Fluxes theory in experiments with random distributed channels on vesicles

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When channels are randomly distributed in a population of vesicles, disregarding the number of channels per vesicle, these channels follow a Poisson distribution. This has been verified in many cases, determining the average of channels per vesicle. However, to determine kinetic parameters in population studies, a mathematical expression for the mean flux of solute through channels per vesicle is necessary. Hence, here, this mean flux is calculated, assuming Poisson distributed channels in a population of vesicle. Moreover, this result has been generalized to any number of different kinds of channels (i.e., channels with different permeabilities). These results, useful for in vitro experiments with mixed both channels and vesicles, can be supplemented with those from other techniques, in order to understanding how the nature of the lipid membrane affects some channel kinetic parameters.

Introduction

There are several in vitro systems in which membrane channels are reconstituted into lipid membranes. In these systems, the activity of channel is studied and, including results from other methods, in vitro regulatory mechanisms are proposed. In aqueous solution, vesicles can be mixed with channel-forming proteins, as in kinetic experiments with unilamellar liposomes, where the composition of the lipid bilayer and both intra and extra vesicular media can be determined.1 However, understanding measurements of both fluxes and equilibrium states of the permeant solute (either ionic or non-ionic) requires that the channel distribution model on the vesicles are known.

If we assume equivalent and mutually independent channels, and vesicles of uniform sizes, then, in cases of random distribution, those channels could be considered as Poisson distributed on the individual vesicles. This distribution has been used in analyzing of several experiments.2-6 Thus, the liposome population must be corrected in accordance to Poisson distribution for adequate interpretations of results. Working with transport-specific fractionation of liposomes, into which connexin channels were reconstituted, it was suggested that the channels are Poisson distributed among the liposomes.2 This is in accordance to hypothetical modes of monomeric association to form channels on liposomes.7 Based on channel Poisson distributions and using inside out vesicles, Alvarez et al. developed a method to estimate the number of Ca²⁺-dependent K⁺ channels in the human red cell.8 Moreover, the use of a Poisson distribution of pores in vesicles has been useful in solute emptying models in vesicles: all-or-nothing models4-5 or gradual, regarding dynamic factors.6

Determining, at many times, the permmeant solute concentration on aqueous liposome system, and employing an adequate mathematical model, some channel parameters could be obtained. For this reason, assuming passive membrane transport process, here is calculated the mean of solute flux per vesicle, through Poisson distributed channels on a population of vesicles. Subsequently, the expression is generalized to any number of channels with different permeabilities, a result that could be applied to cases of channel with more than one conformation, or channels with normal distribution of permeability, such as it has been described.9 Thus, this work pretends that mathematical conclusions may be useful for design new vesicular experiments capable to contribute, in addition to other techniques, to the understanding how the nature of the lipid membrane affect some channel kinetic parameters.

Theoretical Framework and Results

Defining: \( N \), number of vesicles of volume \( v \) in a total volume \( V \) of aqueous solution; \( n \), number of channels per vesicle; and \( C \), number of channels in all vesicles. If the vesicles are randomly selected for channel insertion, then, \( n \) is a binomial random variable with parameters \( C \) and \( 1/N \). Assuming that \( C \to \infty \) and \( (1/N) \to 0 \), with \( \alpha = CN \) (the mean of channels per vesicle), the probability \( P_n \) of a vesicle with \( n \) channels follows a Poisson distribution:

\[
P_n = \frac{e^{-\alpha} \alpha^n}{n!}
\]  
(1)
Calculation of the mean of intravesicular solute concentrations

Denoting by \( X_n \) and \( X_{eq} \) the intravesicular and extravesicular concentrations of a permeant solute \( S \), respectively. If \( V > Nv \), then \( X_{eq} \) is assumed a constant. In a passive membrane transport process, for \( X_n \) near to its equilibrium value \( X_{eq} \), and from the Fick's first law, a simple model of first order is given by:

\[
\frac{dX_n}{dt} = nk \cdot (X_{eq} - X_n)
\]

(2)

With \( k \) the single channel flux rate, and \( nk \) the transport coefficient (proportional to the total permeability of the vesicle channels). The solution of the initial value problem, \( X_n(t=0) = X_0 \), is

\[
X_n = X_{eq} - (X_{eq} - X_0) e^{-nk t}
\]

(3)

The mean \( \bar{X} \) of intravesicular concentrations is given by the expected value of \( X_n \):

\[
\bar{X} = \sum_{n=0}^{\infty} X_n P_n
\]

(4)

where \( P_n \) is given by Eq. 1. In series as in Eq. 4, as well as other in this article, the contribution of terms with a very large \( n \) (i.e., an impossible number of channels) must be regarded as insignificant, because the corresponding Poisson probability factor is diminished.

From Eq. 1 and Eq. 3 into Eq. 4:

\[
\bar{X} = X_{eq} - (X_{eq} - X_0) e^{-\alpha} \sum_{n=0}^{\infty} \frac{e^{-\alpha n}}{n!} = X_{eq} - (X_{eq} - X_0) e^{-\alpha}
\]

(5)

Given that the summation is the Taylor's series expansion for the exponential function \( e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \), then the average value is

\[
\bar{X} = X_{eq} - (X_{eq} - X_0) e^{[\exp(-\alpha t) - 1]}
\]

(6)

Thus, in case of random distribution of channels over the vesicles, and with all the above experimental assumptions, the intravesicular concentration of solute \( S \) can be calculated from Eq. 3, and its mean of concentration must be similar to the analytical calculation given by Eq. 6. The agreement between these two modes of calculation is showing in Figure 1.

If \( X_0 = 0 \), then, \( \bar{X} \) at the equilibrium, equal to \( \bar{X}_{eq} \), is calculated from Eq. 6, confirming a well-known result:

\[
\bar{X}_{eq} = \lim_{t \to \infty} \left( \bar{X} \right) = X_{eq} \cdot (1 - e^{-\alpha}) = X_{eq} \cdot \left(1 - P_0\right) = X_{eq} P_{n \neq 0}
\]

(7)

i.e.: \( X_{eq} \) equals the fraction of vesicles with at least one channel \((n \neq 0)\), multiplied by the intravesicular equilibrium concentration of \( S \).

An application of the theory to determine the single channel flux rate (i.e., \( k \))

Parameters \( X_{eq} \) and \( X_n \) can be obtained by conventional methods employing measurements at the equilibrium, and, then, \( \alpha \) can be calculated from Eq. 7, giving

\[
\alpha = \ln \left( \left( \frac{X_{eq}}{X_0 - X_{eq}} \right) \right)
\]

(8)

Once \( \alpha \) and \( X_{eq} \) are known, \( k \) might be calculated from an expression for the initial flux. Thus, deriving Eq. 6

\[
\frac{d\bar{X}}{dt} = \alpha k \cdot (X_{eq} - X_0) e^{-\alpha t} e^{[\exp(-\alpha t) - 1]}
\]

(9)

and reordering Eq. 9, and from \( t = 0 \), we obtain:

\[
\frac{1}{(X_{eq} - X_0)} \frac{d\bar{X}}{dt} = k \alpha
\]

(10)

If \( X_0 = 0 \) and regarding the linear zone of \( \bar{X} \) vs. \( t \) (i.e., with \( t \) ranging from 0 to an enough small value of \( \Delta t \)), then

\[
\frac{d\bar{X}}{dt} (t = 0) = \frac{\Delta \bar{X}}{\Delta t}
\]

Thus, given a lot of different data of

\[
\left( \alpha, \frac{1}{X_{eq}} \frac{\Delta \bar{X}}{\Delta t} \right)
\]
Figure 1. The mean of intravesicular concentration $X$ vs. time $t$, in $X_{eq}$ and $1/k$ units, respectively. The system consists of $10^3$ vesicles and $10^5$ channels, using different values of $\alpha$ (i.e., the ratio between the number of channels and the number of vesicles). Before the channel addition, there is no substrate into the vesicles ($X_0 = 0$). Circles correspond to obtained values from computer simulated random allocation of channels into vesicles, and indicate the subsequent mean of the calculated intravesicular concentrations of $S$ (from Eq. 3), the permeant solute. Curves indicate values directly obtained from Eq. 6, given that the channels are Poisson distributed on the vesicles.

at $t = 0$, a linear plot $\frac{1}{X_{eq}} \frac{\Delta X}{\Delta t}$ vs. $\alpha$ must have a slope equal to $k$. (Different values of $\alpha$ can be obtained from different ratios Lipid/Channel protein). In Figure 2 we can see how this method finds a value of 0.3230 min$^{-1}$ for a theoretical $k$ equal to 0.3230 min$^{-1}$ (relative error $\approx 8.7\%$)

Moreover, Eq. 10 could be useful to obtain the relative permeabilities of different solutes:

$$\frac{X_{eq,n_1}}{X_{eq,n_2}} \frac{dX_{1}/dt}{dX_{2}/dt} = \frac{k_1}{k_2}$$

(11)

Parameter and variable indexes are regarding indexes of solutes $S_1$ and $S_2$.

Generalizing to a model of multi-channel distribution in a population of vesicles

We could assume that there is a set of different kinds of channels to be distributed over the vesicles, each one following a particular Poisson distribution. In this case, it would be useful to generalize the previous calculations.

Let’s have $R$ different kinds of channels, each one named the $i$-th channel ($i = 1, 2, ..., R$) and with a particular Poisson distribution over the vesicles. The $i$-th channel has associated a single pore flux rate $k_i$, a number $n_i$ of repeated channels in a vesicle, and a mean of the number of $i$-th channels per vesicle, called $\alpha_i$ (the mean of $n_i$). Thus, a vesicle may have: $n_1$, $n_2$, ..., $n_R$, and $n_\alpha$ channels of the types 1-th, 2-th, ..., $i$-th, ..., and $R$-th, respectively. Then, a realization of channels in a vesicle can be denoted by a specific sequence of $R$ positive integers $(n_1, n_2, ..., n_R)$, called $D$. The concentration of solute into a vesicle with a channel realization $D$ is named $X_{eq,D}$, which satisfies the following dynamical system

$$\frac{dX_{eq,D}}{dt} = \sum_{i=1}^{R} n_i k_i \left( X_{eq} - X_{eq,D} \right)$$

(12)

where $X_{eq,D}$ is the intravesicular solute concentration at the equilibrium and $n_i$ is an event Poisson distributed, with probability $P_{n_i}$

$$P_{n_i} = e^{-\alpha_i} \alpha_i^{n_i} / n_i !$$

$$\alpha \equiv \sum_{i=1}^{R} \alpha_i$$

Parameter and variable indexes are regarding indexes of solutes $S_1$ and $S_2$.
with parameter $\alpha > 0$. Noting that equals the mean of the number of channels on the vesicles. Then, we can prove that the expectation value of $X_D$ due to all the realizations of $D$ is given by

$$\bar{X} = X_{eq} - \left( X_{eq} - X_{D0} \right) e^{\frac{\alpha}{\alpha} \left( \exp(-k,t) - 1 \right)}$$

with

$$\langle \exp(-k,t) \rangle = \sum_{i=1}^{g} \exp(-k,t) \frac{\alpha_i}{\alpha}$$

(i.e., the mean of $\exp(-k,t)$ regarding all different kinds of channels in the vesicles, and where $\frac{\alpha_i}{\alpha}$ equals to the probability of having an $i$-th channel on a vesicle). The proof is in Appendix A.

**Discussion**

Here, for experiments of reconstitution of channels into vesicular membrane, a theory has been developed regarding solute fluxes due to passive transport. Given $A_\perp$, a transverse area of channel on lipid membrane, and $A_\parallel$ equal to the area of the surface of lipid vesicle, we have that $\alpha A_\perp$ equals the average of total cross-sectional area of channels per vesicle and that $\alpha A_\perp < A_\parallel$ is a necessary condition for an insertion of channel into the vesicular membrane regardless of any previous channel insertion, such that the number of channels per vesicle can follow a Poisson distribution. Thus, very large values of $\alpha$ might cause a deviation from the Poisson distribution.

Vesicles without channels, under a Poisson distribution of channels, are possible because an irreversible binding of channel proteins with the lipid membrane. There are studies in which it has been assumed a rapid peptide binding in the vesicle membranes and, once a channel is formed in a vesicle, all this content would leak within less than 1 s, and, then, the kinetic leakage has been equated to the kinetic of aggregation of critical or supercritical peptides in the membrane. In such approach, kinetic aspects of leaking per vesicle are not considered. Differently, in our analysis we assumed that, before the flux induction (e.g., by adding some antagonist or the necessary solute), the channels are both formed and random distributed. Therefore, in this case, changing in fluxes indicate kinetic aspects of channel.

Here, a mathematical expression for the expectation of solute, flowing through Poisson distributed channels on the vesicles, has been obtained. According to the results, the solute concentration should not follow a single exponential. Alvarez et al. have reported, in kinetic experiments employing inside-out-vesicles, that $\mbox{86Rb}^+$ uptake through Ca$^{2+}$ dependent K$^+$ channels does not follow single exponentials. Besides this could reflect size heterogeneity in the vesicle population, it would also be expected from random distribution of channels in vesicles with uniform sizes.

Some channels with more than one configuration has been described, each one with different conductance. One of the simplest cases is when the channel has a Poisson distribution for both open and closed conformations. In order to study more
complex profile of permeabilities for a population of channels, we have obtained a general expression for a number \( n \) of channels with different non interconvertible permeabilities (Eq. 14 and 15). This general expression would allow modeling of multiple channels, or also fluxes due to a probability of distribution for the values of a single pore flux rate \( k \). This distribution of \( k \) has been founded in experiments with membranes of resealed human erythrocyte ghosts by incubation with perforin (cytolysin), in which the measurements of single-ghost flux of the polar fluorescent probe Lucifer Yellow were by fluorescence microphotolysis (photobleaching) technique. These experiments have shown that pores are distributed in the vesicles according to Poisson and, however, the single pore flux rate \( k \), associated to a single pore, is Gaussian distributed.\(^8\)

These results could also apply to experiments with suspended cells. This is because the Poisson distribution of channels also may be evident in some patch experiments.\(^{14}\) However, also some cases have been reported in which the cumulative distribution of the number of channels in patches does not follow a Poisson distribution, suggesting channel clusters.\(^{15}\) To explain nonrandom distribution of channels in plasma membranes, at least two mechanisms should be considered: First, the association of ion channels to specialized microdomains in the membrane, commonly referred as lipid rafts. Second, by mean of direct protein–protein interactions, channels can be clustered by interacting with the extracellular matrix and/or by anchoring to cytoskeletal elements.\(^{16}\)

Experimentalists could apply this theory specially to obtain the parameter \( k \) (i.e., the single channel flux rate), and besides, the relative permeabilities of different solutes. However, starting the fluxes, the initial linearity of the mean of intravesicular concentration vs. time is essential to decrease the error in the estimation of the parameter \( k \), in such a way that

\[
\frac{d \bar{X}}{dt} (t = 0) = \frac{\Delta \bar{X}}{\Delta t}
\]

In addition, other parameters could be obtained at the equilibrium, either by this theory or by the already known methods.

On the other hand, the case of many mixed kinds of solutes in solution, all of them regarding the same channel, should be similar to the case of only one solute, here studied, since our model does not include solute competition for the channel.

The theory developed here also could allow obtaining channel permeability from experiments with liposomes, by performing measurements at different times, and not only at initial conditions. These results could be compared with those from single channels, obtained by experiments of patch or planar bilayers. The Poisson distribution of channels into vesicles is a part of a broader distribution problem of molecules into lipid membranes, whatever their activity associated (e.g.: enzyme, receptors, ligands, fluorescent probe or channel), an aspect that is largely overlooked in the literature.\(^{17,18}\) Each time a protein on the membrane is inserted regardless of previous events insertion, the Poisson distribution could be considered in the development of response models.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Appendix**

Proving that the average of solute concentration into a vesicle, with \( R \) kinds of Poisson distributed channels, is given by

\[
\bar{X} = X_{eq} - \left(X_{eq} - X_{D0}\right) e^{\frac{\sum \eta_i k_i}{\alpha}}
\]

with

\[
\left< \exp(-k_i) \right> \equiv \sum_{i=1}^{R} \exp(-k_i \alpha) \frac{\alpha_i}{\alpha}
\]

The solution of Eq. 12, by mean of integration, with the initial value problem \( X_D (t = 0) = X_{D0} \), is

\[
X_D = X_{eq} - \left(X_{eq} - X_{D0}\right) e^{-\sum \eta_i k_i / \alpha}
\]

Since \( \eta_i \) is an event Poisson distributed, with probability \( P_{n_i} \), the mean \( X \) of \( X_D \) is equal to the expected value of \( X_D \).

\[
X = \sum_D X_D P_D = \sum_{n_1=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} \left[ X_{eq} - \left(X_{eq} - X_{D0}\right) e^{-\sum \eta_i k_i / \alpha} \right] \prod_{i=1}^{R} P_{n_i} =
\]

\[
X = X_{eq} \sum_{n_1=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} \prod_{i=1}^{R} P_{n_i} - \left(X_{eq} - X_{D0}\right) \sum_{n_1=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} e^{-\sum \eta_i k_i / \alpha} \prod_{i=1}^{R} P_{n_i} =
\]

(17)

From

\[
\sum_{n_1=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} \prod_{i=1}^{R} P_{n_i} = \sum_{n_1=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} P_D = \sum_{D} P_D = 1
\]

(18)

(from the normalization of the probability \( P_D \))
Using Poisson expression for $P_{n_i}$ (Eq. 13):

\[
\bar{X} = X_{eq} - (X_{eq} - X_{D0}) \sum_{n_i=0}^{\infty} \sum_{n_j=0}^{\infty} \ldots \sum_{n_R=0}^{\infty} \prod_{i=1}^{R} (e^{-n_i} P_{n_i})
\]

(20)

From definitions of $\alpha$ and $(\exp(-k))$ (Eq. 15), the above expression for $\bar{X}$ may be written as in Eq. 14.

Recognizing that summations in $n_i$ is the Taylor series expansion of the exponential function $\left( e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \right)$, the average value is:

\[
\bar{X} = X_{eq} - (X_{eq} - X_{D0}) e^{-\sum_{i=1}^{R} \alpha_i} \sum_{i=1}^{R} e^{\frac{\alpha_i}{n_i!}}
\]

(22)

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