Published in final edited form as:

Adv Exp Med Biol. 2018 ; 1072: 405–411. doi:10.1007/978-3-319-91287-5_65.

Reduced Oxygen Permeability Upon Protein Incorporation Within Phospholipid Bilayers

Rachel J. Dotson and Sally C. Pias

Department of Chemistry, New Mexico Institute of Mining and Technology (New Mexico Tech), Socorro, NM, USA

Abstract

Intracellular oxygenation is key to energy metabolism as well as tumor radiation therapy. Although integral proteins are ubiquitous in membranes, few studies have considered their effects on molecular oxygen permeability. Published experimental work with rhodopsin and bacteriorhodopsin has led to the hypothesis that integral proteins lessen membrane oxygen permeability, as well as the permeability of the lipid region. The current work uses atomistic molecular dynamics simulations to test the influence of an ungated potassium channel protein on the oxygen permeability of palmitoylcholine (POPC) bilayers with and without cholesterol. Consistent with experiment, whole-membrane oxygen permeability is cut in half for membranes comprised of 30 wt% potassium channel protein in POPC, and the apparent permeability of the lipid portion decreases by 40%. Unexpectedly, oxygen is found to interact directly with the protein surface, accompanied by a 40% reduction of the apparent whole-membrane diffusion coefficient. Similar effects are seen in 1:1 POPC/cholesterol, but the magnitude of permeability reduction is smaller by ~30%. Overall, the simulations indicate that integral proteins can reduce oxygen permeability by altering the diffusional path and the local diffusivity. This effect may be especially important in the proteindense membranes of mitochondria.

1 Introduction

The permeability of cellular membranes to molecular oxygen (O_2) is a critical parameter for understanding metabolic flux in healthy and diseased cells. Insight into cellular oxygenation at the membrane level may improve clinical prediction and monitoring of tumor oxygenation, a key parameter affecting radiotherapy outcomes [1]. Nonetheless, membranes are highly complex, organized, and dynamic structures, composed of diverse mixtures of lipid and protein. Simplified experimental and simulation models have provided valuable estimates of lipid bilayer O_2 permeability and related properties, as influenced by a few types of phospholipid and cholesterol [2–6]. Limited work has addressed the effect of integral membrane proteins, although it is expected to be significant [5,7,8], especially where the protein density is 65–75 wt%, as in mitochondrial inner membranes and eye lens fiber cell plasma membranes [5].

The permeability coefficient (or ‘permeability’) functions as a rate constant for diffusional processes and helps to describe the diffusional flux, J , for a solute in response to a concentration gradient. In the context of a membrane, the permeability, P_M , relates to the flux according to Fick’s law: $J = -P_M \nabla C_w$ where ∇C_w is the concentration gradient, as measured in the bulk water on either side of the membrane. The well-established solubility-diffusion model, or Meyer-Overton rule, predicts a molecule’s membrane permeability to be directly proportional to its solubility in the membrane. The rule can be expressed mathematically as

$$P_M \equiv \frac{K_P D_M}{h}, \quad (1)$$

where K_P is the equilibrium concentration of O_2 in the membrane, relative to bulk water; D_M is the diffusion coefficient for the membrane; and h is the membrane thickness [9].

Assuming that the rate of oxygen diffusion across a membrane is first-order (dependent on the concentration of O_2 but not other species), the permeability coefficient can be calculated readily from unrestrained molecular dynamics simulations [10,11]. To do so, the trajectories of individual O_2 molecules are tracked, enabling assessment of the rate of O_2 molecule escape from the bilayer at equilibrium, Φ_{esc} . This value is divided by the bulk-water O_2 concentration, C_w , and the interfacial area of the membrane, A_I which is twice the area of a single leaflet. The resulting equation is

$$P_M = \frac{\Phi_{esc}}{A_I C_w}. \quad (2)$$

Prior experimental studies examined oxygen transport in the lipid portion of bilayers incorporating either rhodopsin or bacteriorhodopsin. Based on electron paramagnetic-resonance (EPR) spin-label oximetry measurements, the researchers concluded that integral membrane proteins are essentially impermeable to oxygen [5,7,12]. As such, the permeability assessed for the lipid portion of the membrane should be reduced by a factor proportional to the surface area of the bilayer region (omitting the area occupied by protein), divided by the entire surface area of the membrane. To a first approximation, then, the permeability averaged over the whole membrane can be expressed as a function of P_L , the permeability of the lipid region:

$$P_M = a_L P_L, \quad (3)$$

where a_L is the fractional surface area occupied by lipid. The EPR studies additionally found that proteins lower the permeability of the lipid portion of the membrane, perhaps due to reduced rotational motion of the lipid hydrocarbons in protein-lipid interfacial ‘boundary regions’ [8].

The current work uses atomistic molecular dynamics simulations to investigate the effect of another integral membrane protein on oxygen permeability. Based on the previous experimental findings, we hypothesize that an ungated potassium channel protein will lower whole-membrane oxygen permeability by diverting the oxygen diffusional path to circumvent the protein and also by lowering the permeability of the surrounding bilayer.

2 Methods

Model hydrated membranes were constructed using the CHARMM-GUI Membrane Builder [13]. A crystallographic structure of an ungated mouse potassium channel (PDB identifier 1R3J) was embedded within bilayers composed of palmitoylcholine (POPC) phospholipid or POPC with cholesterol (Chol) in a 1:1 molar ratio (50 mol%). POPC and cholesterol are ubiquitous in eukaryotic plasma membranes and have been studied extensively [5,14,15]. Due to asymmetry of the potassium channel across the bilayer leaflets, more lipid molecules were placed in one leaflet than in the other. Control systems without protein were also constructed, using 128 lipids distributed evenly across the leaflets.

The fractional surface area occupied by lipid, a_L , was computed according to the relation $a_L = (A_I - A_P)/A_I$, where A_P is the average total interfacial area of the protein. Because the protein faces are roughly square-shaped, the interfacial area of the protein was approximated by defining ‘corners’ for each face, at a depth where the protein first contacts the POPC headgroups (water-bilayer interface). The outermost residues Y24, Y127, and Y230 (hydroxyl O atoms) were used as corners for the larger face and R6, R109, and R212 (*zeta* C atoms) as corners for the smaller face. Distances between pairs of corner residues were tracked over time and used to calculate an average area over time for each face. The two areas were then summed to estimate the total interfacial area for the protein. (See Figure 1b, below, for visualization of the residue positions and edges of the square for one face.) The transmembrane region of the potassium channel is cone-like, with a linear transition between the two faces. Thus, we have assumed that the sum of the areas of the two faces should provide a meaningful approximation of the fraction of the membrane occluded by the protein, even though the protein is asymmetric.

The model membranes were hydrated with a minimum of 35 waters per lipid or a 10-Å water layer flanking the extramembrane surfaces of the protein. An additional system was constructed with POPC, the potassium channel (K^+ chan), and KCl salt to neutralize the protein’s charge and generate a neutral 150 mM KCl solution in the aqueous layer. O_2 molecules were added to reach a concentration of approximately 150 mM, based on the equilibrated whole-system volume (including the protein, the membrane, and the water). Such a high O_2 concentration enhances sampling of the thermodynamic ensemble and has been previously verified not to influence the free energy landscape for oxygen diffusion [11]. Although placed initially in the water layer, the O_2 molecules diffused rapidly throughout the system. Table 1 provides compositional details of all the simulation systems.

Isothermal-isobaric molecular dynamics simulations were performed with conditions identical to those described in our recent publication [11], with addition of the ff14SB force field for protein modeling [16]. The systems were pre-equilibrated at 37°C for 200 ns,

followed by introduction of oxygen and subsequent 300-ns production simulations at 37°C. Analyses other than those discussed above were carried out as reported previously [11]. Uncertainty was estimated for P_M and P_L by applying common rules of error propagation to the standard deviations for the terms of Eqs. 2 and 3.

3 Results and Discussion

Figure 1 shows a simulation snapshot of the potassium channel protein, embedded in a hydrated POPC bilayer. The approximate bilayer thickness used in this work is indicated as $\sim h$ in Fig. 1a. Wherever relevant, the boundaries of the membrane were defined as $\pm h/2$, referenced from the center of the bilayer. Table 2 reports values calculated from the simulations, featuring whole-membrane O_2 permeability (P_M) and associated physical properties.

The first-approximation model in Eq. 3 predicts that the average permeability of the whole membrane will diminish relative to the lipid permeability (P_L) by a factor related to the interfacial surface area occluded by protein. The model assumes the permeability of the lipid region to be independent of the presence of protein, while treating integral membrane proteins as virtually impermeable to oxygen [5]. Applying the Eq. 3 model and also adjusting for the changes in bilayer thickness observed with protein incorporation gives predicted P_M values of 37 cm/s for the K^+ chan/POPC membrane and 33 cm/s for K^+ chan/POPC/Chol.¹ The observed simulation P_M values based on O_2 trajectories (Eq. 2) are 30-35% lower, at 24 and 23 cm/s (Table 2). This finding is consistent with a previously observed lipid permeability reduction by a factor of ~ 1.5 (33%) in bilayers incorporating monomeric bacteriorhodopsin [8].

To discern whether increased lipid tail order may be responsible for the apparent loss of lipid permeability in the presence of protein, we examined lipid order parameter, $\langle S \rangle$. The difference in order due to cholesterol is much greater than the differences due to protein incorporation (Table 2). Moreover, adding the potassium channel to POPC leads to a modest increase in $\langle S \rangle$, from 0.19 to 0.23, while adding it to POPC/Chol leads to a modest decrease, from 0.38 to 0.34. Even though the order parameter values are very different for the potassium channel with POPC and with POPC/Chol (0.23 and 0.34), the whole-membrane permeabilities for these systems are quite similar (24 and 23 cm/s). Thus, changes in tail order do not seem to explain why the apparent permeability of the lipid, P_L , is lower in the protein-incorporating systems.

The Meyer-Overton rule (Eq. 1) predicts the permeability to be directly proportional to the partition coefficient, K_P . Thus, examining the response of K_P to protein incorporation may be informative. If O_2 were soluble only in the lipid and water components of the system, we would expect K_P to decrease by a factor proportional to the volume of the membrane-spanning part of the protein. The fractional membrane volume occupied by lipid is

¹ P_L for POPC or POPC/Chol was adjusted to reflect the thickness of the respective protein-incorporating bilayer, using the relation in Eq. 1 under the condition $P_M = P_L$ for lipid alone. Each thickness-adjusted P_L value was then used with Eq. 3, along with a_L from Table 2, to predict P_M for the protein-incorporating membrane. For example, for K^+ chan/POPC:

$P_{L,KchanPOPC} = (K_{P,POPC} \cdot D_{M,POPC}) / h_{KchanPOPC} = (7.8) (3.8 \times 10^{-6} \text{ cm}^2/\text{s}) / 6.2 \text{ nm} = 47.8 \text{ cm/s}$,
 $P_{M,KchanPOPC} = a_{L,KchanPOPC} \cdot P_{L,KchanPOPC} = (0.77) (47.8 \text{ cm/s}) = 37 \text{ cm/s}$.

approximately equal to the fractional area, a_L . Using the a_L values in Table 2 with the K_P values for the lipid-only systems gives predicted K_P estimates of 6.0 for K⁺chan/POPC and 4.1 for K⁺chan/POPC/Chol. The values calculated from the protein-incorporating simulations are higher than these estimates, suggesting that the protein volume also contributes to the solubility of oxygen in the membrane.

Inclusion of K⁺ and Cl⁻ ions, along with the potassium channel, further raises the whole-membrane partition coefficient, and a corresponding increase in P_M is observed. While intensified membrane/water partitioning might reflect lower solubility of O₂ in salt water compared with pure water [19], simulations of POPC bilayers at 300 K indicate that K_P and P_M do not respond to aqueous KCl at the concentration used here (150 mM; data not shown). Increased O₂ solubility in the lipid region seems unlikely, as KCl has no appreciable effect on the lipid interfacial area (data not shown), the bilayer thickness, h , or the lipid order parameter, $\langle S \rangle$ (Table 2). Thus, the reason for increased membrane/water O₂ partitioning in the presence of KCl is not yet apparent. Moreover, we note that the P_M value for the system with salt and protein is far lower than the permeability of the pure POPC bilayer, although the partition coefficients are not different.

Figure 1c provides additional evidence that the ‘solubility’ of O₂ in the protein-occupied volume is substantial. Namely, in the simulation snapshot featured, nearly half of the O₂ molecules in the membrane are found to be in contact with the protein’s surface (red spheres). A few others are found in the core of the protein (lower three blue spheres) or in the water-filled ‘vestibule’ characteristic of potassium channel (upper two blue spheres). Oxygen molecules are similarly observed to be in contact with the potassium channel in a snapshot from the simulation with ions (image not shown).

Additional investigation will be needed to clarify the nature of the O₂-protein interactions and their lifetime. As a first step, we calculated an apparent average diffusivity of O₂ over the whole membrane, D_M , using Eq. 1 with the K_P , P_M , and h values in Table 2. The apparent diffusivity is reduced with protein incorporation, converging on a value of $\sim 2.3 \times 10^{-6}$ cm²/s in all the systems incorporating the potassium channel. This finding is unexpected, as solute diffusion coefficients have been found to be largely unresponsive to cholesterol content [11,19] and are, thus, unlikely to vary with the relatively small changes in lipid order observed here in response to protein incorporation.

The reduced apparent diffusivity suggests that the residence time of O₂ in the membrane interior is increased by interaction with the protein. We anticipate the presence of transient volume pockets along the protein’s surface and among the *alpha*-helices that accommodate transient, weak-adhesive nonpolar interactions (dispersion forces) along the membrane-spanning protein surface. Other researchers predicted that reduced lipid tail motion (*trans-gauche* isomerizations) at the protein-lipid interface may be responsible for reduced lipid permeability in protein-incorporating experimental models [5,8]. Rather, we surmise that the rotations of the protein sidechains may be slow, relative to lipid tail rotations, leading to reduced diffusivity along the protein’s surface.

A limitation of the current work is the use of an oxygen model with a known tendency to overpartition into lipid, as discussed in our recent work [11]. Further, the occupancy of oxygen in the protein interior (channel and vestibule) has not been analyzed in detail. Likewise, the O₂ populations ‘inside’ the protein were not separated from others positioned within the spatial boundaries of the membrane interior, defined by a threshold distance along the bilayer normal. We do not expect this approximation to impact the permeability or other properties significantly, but it may promote overestimation of K_P for the protein-incorporating membranes. We expect that differentiating K_P for the lipid region, the lipid-protein boundary region, and the protein region will be informative. Finally, we note that the assumption of first-order kinetics for the simulation P_M calculations (Eq. 2) may turn out not to be valid where the potassium channel is present, due to O₂-protein interactions. Even so, this study’s semiquantitative comparison of permeability and related properties provides valuable predictions and a foundation for further work.

4 Conclusions

The simulations indicate that 30 wt% ungated potassium channel protein incorporation with POPC phospholipids reduces whole-membrane oxygen permeability by a factor of two. The permeability of the lipid region is, likewise, diminished by about 40%. Direct interaction of O₂ molecules with the protein surface appears to reduce the average diffusivity of oxygen within the membrane by 40%. POPC/cholesterol bilayers are similarly affected, but the relative magnitude of the permeability decrease is roughly 30% smaller than in POPC bilayers, while the diffusivity change is greater.

We anticipate that differences in tertiary structure among integral membrane proteins may modulate the extent of their influence on oxygen permeability. In particular, it will be interesting to compare membrane-spanning segments dominated by *alpha*-helix bundles with those dominated by beta-barrels. Taken together with previous work (reviewed in [5]), the findings of the current study imply that the influence of integral proteins on oxygen permeability will be especially interesting in the context of protein-crowded membranes, such as those found in mitochondria and eye lens fiber cells.

Acknowledgments

The authors thank Harold Swartz for encouraging our inclusion of membrane proteins in the simulation models, as well as Richard Pastor and James Kindt for valuable comments on the manuscript. The work was funded by the National Institutes of Health (NIH) under National Institute of General Medical Sciences (NIGMS) grant P20GM103451 and by a gift from the Glendon Foundation.

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Fig. 1. Simulation snapshot of the K⁺chan/POPC system, imaged at the end of the 300 ns production trajectory. (a-b) Side view and top view. Approximate thickness of the membrane, h , is indicated. Protein shown as black ribbons. POPC as white licorice/gray lines, water as small cyan spheres, and O₂ as larger red spheres. H atoms omitted for clarity. Blue circles and lines in *panel b* indicate approximate positions of “corner” residues and pairwise distances used to estimate interfacial area for this face of the protein. (c) Side view of protein in surface representation, highlighting the O₂ molecules that contact the protein surface (red spheres). Blue spheres are O₂ molecules in the core of the protein or its water-filled “vestibule.” Imaged with PyMOL [20].

Table 1.

Simulation system compositions (number of molecules; lipid distribution by leaflet)

System	POPC	Cholesterol	Salt	Water	O ₂	wt% protein
POPC	64/64	—	—	4454	26	0
POPC/Chol	32/32	32/32	—	4457	23	0
K ⁺ chan/POPC	64/79	—	—	7820	42	29
K ⁺ chan/POPC/Chol	32/42	32/42	—	6901	37	35
K ⁺ chan/KCl/POPC	64/79	—	22 K ⁺ , 30 Cl ⁻	7815	43	29

Table 2.Oxygen permeability (mean $\pm$ SD), partitioning, and related values.

System	P_M (cm/s) ^a	a_L ^b	P_L (cm/s) ^c	$h \mid l$ (nm) ^d	$\langle S \rangle$ ^e	K_P ^f	$D_M (\times 10^{-6} \text{ cm}^2/\text{s})$ ^g
POPC	53 $\pm$ 2	1	53 $\pm$ 2	5.6 7.1	0.19	7.8	3.8
POPC/Chol	42 $\pm$ 2	1	42 $\pm$ 2	6.2 9.4	0.38	5.6	4.7
K ⁺ chan/POPC	24 $\pm$ 1	0.77	31 $\pm$ 1	6.2 8.4	0.23	6.6	2.3
K ⁺ chan/POPC/Chol	23 $\pm$ 1	0.73	32 $\pm$ 2	5.8 9.0	0.34	5.5	2.4
K ⁺ chan/KCl/POPC	29 $\pm$ 2	0.77	37 $\pm$ 2	6.2 8.4	0.22	7.7	2.3

^aWhole-membrane permeability, estimated according to Eq. 2, as in our prior work [11].^bFractional area occupied by lipid.^cActual or apparent permeability of the lipid bilayer region, estimated from Eq. 3.^dMembrane thickness, h , based on O₂ free energy profiles, determined as in [11] (data not shown). Average total box length, l , normal to the bilayer (standard deviation = 0.1 nm).^eAverage lipid order parameter for carbons 4-8 of all POPC palmitoyl tails, as described in [17].^fMembrane/water O₂ partition coefficient, from depth-dependent O₂ distribution profiles, averaged over the whole membrane.^gApparent whole-membrane diffusion coefficient, estimated from the relation in Eq. 1.