during the initial 5–10 min, but revealed some spontaneous efflux later on. This turned out to be more

pronounced and less reproducible with the SUV (5–10% fluorescence increase after 20 min) than with the LUV_{100} (only 2–5% after 30 min).

3.5. Peptide mediated dequenching

We have suspended an appropriate amount of dye loaded vesicles in about 2.5 ml buffer at 20°C and measured the respective value of F_0. Immediately thereafter some peptide (0.1–4 μM) had been added and the increase of fluorescence intensity due to efflux of CF was registered continuously. The rate of fluorescence change slowed down substantially in the course of about 30 min (to less than 3% in 5 more minutes), so that the apparent efflux virtually stopped at a level somewhere below the 100% mark depending on the peptide to lipid ratio. Then 1 ml of the sample was eluted with the same buffer on a Sephadex G-50 column in order to remove the so far released dye. Some 2 ml of the resulting vesicle suspension was then taken to determine the apparent 'dynamic' quenching factor $Q_t = F_0'/F_\infty'$ of dye in liposomes which are more or less depleted of CF due to the action of MPX. In some cases of SUV where the original Q_0 of the stock solution had been increased due to spontaneous efflux, the measured Q_t was corrected for this effect. By means of the present theory, ρ was calculated with the apparent (lower) initial dye concentration (as determined from the 'static' quenching curve) and then used to compute the appropriate Q_t for an initial $c_0 = 50$ mM.

In an alternative series of experiments we did not wait until the efflux subsided of itself. At some time when the efflux rate was still of appreciable magnitude we added a 3 to 5 times excess of unloaded vesicles to bring about a fast stop of the release process [12]. The Q_t measured under these circumstances turned out to be essentially equal to those determined at a spontaneous stop provided the respective values of $E(t)$ were the same.

4. Results

4.1. Vesicle sizes

The DLS measurements have been carried out in some detail with the LUV_{100}. A practically monodisperse distribution was found that exhibited a mean external radius $R_v = 49(\pm 3)$ nm, being practically independent of the lipid, dye or peptide concentrations used as well as of the time elapsed after preparation (up to 10 days).

In the case of the SUV two peaks in the size distribution have been observed. One of them was well reproducible exhibiting a $R_v = 13(\pm 2)$ nm while the other one at approx. 50 nm was broader and could be less well reproduced. This latter fraction of liposomes was always separated off before the experiments as mentioned already above.

At any rate, the low concentration of MPX used in this study implied peptide/lipid ratios that were definitely too

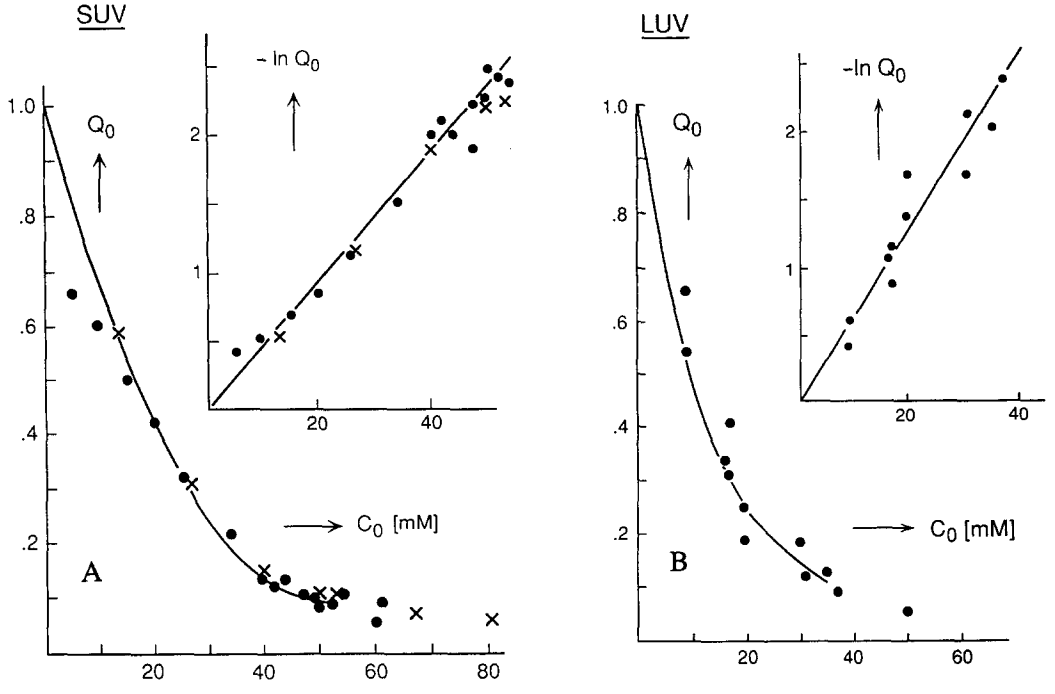

Fig. 1. The 'static' quenching factor Q_0 of carboxyfluorescein entrapped in POPC unilamellar vesicles as a function of the internal dye concentration c_0. (A) Results for SUV (×: unpublished data of T. Popescu in this laboratory). The solid curve in the lower diagram is a quadratic power series fit according to Eq. (7), expressed by $q(y) = 1 - 1.8y + 0.9y^2$ (where $y = c_0/50$ mM). The measured data may also be fitted quite well to an exponential function $Q_0 = \exp(-ac_0)$ with $a = 47\ M^{-1}$ as demonstrated by the semi-logarithmic plot in the inset. (B) Results for LUV_{100}. The present data have been fitted by the power series $q(y) = 1 - 2.54y + 2.73y^2 - 1.1y^3$ ($y = c_0/37$ mM). The exponential fit in the upper diagram has been done with $a = 65\ M^{-1}$.

small to impair the integrity of both vesicle species under investigation. Only adding rather large quantities of detergent (such as 0.1% Triton X-100) actually decomposes the liposomes completely. This could be confirmed by pertinent DLS measurements showing that mixed micelles of 3 nm radius are created.

4.2. Dequenching of internal dye

Measured 'static' quenching factors Q_0 versus the entrapped dye concentrations c_0 are presented in the Fig. 1.

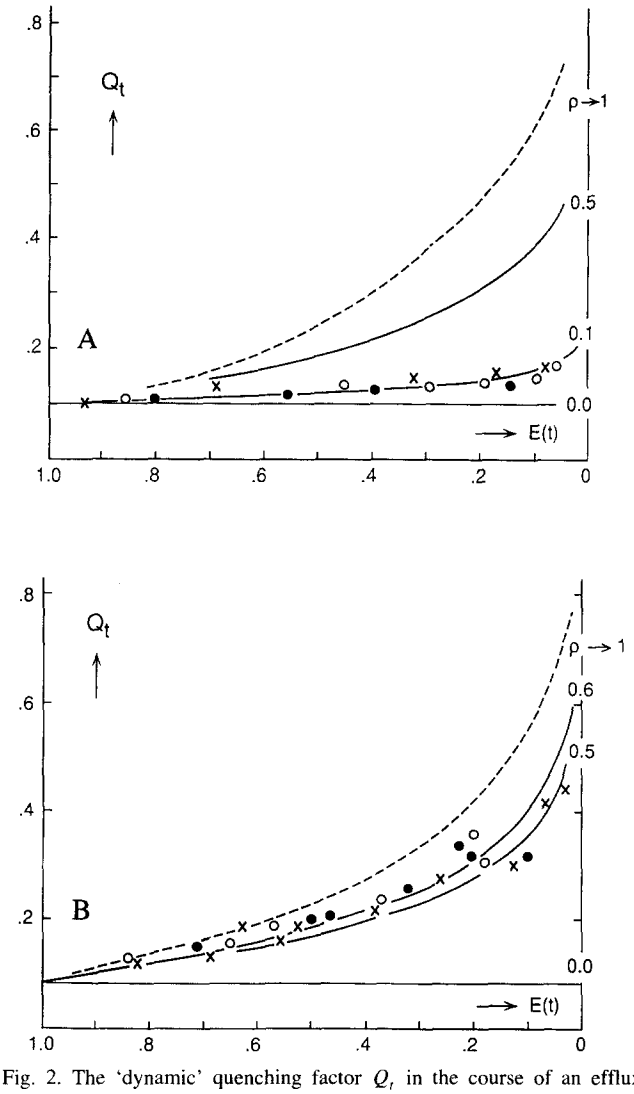

Fig. 2. The 'dynamic' quenching factor Q_t in the course of an efflux process induced by MPX added to POPC unilamellar vesicles in the course of dye release as expressed by the decreasing efflux function $E(t)$. (A) Results for SUV where the internal CF concentration was initially set to a value of 50 mM ($Q_0 = 0.1$). The curves shown have been calculated by means of Eq. (9) for various values of ρ using the $q(y)$ fit of Fig. 1A. The 'all-or-none' release (with $\rho = 0$) implies $Q_t = Q_0$ at any $E(t)$. The dashed curve ($\rho \rightarrow 1$) stands for the other extreme case where the pore lifetime is so small that very many pore openings are required for a complete release of the entrapped dye. The data points (indicated by ×, ○, ●) have been measured in three different series of experiments. (B) Results for LUV_{100} involving an initial $c_0 = 37$ mM and the $q(y)$ fit of Fig. 1B.

For the SUV the data can apparently be fitted quite well up to 50 mM by a quadratic expression according to Eq. (7). Regarding the LUV_{100} there is a good fit by a third degree polynomial up to 37 mM. These fits have been used in order to calculate dynamic quenching factors Q_t as a function of $E(t)$ under our experimental conditions by means of the Eqs. (4) and (9). The curves obtained in the case of various parameters ρ are plotted in Fig. 2A (SUV) and Fig. 2B (LUV_{100}), respectively. Relevant experimental values are also shown indicating that we may set $\rho = 0.10$ (± 0.02) for the SUV and $\rho = 0.55$ (± 0.05) for the LUV_{100}. Apparently the Q_t measured at a more advanced stage of efflux, i.e., small $E(t)$, will be more significant regarding the possible range of uncertainty of ρ determined in this way.

5. Discussion

It could be clearly shown that the addition of the peptide MPX to POPC vesicles causes a release of entrapped CF molecules. This phenomenon is certainly not the result of a peptide-induced disintegration of the liposomes. The peptide/lipid ratio is by orders of magnitude too small to allow a formation of mixed micelles so that the lipid may be solubilized. Furthermore, our DLS experiments reveal no apparent change of vesicle shape in the presence of the given low peptide concentrations. Accordingly we attribute the observed leakage of the vesicles to the formation of some kind of pores, i.e., peptide-induced structural defects in the lipid bilayer where the diffusion of hydrophilic material across the hydrophobic membrane core is very much facilitated. It stands to reason that such a pore must be composed of a greater number of monomeric peptide molecules if the small size of a monomer is taken into account. A pore that is large enough to let CF molecules pass should have a diameter of about 1 nm or more. For a corresponding water-filled channel in a SUV membrane the single pore release time τ_0 can be expected to be less than a millisecond [2]. This and the practically uniform vesicle sizes comply with our theoretical assumptions pointed out above.

Our experimental Q_t vs. $E(t)$ data as presented in Fig. 2 are indeed rather well described by the proposed theory if an appropriate value of ρ is adjusted. Because of Eq. (5) we may then determine the ratio $\tau_0/\tau_p = \rho/(1-\rho)$. For the SUV (where $\rho \approx 0.10$) one so obtains an average pore lifetime τ_p that is about 9-times larger than τ_0, in other words the situation comes close to the 'all-or-none' case of marker release. Assuming the same kind of pores in the LUV_{100} membrane, the τ_0 would linearly increase with the vesicle volume, i.e., in proportion to the third power of the inner radius [2]. With the outer radii given above and a membrane thickness of 3.7 nm [13] this makes a factor of $(45.3/9.3)^3 = 116$ so that according to Eq. (5) a value of $\rho = 0.93$ ($\tau_0/\tau_p \approx 13$) has to be expected. Actually we

have found $\rho = 0.55$ corresponding to $\tau_0/\tau_p \approx 1.2$. Therefore, we must conclude that the pores formed by MPX in the LUV_{100} are substantially different from those in the SUV made of the same lipid. This may be due to the reduced curvature of the bilayer, possibly resulting in a larger pore cross section (which implies a decrease of τ_0) and/or a higher pore stability (so that the pore life time is increased).

Knowing ρ and the calculated Q_t vs. $E(t)$ curve we can evaluate and discuss the pore formation rate on the basis of the measured efflux function. Because of the nearly 'all-or-none' efflux mode with the SUV the deviation of $R(t)$ is comparatively small there. For the LUV_{100} there is, however, a sizable difference.

So far our results are consistent with a quite homogeneous and mutually independent pore mode. Naturally, a more complex situation may be encountered in practice. It could imply a broad distribution of different pore structures and/or vesicle sizes as well as possible cooperative interactions of pores. Taking into account such circumstances, the present theory may be properly extended at the expense of an increase in the computational effort. Furthermore, in cases where τ_0 would be no longer small in comparison with the measuring time (as to be expected with rather large vesicles or cells) our approach must be modified (since ρ will then become time-dependent).

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Appendix A

The theoretical foundation of the present analysis is based on the general approach which has recently been described elsewhere [4]. Equivalent and mutually independent pores are Poisson distributed on the individual liposomes (assumed to be of uniform size). The dye release through a given pore is single exponential with a relaxation time τ_0. This rate is thought to be fast in comparison with the measured overall efflux. Then $p(t)$, the average number of pores per vesicle in the time t after the release experiment was started, and the pore lifetimes will be the rate limiting elements.

First we consider only those vesicles where a certain number of pores, $n \geq 1$, had been active during t. Their respective individual lifetimes are denoted $\tau_i (i = 1,2,..,n)$ The fraction of dye being retained in such a liposome would be equal to $y = \exp(-\tau/\tau_0)$ with τ being the sum of all the individual lifetimes. In the envisaged first order kinetics of the pore closing the probability of a single pore lifetime between τ_i and $\tau_i + d\tau_i$ turns out to be $\exp(-\tau_i/\tau_p)\,(d\tau_i/\tau_p)$ which involves τ_p, the average lifetime of a pore (being equal to the reciprocal first order rate constant of the closing reaction). If there are n pores, the probability of a combined lifetime between τ and $\tau + d\tau$ can be formulated as

$$\exp(-x) \cdot d\Phi_n(x) = \omega_n(x) \cdot dx \text{ with } x = \tau/\tau_p \text{ and}$$

$$\Phi_n(x) = \int\int \cdots \int (d\tau_1/\tau_p)(d\tau_2/\tau_p) \cdots (d\tau_n/\tau_p)$$

integration for $\tau_1 + \tau_2 + \cdots + \tau_n \leq \tau = x \cdot \tau_p$

This multiple integral can be easily calculated by total induction starting from $n = 1,2,..$ resulting in

$$\Phi_n(x) = \int_0^\infty \Phi_{n-1}(x - x')\,dx' = x^n/n!$$

so that eventually

$$\omega_n(x) = \left(x^{n-1}/(n-1)!\right)\exp(-x)$$

is obtained.

The contribution towards F_{in} originating in vesicles with n (≥ 1) pores and a combined lifetime interval τ, $\tau + d\tau$ may now be written as

$$dF_{in}^{(n)} = w_n \cdot q(y) \cdot y \cdot F_\infty \cdot \omega_n(x)dx$$

This involves $w_n = (p^n/n!) \cdot \exp(-p)$, i.e., the probability of liposomes so far infected by n pores, and $y = \exp(-(1-\rho) \cdot x/\rho)$ standing for the ratio of the retained to the initial dye content. Furthermore, $q(y)$ is the quenching factor applicable to the reduced internal dye concentration. Taking into account also vesicles without pores ($n = 0$, $w_0 = \exp(-p)$) and summing up all possible contributions of infected liposomes we find that

$$F_{in}/F_\infty = \exp(-x) \cdot \left(Q_0 + \sum_{n \geq 1} (p^n/n!) \cdot \int_0^\infty q(y) \cdot \left(x^{n-1}/(n-1)!\right) \cdot \exp(-x/\rho)dx \right)$$

When dividing this expression by $R(t)$ according to Eq. (2), the Eqs. (6a) and (6b) are readily derived.

References

[1] Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A. (1977) Science 195, 489–492.
[2] Schwarz, G. and Robert, C.H. (1990) Biophys. J. 58, 577–583.
[3] Schwarz, G., Zong, R. and Popescu, T. (1992) Biochim. Biophys. Acta 1110, 97–104.
[4] Schwarz, G. and Robert, C.H. (1992) Biophys. Chem. 42, 291–296.
[5] Weinstein, J.N., Klausner, R.D., Innerarity, T., Ralston, E. and Blumenthal, R. (1981) Biochim. Biophys. Acta 647, 270–284.
[6] Parente, R.A., Nir, S. and Szoka Jr., F.C. (1990) Biochemistry 29, 8720–8728.
[7] Böttcher, C.J.F., Van Gent, C.M. and Fries, C. (1961) Anal. Chim. Acta 24, 203–204.
[8] Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
[9] Mayer, L.D., Hope, M.J. and Cullis, P.R. (1986) Biochim. Biophys. Acta 588, 161–168.

[10] Berne, B.J. and Pecora, R. (1976) Dynamic Light Scattering, J. Wiley, New York.
[11] Wakamatsu, K., Higashijima, T., Fujino, M., Nakajima, T. and Miyazawa, T. (1983) FEBS Lett. 162, 123–126.
[12] Grant Jr., E., Beeler, T.J., Taylor, K.M.P., Gable, K. and Roseman, M.A. (1992) Biochemistry 31, 9912–9918.
[13] Huang, C. and Mason, J.T. (1978) Proc. Natl. Acad. Sci. USA 75, 308–310.