Drug Transport in Responding Lipid Membranes Can Be Regulated by an External Osmotic Gradient

Fátima O. Costa-Balogh,*,†,‡ Christoffer Åberg,† João J. S. Sousa,‡ and Emma Sparr†

Department of Physical Chemistry 1, Chemical Center, Lund University, P.O. Box 124, SE-22100 Lund, Sweden, and Centro de Estudos Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra, Coimbra codex P-3004-535, Portugal

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In this paper, we demonstrate, for the first time, how an external osmotic gradient can be used to regulate diffusion of solutes across a lipid membrane. We present experimental and theoretical studies of the transport of different solutes across a monoolein membrane in the presence of an external osmotic gradient. The osmotic gradient introduces phase transformations in the membrane, and it causes nonlinear transport behavior. The external gradient can thus act as a kind of switch for diffusive transport in the skin and in controlled release drug formulations.

Introduction

In the simple description of diffusion transport, one considers a system with a concentration gradient of the diffusing species. However, in a living system, diffusion often occurs between regions where the properties differ in a more profound way, which implies the presence of more than one gradient in the system. Moreover, the membrane itself cannot always be regarded as a simple barrier with given properties, since the barrier properties can be altered by changes in the surroundings. This can give rise to a rich variety of different possible effects. A phenomenon particularly relevant for lipid systems in, for example, biological membranes is the possibility of phase transformations along the gradients. It is typical for the rich phase behavior of lipids that small changes in the external conditions can trigger large structural changes, e.g., between lamellar, hexagonal, cubic, or gel states. Phase changes are cooperative phenomena and they introduce a highly nonlinear element into the description of the diffusive transport. The structural transformations between these phases can, for example, be induced by variations in osmotic pressure.[1−3]

One situation where the effects of osmotic pressure on lipid phase behavior is of utmost importance is found in the stratum corneum (the upper layer of the skin), due to the large gradient in water between the water-rich tissue on the inside, and the relatively dry environment on the outside of the body. The extracellular lipids in stratum corneum constitute the sole continuous regions of the stratum corneum, and molecules passing the skin barrier must be transported through them.[4−6] The osmotic gradient is expected to affect the lipid structure and permeability, and the understanding of how the permeability of lipid membranes can be regulated by external gradients can have a large impact on, for example, transdermal drug delivery.

We have previously presented a theoretical model for transport in responding lipid membranes,[7−8] demonstrating that the external osmotic gradient can lead to nonlinear transport behavior. Here, we present the first experimental demonstration of the phenomenon combined with an application of the theoretical approach. We specifically study solute flux in a model system with monoolein in the presence of an osmotic gradient that can induce the transformation between an inverted bicontinuous *Ia*3*d* cubic phase and a liquid crystalline L_α lamellar phase.[9−10]

Materials and Methods

We use a setup of Franz diffusion cells (8 mL receptor volume, 0.636 cm^2 diffusion area) from Permegear, Inc. (Bethlehem, PA, USA). The membranes separating the two compartments are composed of monoolein (Danisco Ingredients, Denmark, ID98−027, 95% purity). Each compartment (donor and receptor chambers) contains an aqueous solution with different concentrations of poly(ethylene glycol) 8000 (PEG 8000) (Aldrich-Chemie, Germany, Lot0808114. PEG is a commonly used polymer in osmotic stress experiments. Within this setup, we can control the osmotic gradient over the lipid membrane.

Diffusion studies were made for two different model solutes (1 mg/ml); eosin (Kebo, Sweden, A1344) and metoprolol tartrate (Sigma-Aldrich, Inc USA, Lot 064K1197). The amount of the model drug that diffused through the membrane was continuously measured by UV/visible spectrophotometry (Perkin-Elmer UV/VIS Spectrometer Lambda 14; Peristaltic pump Ismatec, 4 channels).

The membranes were prepared as oriented thin layers of monoolein on a supporting hydrophilic porous membrane (GH Polypro, 0.45μm Pall Life Sciences, USA). A solution of monoolein in chloroform:methanol (1:1) (0.5 mg in 5 μL) was spread over the support membrane by spin coating. After the evaporation of the solvent (>5 h in a vacuum), the lipids were carefully hydrated in 20 μL of water. The membrane was thereafter placed on the receptor compartment, which was filled with aqueous solutions with PEG 8000 to give an osmotic pressure of 1.22MN/m^2. Care

* Corresponding author. Phone: +46 46 222 8154. Fax: +46 46 222 4413. E-mail address: Fatima.Costa@fkem1.lu.se.
† Lund University.
‡ Universidade de Coimbra.

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Table 1. PEG 8000 Percentages (w/v) and Correspondent Osmotic Pressure Values (MN/m²) of the Solutions in the Diffusion Cell Donor Chamber for Each Experiment and Correspondent Phase Behavior of the Monoolein Membrane

PEG 8000 (% w/v)	Π (MN/m²)	monoolein equilibrium phase behavior
25	1.22	cubic (Q *Ia*3*d*)
30	2.02	cubic (Q *Ia*3*d*)
32.5	2.94	cubic (Q *Ia*3*d*)
35	3.17	lamellar (L_α)
40	4.88	lamellar (L_α)

was taken to ensure that no air bubbles were entrapped under the membrane. A support membrane was placed on top of the lipid membrane to prevent incorporation of PEG 8000 in the monoolein phases that otherwise could occur.[11] Finally, the donor chamber was placed on the top of the membrane, and it was filled with aqueous PEG 8000 solutions. The concentration of PEG 8000 in the donor solution, and thus the osmotic pressure, was varied between the experiments, according to Table 1.[12] The membranes were in this way left for 15−20 h before starting the experiment. The solutions in the donor chambers were then replaced by aqueous solutions containing the model solutes and PEG 8000 with the same osmotic pressure. To maintain the solute and osmotic gradients, the solution in the donor cell was exchanged during the experiment to fresh solution with same composition. The volume of the receptor solution was large enough to maintain sink conditions. In this way, we can introduce osmotic and solute gradients over the lipid membrane. The osmotic stress causes dehydration of the lipid membrane, and under these conditions we have an inhomogeneous system. Hence, this system is defined by the osmotic gradient and not by the composition (water content) of the membrane. The amount of water that is released from the membrane due to the dehydration is negligible compared to the volumes of the donor and receptor cells, and we can therefore assume that it does not affect the overall osmotic gradient.

The absorbance of the solutes in the acceptor solution was recorded automatically at 521 (eosin) and 223 nm (metoprolol tartrate) every minute for 10 h. The solutions in the receptor chambers were magnetically stirred to ensure adequate mixing during the entire experiment. The experiments were performed at 23 ± 2 °C, and each experiment was carried out in triplicate. The flux was deduced from the spectrophotometric measurements of the concentration of the model drug in the receptor solution as a function of time during steady state. The presented data are the effective permeability values, $P_{eff} = J/\Delta C$, obtained under steady-state conditions (m s^{-1}), where J is the flux and ΔC is the concentration gradient of the model drug. The lipid phase behavior was also characterized by small-angle X-ray scattering (SAXS) for monoolein equilibrated in aqueous PEG 8000 solutions, confirming the data in Table 1. We have used technical monoolein (95% purity), so the positions of the phase transition are slightly different from the purified one observed in the literature.[9,10,13]

Results and discussion

Figure 1 shows how the effective permeability of the membrane for two different solutes, eosin and metoprolol tartrate, is regulated by variations in an external osmotic gradient. Here, the osmotic pressure in the receptor cell (Π_r) was kept constant in all experiments to 1.22 MN/m², at which the monoolein forms the *Ia*3*d* cubic phase. The osmotic pressure in the donor cell (Π_d) was varied from 1.22 to 4.88 MN/m². Under these conditions, a phase transformation from the *Ia*3*d* cubic phase to the L_α lamellar phase (Figure 2) can be induced in the layer of the membrane that is in contact with the donor cell. Our data clearly show that there is a stepwise decrease in solute flux at Π_d around 3 MN/m² for both solutes, which coincide with the *Ia*3*d*−L_α phase transition (Table 1). The control experiments, where transport across support membranes without the monoolein was studied, showed no variation in solute flux at different osmotic gradients, confirming that the observed effects are due to the response in the monoolein membrane to the osmotic gradient.

The nonlinear transport behavior can be explained by the induced phase transition, as the different structures have distinctly different diffusion characteristics. In

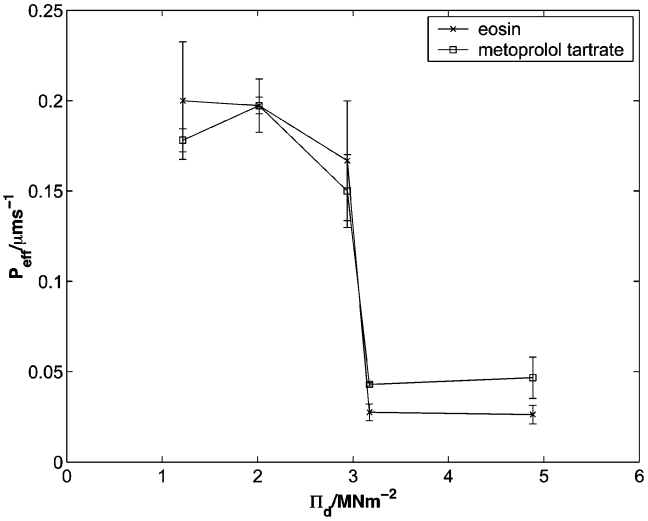

Figure 1. Effective permeability in the presence of osmotic pressure gradients. The osmotic pressure in the donor chamber (Π_d) was varied while the osmotic pressure in the receptor chamber was kept constant to 1.22 MN/m². Experiments were carried out in triplicate and data is expressed as mean ± standard deviation.

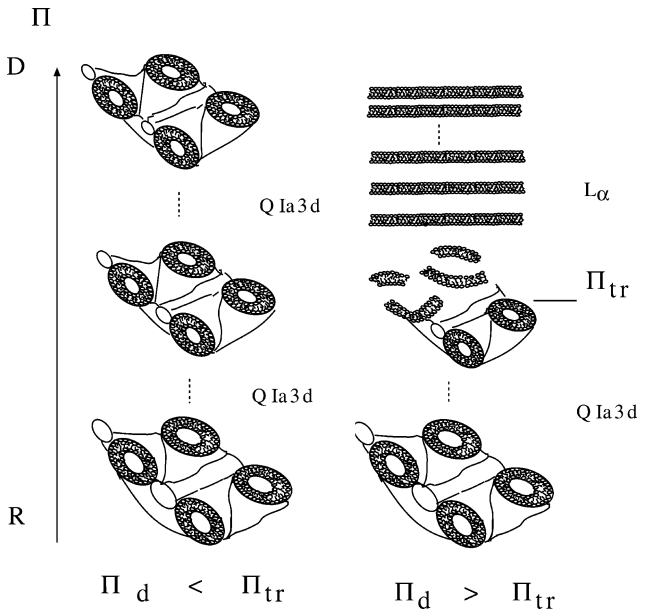

Figure 2. Schematic representation of phase transitions that occur in the membrane in the presence of osmotic pressure gradients. D, Franz cell donor chamber; R, Franz cell receptor chamber; Π_d, osmotic pressure in donor chamber; Π_{tr}, osmotic pressure where phase transition occurs. When the osmotic pressure in the donor chamber is lower than Π_{tr} there is no phase transition (left image). When it is higher than Π_{tr}, then the membrane upper layer is in the lamellar phase (right image). The osmotic gradient also leads to a variation in swelling of the cubic and lamellar phases within the membrane.

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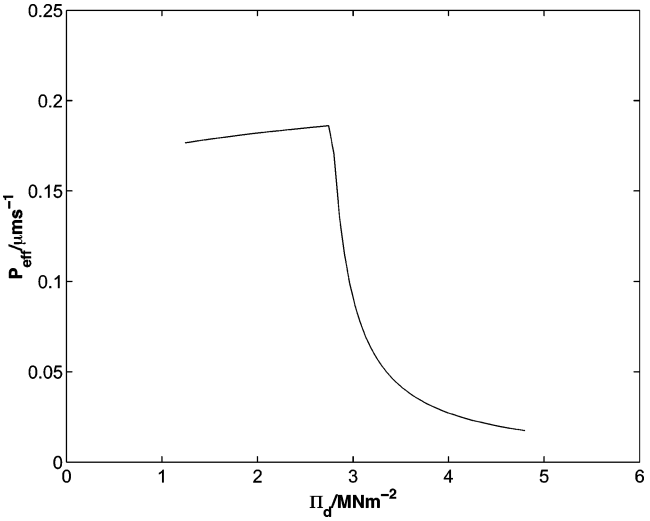

Figure 3. Effective permeability as a function of the osmotic gradient. The parameters of the Volke model were set to $f = 21.76$ and $N_{c,d} = 4.06$, and the bulk water diffusion coefficient to $D^0_{H_2O} = 2.30 \times 10^{-9}$ m²/s.[20] For the lamellar phase, the permeability of water of a single lipid bilayer $P^{lip}_{H_2O} = 3 \times 10^{-5}$ m/s.[21] The parameters for the diffusion of the solute were set to $K^C_i D^C_i = 1.5 \times 10^{-12}$ m²/s and $P^{lip}_i = 4 \times 10^{-8}$ m/s.

lamellar liquid crystalline systems, the diffusion is highly anisotropic, and the diffusion in the perpendicular direction depends strongly on the partitioning of the diffusing species between apolar and aqueous environments. For the bicontinuous cubic systems, on the other hand, diffusion is typically rapid in all directions. This implies that the introduction of a small fraction of the lamellar phase in the membrane dramatically reduces the effective permeability (with the exception of solutes that partition equally between the lipid and the aqueous layers). This effect becomes more pronounced for more polar or more apolar substances, i.e., for solutes with partition coefficients $K \gg 1$ and $K \ll 1$. The solutes used in the present study are both preferentially soluble in water, and the lipid bilayers in the lamellar phase constitute the main barrier to the diffusion. We observe that the membrane effective permeability for eosin is lower than that for metoprolol tartrate at $\Pi_d \geq 3.17$ MN/m², i.e., when the fraction of the monoolein membrane facing the donor cell is in the lamellar phase. This is consistent with the fact that eosin has a lower octanol/water partition coefficient than metoprolol tartrate ($K = 6.3 \times 10^{-3}$ and 6.3×10^{-1}, respectively, as experimentally determined).

By further developing the previously presented model,[7,8] we obtain a very good description of the experimental observations (Figure 3). In the theoretical model, we consider the situation of an oriented monoolein membrane in the presence of a solute gradient as well as an osmotic gradient. The model allows for a coupling between the steady-state flux of water and the thermodynamic response to the local osmotic pressure of water. Here, we take advantage of the fact that the monoolein−water system is very well characterized, and the large body of experimental data needed for making the numerical interpretations is available from the literature.[2,13−15] This circumstance provides a rationale for the choice of the model system.

In the model, we consider the osmotic gradient that causes water flux over the membrane and also induces variations in the structure within the membrane (Figure 2). First, the osmotic gradient implies heterogeneous swelling of the membrane, where the water content in each unit cell (in the cubic phase) or each aqueous layer (in the lamellar phase) is determined from the local osmotic pressures (Π) using the analysis and experimental data from previous characterization of the monoolein system at low water contents.[2,14,16,17] Second, a phase transition from the cubic to the lamellar phase is induced in the fractions of the membrane where $\Pi > 2.8$ MN/m² (for pure monoolein at 25 °C)[2]. To obtain the vertical position of this transition within the membrane, we make the analysis where the structure/swelling at varying osmotic pressures is coupled to the steady-state flux of water.

Assuming that Π is constant in each unit cell in the cubic phase, the water flux (J_{H_2O}) through the same unit cell is given in terms of the gradient of water chemical potential across the cell ($d\mu_{H_2O}/dn$) and the concentration (c_{H_2O}) by the generalized Fick's law[18]

$$J_{H_2O} = -\frac{D^C_{H_2O}}{a}\frac{c_{H_2O}}{RT}\frac{d\mu_{H_2O}}{dn} \quad (1)$$

where both the diffusion coefficient ($D^C_{H_2O}$) as well as the thickness of the unit cell (the lattice constant) (a) are dependent on the Π in the given unit cell, due to the obstruction and the swelling effects. The relation between $D^C_{H_2O}$ and Π is given by the model presented by Volke et al.,[19] where the ratio between $D^C_{H_2O}$ and the bulk water diffusion coefficient ($D^0_{H_2O}$) is related to the ratio between the number of water molecules per lipid (n) in terms of the two parameters f and $N_{c,d}$ as

$$\frac{D^C_{H_2O}}{D^0_{H_2O}} = 1 + \frac{N_{c,d}}{n}\ln\left(\frac{1 + fe^{-n/N_{c,d}}}{1+f}\right) \quad (2)$$

The dependence of $D^C_{H_2O}$ on temperature at different n has been measured by NMR diffusion, resulting in a linear trend between $\log(D^C_{H_2O})$ and inverse temperature.[15] The parameters here were chosen such as to reproduce the points that these lines cross at a temperature of 25 °C.

To find the relation between the dimensions of the unit cell of the cubic phase and Π, we assume that the free energy per unit cell (ΔG_C) is given by the curvature elastic energy per unit area[14]

$$2k_c(\langle H_l^2\rangle - 2H_0\langle H_l\rangle + H_0^2) + k_g\langle K_l\rangle \quad (3)$$

multiplied by twice the area of one monolayer of one unit cell[17]

$$\sigma a^2 + 2\pi\chi l^2 \quad (4)$$

Here k_c is the rigidity bending constant, k_g is the Gaussian curvature constant, H_0 is the spontaneous curvature, H_l^2, H_l, and K_l represent, respectively, the second moment of mean curvature, mean curvature, and Gaussian curvature

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each evaluated at the distance l from the minimal surface, and $\langle ... \rangle$ denotes the average obtained by integrating over one of the two monolayers in the unit cell. Furthermore, σa^2 and χ are the surface area of the minimal surface and Euler characteristic per unit cell, respectively. This expression is evaluated as in ref 16 with $l = l_n$, the position of the so-called neutral surface area. The constants k_c, k_g, H_0, and l_n are taken from ref 14 and σ and χ from ref 17.

By using the relation[17]

$$\phi_{H_2O} = 1 - 2\sigma\frac{l_m}{a} - \frac{4\pi}{3}\chi\left(\frac{l_m}{a}\right)^3 \quad (5)$$

between the water volume fraction (ϕ_{H_2O}), a and the monolayer thickness (l_m), we obtain the number of mole water per unit cell. By differentiation, the chemical potential, and thus Π, can be calculated.

For the lamellar phase, the water flux is given by the permeability of a single lipid bilayer ($P^{lip}_{H_2O}$), divided by the number of such bilayers (N_L).[7] Assuming steady-state conditions and a constant amount of lipid uniquely determines the number of layers in the cubic phase (N_C) and the lamellar phase, respectively; in other words, we obtain the position of the phase transition in the membrane for a given osmotic gradient. This is then performed for varying osmotic gradients to enable comparison to the experimental data.

When the variation in structure within the membrane is known, we can estimate the effective permeability for any diffusing solute i ($P_{eff,i}$). This gives a calculated relation between $P_{eff,i}$ and the osmotic gradient as shown in Figure 3. Again assuming steady-state conditions, we find $1/P_{eff,i} = 1/P^C_{eff} + 1/P^L_{eff}$, where P^C_{eff} and P^L_{eff} are the effective permeabilities of the cubic and lamellar phases, respectively. The former is similarly given by

$$\frac{1}{P^C_{eff}} = \sum_{n=0}^{N_C(\Pi_d)} \frac{1}{K^C_i D^C_i / a(\Pi_n)} \quad (6)$$

where K^C_i is the partition coefficient between the cubic phase and water for i and D^C_i is the (constant) diffusion coefficient of i in the cubic phase; the effect of swelling is thus, in this approximation, solely manifested in an increase of a. As for water, the permeability of the lamellar phase P^L_{eff} is given by a (different) fixed permeability of a single lipid bilayer P^{lip}_i

$$P^L_{eff} = \frac{P^{lip}_i}{N_L(\Pi_d)} \quad (7)$$

This model gives a relation between the effective permeability and the osmotic gradient that very well reproduce the experimental data (Figures 1 and 3). In the calculations, the parameters for the diffusion of the solute were set to $P^{lip}_i = 4 \times 10^{-8}$ m/s and $K^C_i D^C_i = 1.5 \times 10^{-12}$ m^2/s, which are reasonable values for polar solutes. The permeability of the support membranes has not been included in the calculations of the effective permeability shown in Figure 3. However, taking this into consideration makes no qualitative difference, as the permeability of these is of the same order as the permeability of the cubic phase (not shown).

When the lamellar phase is induced, the effective permeability of the membrane is reduced ca. 8 and 5 times for eosin and metoprolol tartrate, respectively (Figure 1). The magnitude of this step is dependent on the fraction of the membrane that is forming the lamellar phase and on the partition coefficient of the solute. In fact, the theoretical calculations show that only a small fraction of the membrane is transformed to the lamellar state, also at the larger osmotic gradients (0.15% of the lipids, corresponds to 1−3 bilayers). The steady state flux is constant at every layer within the membrane, and the reduced permeability in the lamellar phase compared to the cubic phase is balanced by a larger gradient over fewer layers. This requires that there is a large difference in solute permeability between the two phases. It is striking that the presence of so few lamellar bilayers has such a strongly reducing effect on the effective permeability of the membrane.

In the theoretical treatment, we assume that the lipid membrane is lying on top of the supporting membrane without penetrating into the pores. During the preparation, the lipids were spread from a chloroform:methanol solution by spin coating. In a separate experiment, it was confirmed that the solvent does not completely wet the polar surface of the membrane. Also, the evaporation rate of the solvent is very fast during spin coating. Therefore, the lipids are most likely present as a dry film on the surface of the supporting membrane after the evaporation of the solvent, and this film is then hydrated to form the lipid membrane. Still, we cannot exclude that there is some penetration into the surface layer of the supporting membrane, which would imply a less well-defined interfacial structure. However, the thickness of such layer is considered small compared to the total thickness of the lipid membrane and would therefore only affect the quantitative results marginally. Furthermore, the irregularities induced by the supporting membrane would only occur on the side of the membrane that faces the receptor cell, i.e., the side of the membrane that always form a cubic phase ($\Pi_r = 1.22$ MN/m^2). The phase transition from the cubic to the lamellar phase occurs in a thin layer of the upper part of the membrane that faces the donor cell, which should not be affected by the spreading on the supporting membranes. Small irregularities in the lipid cubic phase structure are not expected to affect the effective permeability in a major way. The theoretical model should therefore provide a satisfactory description of the present situation. By changing the properties of the supporting membrane, we might induce penetration of lipids into the porous system. This could give rise to capillary induced phenomena, which would affect the phase transitions[22] and which is an interesting possibility that deserves further study.

Conclusions

In conclusion, we have shown how an external gradient that modifies the lipid structure in the membrane can be used as a regulating mechanism to control the barrier properties. Beside the basic scientific interest in these mechanisms, several applications in biology and technology can be seen, as exemplified by the barrier properties of stratum corneum and by controlled release systems for drug delivery.

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