Permeability of 5-aminolevulinic acid oxime derivatives in lipid membranes

Emma S. E. Eriksson, Edvin Erdtman and Leif A. Eriksson

The self-archived postprint version of this journal article is available at Linköping University Institutional Repository (DiVA):

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-129615>

N.B.: When citing this work, cite the original publication.

The original publication is available at www.springerlink.com:

Eriksson, E. S. E., Erdtman, E., Eriksson, L. A., (2016), Permeability of 5-aminolevulinic acid oxime derivatives in lipid membranes, *Theoretical Chemistry accounts*, 135(1), 1-9. <https://doi.org/10.1007/s00214-015-1798-0>

Original publication available at:

<https://doi.org/10.1007/s00214-015-1798-0>

Copyright: Springer Verlag (Germany)

<http://www.springerlink.com/?MUD=MP>

Permeability of 5-aminolevulinic acid oxime derivatives in lipid membranes

Emma S. E. Eriksson¹ · Edvin Erdtman² · Leif A. Eriksson¹

Received: 28 August 2015 / Accepted: 17 December 2015 / Published online: 7 January 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract The endogenous molecule 5-aminolevulinic acid (5ALA) and its methyl ester (Me-5ALA) have been used as prodrugs in photodynamic treatment of actinic keratosis and superficial non-melanoma skin cancers for over a decade. Recently, a novel set of 5ALA derivatives based on introducing a hydrolyzable oxime functionality was proposed and shown to generate considerably stronger onset of the photoactive molecule protoporphyrin IX (PpIX) in the cells. In the current work, we employ molecular dynamics simulation techniques to explore whether the higher intercellular concentration of PpIX caused by the oxime derivatives is related to enhanced membrane permeability, or whether other factors contribute to this. It is concluded that the oximes show overall similar accumulation at the membrane headgroup regions as the conventional derivatives and that the transmembrane permeabilities are in general close to that of 5ALA. The highest permeability of all compounds explored is found for Me-5ALA, which correlates with a considerably lower free energy barrier at the hydrophobic bilayer center. The high PpIX concentration must hence be sought in other factors, where slow hydrolysis

of the oxime functionality is a plausible reason, enabling stronger buildup of PpIX over time.

Keywords 5-Aminolevulinic acid · Oxime derivatives · Photodynamic therapy · Lipid membrane · Permeation · Molecular dynamics simulations

1 Introduction

5-Aminolevulinic acid (5ALA; Fig. 1a) and its methyl ester (Me-5ALA; Fig. 1b) can be used as prodrugs in photodynamic therapy (PDT), a method used to treat cancer in which a photoactive agent upon light excitation reacts with molecular oxygen and forms reactive oxygen species. 5ALA is a precursor to several important natural compounds with heme being the most essential. Heme is endogenously synthesized from eight 5ALA molecules via a number of enzymatic reactions in which the last step involving the incorporation of iron into protoporphyrin IX (PpIX) is rate-determining. The endogenous formation of 5ALA is regulated by feedback inhibition by heme concentration, in order to avoid excess PpIX accumulation. The inhibition can, however, be bypassed by addition of exogenous 5ALA, thus resulting in accumulation of PpIX. The accumulation of PpIX upon addition of exogenous 5ALA has furthermore been shown to be particularly high in cancer cells compared to healthy cells, for reasons as yet unknown [1]. PpIX can be activated by exposure to red light whereby it generates reactive oxygen species, primarily singlet oxygen, via excitation energy transfer, which in turn have a deleterious effect on the cancer cells. As the biosynthesis of PpIX takes place in the cytosol and mitochondria, the 5ALA molecules or derivatives must initially be able to penetrate the cellular plasma membrane.

Published as part of the special collection of articles “Health and Energy from the Sun.”

Electronic supplementary material The online version of this article (doi:10.1007/s00214-015-1798-0) contains supplementary material, which is available to authorized users.

✉ Leif A. Eriksson
leif.eriksson@chem.gu.se

¹ Department of Chemistry and Molecular Biology, University of Gothenburg, 405 30 Göteborg, Sweden

² Department of Physics, Chemistry and Biology (IFM), Linköping University, 581 83 Linköping, Sweden

Fig. 1 **a** 5ALA, **b** Me-5ALA, and the 5ALA oxime derivatives studied, **c** “pure” 5ALA oxime (HH), **d** 5ALA oxime esterified at the carboxylic end (MH), **e** 5ALA oxime methylated at the oxime oxygen (HM), **f** 5ALA oxime esterified at the carboxylic end and methylated at the oxime oxygen (MM)

The 5ALA molecule is under normal conditions present as a zwitterion, whereby the polarity of the molecule hinders cell membrane penetration and consequently reduces the efficiency of the PDT treatment. Second-generation 5ALA derivatives with increased lipophilicity have been developed in order to overcome this problem. The methyl ester of 5ALA (Me-5ALA; Fig. 1b) has been approved for actinic keratosis [2–4] and basal cell carcinoma [5–8] (Metvix®). The hexyl ester of 5ALA has been approved for fluorescence-based diagnosis of bladder cancer (Hexvix®) [9–11]. Membrane permeability of 5ALA initially increases with increased length of the alkyl ester, and maximum accumulation of protoporphyrin IX has in studies observed for the pentyl ester [12]. The alkyl ester substituent will eventually become hydrolyzed, thereby regenerating the biochemically active 5ALA.

The permeability of neutral and zwitterionic 5ALA along with its methyl- (Me-5ALA), ethyl-, and hexyl esters has recently been studied in a dipalmitoylphosphatidylcholine (DPPC) lipid membrane model using molecular dynamics (MD) simulations [13, 14]. All 5ALA derivatives were found to accumulate inside the polar head group region of the bilayer. The permeability of zwitterionic 5ALA across the hydrophobic membrane interior is significantly slower

compared to neutral 5ALA and also slower compared to the methyl and ethyl esters of 5ALA. However, if the 5ALA zwitterion becomes neutralized when residing in the head-group region of the membrane, penetration is enhanced due to the reduced free energy barrier across the center of the bilayer for neutral 5ALA. Me-5ALA showed faster diffusion through the membrane than both neutral and zwitterionic 5ALA. Slightly lipophilic second-generation 5ALA derivatives are thus likely to enhance uptake into tissues, thereby making the PDT treatment more efficient.

In an attempt to improve upon the membrane penetration and amplification of PpIX production, a new kind of 5ALA derivatives, based on introducing an oxime functionality at the central carbonyl, has been proposed (cf. Fig. 1) [15]. The advantage with using an oxime is that this functional group in general displays a very slow rate of hydrolysis [16], which thus enables additional cellular accumulation before onset of PpIX production. The aim of the present study is to investigate whether the oxime functionality also provides enhanced permeation properties in lipid membranes, as compared to the different 5ALA derivatives discussed above. Four 5ALA oxime derivatives were included in the current study (Fig. 1): the “pure” 5ALA oxime (HH; Fig. 1c), 5ALA oxime esterified at the carboxylic end (MH; Fig. 1d), 5ALA oxime

methyated at the oxime oxygen (MH; Fig. 1e), and 5ALA oxime both esterified at the carboxylic end and methyated at the oxime oxygen (MM; Fig. 1f). Throughout, the neutral forms of the molecules were studied, as the charged (protonated/deprotonated/zwitterionic) forms will have considerably higher barriers to transversion.

2 Methodology

The same methodology as previously applied in studies of potential drug compounds in membranes was used [13, 14, 17–19]. The GROMACS program (version 4.0) [20] was used throughout the study, together with the united atom GROMACS force field. As membrane model, a dipalmitoylphosphatidylcholine (DPPC) bilayer consisting of 64 lipids and 3846 water molecules was employed, previously equilibrated and verified [21].

The 5ALA oximes (HH, MH, HM, MM) were first geometry-optimized at the B3LYP/6-31G(d,p) level of theory in implicit water solution using the Gaussian 09 program [22]. Using the coordinates obtained in the quantum optimizations, the molecular topologies were determined with the PRODRG software [23] through its Web server [<http://davapc1.bioch.dundee.ac.uk/prodrg/>], which generates topologies based on the GROMOS87 force field. Mulliken charges obtained from the optimization were assigned to the 5ALA derivatives, using small charge groups with total charges close to zero. For the DPPC phospholipids, a standard united atom force field was applied, and for water, we used the SPC model [24, 25]. Parameters used for the different 5ALA derivatives are provided in Supporting Information (S1–S4).

The membrane was initially equilibrated for 1 ns, followed by eight independent simulations with 5ALA oximes in the membrane, two for each derivative. In four of these simulations, a molecule of the 5ALA oxime derivative was inserted in the outer region of the water phase, and the systems were equilibrated for 10 ns, followed by a 40 ns production run. Four simulations were also performed by initially placing the corresponding 5ALA derivative in the center of the lipid bilayer, following the same simulations protocol. System trajectories were collected every 0.5 ps. All simulations were performed using a time step of 2 fs.

The isothermal–isobaric ensemble (NPT) at $T = 323$ K and $p = 1$ bar was used in all simulations. The temperature and pressure were held constant using a Nosé–Hoover thermostat [26, 27] with a coupling constant of 0.1 ps and a semi-isotropic Parrinello–Rahman barostat [28, 29] with a coupling constant of 1 ps. A particle mesh Ewald scheme [30, 31] was used to calculate the electrostatic interactions with a real space cutoff of 10 Å. The same cutoff was used for van der Waals interactions (Lennard-Jones terms). Bond lengths were constrained using the LINCS algorithm [32].

A potential of mean force formalism was used to calculate free energy profiles for the 5ALA derivatives across the lipid bilayer (the direction of the z -axis). The z -component of the force, F_z , acting on the molecule at certain constrained distances between the molecule and the bilayer center of mass was collected at different positions along the z -axis. The free energy for the transfer process between z_i and z_f is written as

$$\Delta G = G_{z_f} - G_{z_i} = - \int_{z_i}^{z_f} \langle F_z \rangle_z dz \quad (1)$$

where the bracket means an average over the forces collected at each constrained distance. To calculate the free energy profile for the translocation of each molecule, 41 constrained simulations were performed for each derivative at positions differing by 0.1 nm along the z -axis direction. At each point in water (2.6–4.0 nm from the bilayer center), equilibration was performed for 3 ns, followed by a production run of 4 ns. Inside the lipid bilayer (0.0–2.5 nm from the bilayer center), increased sampling was required due to the slower motion of the molecules, and therefore, each point was equilibrated for 5 ns followed by a production run of 10 ns. The starting points for the simulations were sampled from the previous non-constrained equilibration simulations in which the 5ALA derivatives were initially inserted either in the outer region of the water phase or in the center of the lipid bilayer. The force acting on the molecules center of mass was collected at every time step during the production run. A SHAKE algorithm [33] was used to constrain the distance between the center of mass of the bilayer and the 5ALA derivative (the molecules were constrained in the z -direction, but allowed to rotate).

The permeability is defined as the current density divided by the concentration gradient across the membrane. The procedure developed by Marrink and Berendsen [34] was adopted to calculate the permeability coefficients, based on the fluctuation dissipation theorem and using the deviation of the instantaneous force, $F(z, t)$, from the average force acting on the molecule obtained during the constrained dynamics:

$$\Delta F(z, t) = F(z, t) - \bar{F}(z, t) \quad (2)$$

The local time-dependent friction coefficient, ξ , can be calculated from the following autocorrelation function:

$$\xi(z, t) = \frac{\Delta F(z, t) \Delta F(z, 0)}{RT} \quad (3)$$

where T is the absolute temperature and R is the gas constant. By integrating the friction coefficient, one can obtain the diffusion coefficient, D ,

$$D(z) = \frac{RT}{\xi(z)} = \frac{(RT)^2}{\int_0^\infty \langle \Delta F(z, t) \Delta F(z, 0) \rangle dt} \quad (4)$$

Fig. 2 Snapshots from the simulations of (left) 5ALA [13, 14], and (right) HH in the DPPC membranes

This function was fitted to a double exponential using a nonlinear fitting procedure [34] in order to integrate the autocorrelation of the force fluctuations,

$$C(t) = A_0 \exp\left(-\frac{t}{\tau_0}\right) + A_1 \exp\left(-\frac{t}{\tau_1}\right) \quad (5)$$

This translates to that the molecules move inside the lipid bilayer in two distinct time scales, corresponding to the two decay times τ_0 and τ_1 , one fast (z -direction) and one slow (in xy -plane). The permeability coefficient P , finally, is calculated by integrating over the local resistances, $R(z)$, across the membrane. $R(z)$ is obtained by dividing the exponential of the previously calculated free energies, $\Delta G(z)$, by the diffusion coefficients, $D(z)$,

$$\frac{1}{P} = R' = \int_{z_i}^{z_f} R(z) dz = \int_{z_i}^{z_f} \frac{\exp(\Delta G(z)/kT)}{D(z)} dz \quad (6)$$

3 Results and discussion

The 5ALA oxime derivatives initially placed in the water phase were all found to diffuse into the lipid bilayer during the equilibration simulations. During the production runs, the solute molecules stay in the lipids and do not move into the water phase or cross the bilayer center. The molecules are located close to the polar headgroups of the lipids, as illustrated in the simulation snapshots displayed in Fig. 2.

The data for the 5ALA oxime derivatives are compared to data for neutral 5ALA and Me-5ALA reported

previously [13, 14]. In this context, it should be mentioned that the production runs for 5ALA and Me-5ALA were 20 ns, as compared to 40 ns for the 5ALA oxime derivatives. This is, however, not expected to affect the results. In the constrained simulations, 1 ns production runs were used for 5ALA and Me-5ALA, whereas for the 5ALA oxime derivatives productions runs of 4 and 10 ns in water and lipid phases, respectively, were used.

3.1 Density

In Fig. 3, we display the partial densities of the solute molecules along with the densities of the full system, DPPC, and water. Small variations in the preferred location of the 5ALA derivatives are observed, albeit they all locate with a maximum density peak in the range of 1–1.5 nm from the bilayer center. The molecules hence reside close to the very dense headgroup region, but with the peak densities shifted slightly toward the center of the bilayer, highly similar to that observed for psoralen derivatives [16]. As a comparison, hypericin derivatives, photoactive agents that also have been studied in our group, were found to locate within the headgroup region but closer to the interface between the lipids and water, mainly due to strong interactions with water molecules [17, 18].

Of the four oxime derivatives, the HM molecule displays its maximum density at the same distance from the bilayer center as neutral 5ALA and Me-5ALA, although with a more narrow density profile. These three molecules

Fig. 3 Total mass density (*solid line*) and mass densities for DPPC (*dotted line*), water (*dashed line*), and for 5ALA derivatives (*gray line*) **a** 5ALA [13, 14]; **b** Me-5ALA [13, 14]; **c** HH; **d** MH; **e** HM; **f** MM. Densities for the 5ALA derivatives are multiplied by 30

have their maximum densities closer to the bilayer center than the other derivatives included in the study. Me-5ALA has a wider density profile than 5ALA and the 5ALA oxime derivatives, which indicates that Me-5ALA moves both closer to the interface between the lipids and water, and closer to the center of the bilayer during the simulation. HH and MH display similar density profiles, and their maximum peaks are located slightly further away from the bilayer center compared to the profiles for 5ALA, Me-5ALA, and HM. MH, however, has a slightly wider density profile than HH, with a more slowly decaying slope toward the center of the bilayer. MM is according to the density profiles located closest to the interface between the lipids and water during the simulation.

3.2 Radial distribution functions

Radial distribution functions (RDF) were computed from the un-constrained production runs. RDFs between oxygen atoms on the 5ALA derivatives and hydrogen atoms in the surrounding water are displayed in Fig. 4a, and between

Fig. 4 Radial distribution functions between **a** oxygen atoms on 5ALA, [13, 14] Me-5ALA [13, 14], and the 5ALA oxime derivatives, and hydrogen atoms in the surrounding water; **b** polar hydrogen atoms on 5ALA [13, 14] and its derivatives and oxygen atoms in the surrounding water

polar hydrogen atoms on the 5ALA derivatives and oxygen atoms in the surrounding water in Fig. 4b. The first peak in the figures (at ~ 0.18 nm) corresponds to a normal hydrogen bond. The second peak in Fig. 4a corresponds to a second hydrogen atom in the same water molecule or a second solvation shell, and in Fig. 4b, the second peak corresponds to an oxygen in the second solvation shell. The increased amplitude in the RDFs after the second peak is due to the increased number of water molecules that are included in shells of higher order. All 5ALA derivatives show a hydrogen bond between their oxygen atoms and hydrogen atoms in water molecules that penetrate into the lipids (Fig. 4a). Me-5ALA and 5ALA clearly display the highest peaks, which is most likely due to the fact that these molecules move closer to the interface between the lipids and water in part of the simulations, as indicated by the wider density profiles. The four 5ALA oxime derivatives display highly similar amplitudes of the peak corresponding to the first hydrogen bond.

For the RDFs between polar hydrogens on the 5ALA derivatives and oxygen atoms in water, HM clearly displays the highest probability for a hydrogen bond, followed by MH. HM and MH comprise three polar hydrogen atoms

each that can form hydrogen bonds with oxygen atoms in water. Surprisingly, HH that has four polar hydrogen atoms displays the lowest radial distribution function for a hydrogen bond. Most likely, this is related to rotational movement of the molecule, such that each hydrogen only forms a hydrogen bond in a small fraction of the time. 5ALA, Me-5ALA, HH, and MM have the first solvation shell at slightly longer distance than HM and MH, at ~ 0.21 nm. Overall, however, the differences between the six 5ALA compounds are in this respect very small.

3.3 Mean square displacement

The mean square displacement (MSD) [35] reveals details about the movements of the molecules inside the bilayer. The MSD is defined by

$$\text{MSD}(t) = |\vec{r}(t) - \vec{r}(0)|^2 \quad (7)$$

where $\vec{r}(0)$ and $\vec{r}(t)$ are the positions of the particle at time $t = 0$ and at a certain time t . The brackets indicate a time average over all similar particles and over different time origins along the simulation. The Einstein relation allows for the calculation of the diffusion coefficient, D , at sufficiently long simulation times: [35]

$$D = \lim_{t \rightarrow \infty} \frac{1}{2dt} |\vec{r}_i(t) - \vec{r}_i(0)|^2 \quad (8)$$

where d is the dimensionality of the space. This way, one can obtain the MSD for the molecules moving in the bilayer plane $d = 2$ and moving along the bilayer normal $d = 1$, respectively. The MSD provides a measure of the average distance a molecule travels in the system, and the growth rate of the MSD depends on how often the molecules collide, i.e., a measure of the ease of diffusion of the molecule.

Like other molecules diffusing in confined media, the 5ALA derivatives never reach the Einsteinian limit of proper diffusion within the limited time of the simulation, and anomalous diffusion occurs where the MSD is proportional to t^n , with $0 < n < 1$ [36]. The implication is that a direct comparison with experimental diffusion coefficients cannot be made. However, based on the MSD, one can state which molecules have a higher or lower diffusion regime.

MSD for 5ALA [13, 14], Me-5ALA [13, 14], and the 5ALA oxime derivatives in the xy -plane and the direction normal to the bilayer (z -direction) are displayed in Fig. 5. The MSDs for the 5ALA oxime derivatives were calculated for the first 20 ns of the un-constrained production runs in order to compare the data with 5ALA and Me-5ALA.

5ALA, Me-5ALA, and MH display the easiest diffusion in the xy -plane of the bilayer (Fig. 5a). The MSD along the bilayer normal is not finite, as opposed to that in the xy -plane, and should therefore be interpreted with caution.

Fig. 5 MSD for 5ALA [13, 14], Me-5ALA [13, 14], and the 5ALA oxime derivatives in (a) the xy -plane, and (b) the direction normal to the bilayer

The MSD in this direction should level off independently of any characteristics of the molecule. This MSD is significantly lower than in the xy -plane as the molecules prefer to stay at a specific level in the bilayer and thus display negligible movement in the z -direction (Fig. 5b). The different derivatives behave more similarly along this direction.

3.4 Free energy

Free energy profiles for the transport process of the 5ALA oxime derivatives from water and into the lipids, as a function of the distance to the bilayer center, are shown in Fig. 6a. The free energy profiles for 5ALA and Me-5ALA [13, 14] are included for comparison. All molecules display local minima in the region 1–1.5 nm from the bilayer center, the region in which the molecules were found to locate in the un-constrained simulations. HH displays the largest gain in free energy (49.3 kJ/mol) when moving from the water phase into the lipid region, followed by Me-5ALA (45.3 kJ/mol) and MH (38.7 kJ/mol). 5ALA, MM, and HM display more shallow free energy minima (30.5–31.7 kJ/mol). The location of the free energy minima along the z -direction also differs slightly between the different molecules. MM displays the minimum furthest away from the bilayer center, indicating that this molecule prefers to

Fig. 6 **a** Free energy profiles and **b** local diffusion coefficients of 5ALA [13, 14], Me-5ALA [13, 14], and the 5ALA oxime derivatives inside the membranes

Table 1 Calculated resistance (R') and permeability coefficients (P) of 5ALA, Me-5ALA [13, 14], and the 5ALA oxime derivatives inside the membrane

Molecule	R' (s cm ⁻¹)	P (cm s ⁻¹)
5ALA [13, 14]	5.28×10^{-2}	1.89×10^1
Me-5ALA [13, 14]	1.89×10^{-2}	5.28×10^1
HH	3.42×10^{-2}	2.93×10^1
MH	3.23×10^{-2}	3.10×10^1
HM	20.47×10^{-2}	0.49×10^1
MM	8.25×10^{-2}	1.21×10^1

locate closer to the interface between the lipids and water than the other molecules—as was also seen in the density profiles.

In the center of the bilayer, the free energies display maxima, indicating that this region is unfavorable for the molecules to reside in and to pass through and hinders the molecules from smoothly translocating across the membrane. HH, which has the deepest free energy minimum close to the headgroup region, also shows the highest barrier in the middle of the bilayer (48.9 kJ/mol), indicating that this molecule would experience large problems to pass through the bilayer center and would therefore prefer to

accumulate in the lipid region, close to the headgroups. Me-5ALA, on the other hand, which also displayed a reasonably deep free energy minimum, has a significantly lower barrier for transport across the bilayer center (26.0 kJ/mol). The smallest barrier height for the 5ALA oxime derivatives is found for MM (32.4 kJ/mol), which is comparable to the one for 5ALA (33.0 kJ/mol). The barrier heights for MH and HM are intermediate, at 35.5 and 38.1 kJ/mol, respectively. HH, MH, and Me-5ALA have negative free energy changes for the overall transport process from water and into the center of the bilayer, whereas 5ALA, HM, and MM display positive values.

Errors in free energy in the middle of the bilayer have previously been estimated to be in the range of 0.7–4 kJ/mol from shorter MD simulations [37], and we expect the errors from the longer simulations in the present study to be smaller.

3.5 Diffusion coefficients

The diffusion coefficients along the bilayer normal are shown in Fig. 6b, together with data for 5ALA and Me-5ALA [13, 14]. In the water phase (>3 nm from the bilayer center), the diffusion is the highest as the molecules are free to move without any interactions with lipids. In the very center of the bilayer, in the lipid tails, the diffusion also increases due to the lower density compared to the region closer to the headgroups. 5ALA and Me-5ALA display the highest diffusion peak in the center of the bilayer and also the highest overall diffusion inside the lipid region.

3.6 Resistance and permeability coefficients

In Table 1, the permeability coefficients (P) for the 5ALA oxime derivatives are listed along with the values for 5ALA and Me-5ALA [13, 14], calculated from Eq. 6. None of the 5ALA oxime derivatives display higher permeability coefficient than Me-5ALA; the highest permeability coefficient among the oxime derivatives is found for MH followed by HH, MM, and HM in decreasing order. HH and MH display faster permeation through the membrane compared to 5ALA, whereas the permeation of HM and MM is slower. All 5ALA oxime derivatives display significantly faster diffusion than the ethyl ester of 5ALA, for which permeability has also been studied [13, 14]. In a similar study of small solute molecules, the permeability coefficients of ethane, benzene, and methyl acetate were calculated and fall in the same range as those for HM and MM in the present study [37]. The permeability coefficient for 5ALA is slightly higher than for these molecules, whereas Me-5ALA, HH, and HM display significantly higher permeability coefficients.

Fig. 7 **a** Local resistance profiles for 5ALA [13, 14], Me-5ALA [13, 14], and the 5ALA oximes in the membrane. **b** Magnifications of the low-amplitude resistance profiles

An increase in permeability follows a decrease in resistance, which is seen by the reversed order of resistances (R') in Table 1. Local resistance profiles for the 5ALA molecules are shown in Fig. 7. The resistance in the center of the bilayer is extremely high for HM. For 5ALA and MM, the resistance in the center is higher than in water, whereas for HH and MH the resistance is in the same order of magnitude as in water. Me-5ALA displays negligible resistance in the center, which contributes to the enhanced permeability of this molecule in the membrane.

4 Conclusions

Four different 5ALA oxime derivatives have been studied, with respect to their capability to penetrate a DPPC lipid bilayer model system, and the results are compared with the corresponding data for 5ALA and its methyl ester derivative. All 5ALA oxime compounds were found to accumulate in the lipid region of the membrane, close to the polar headgroups, consistent with the preferred location of 5ALA and Me-5ALA. Me-5ALA displayed enhanced diffusion through the membrane in a previous study. The 5ALA oxime derivatives showed higher energy barriers in the center of the membrane compared to Me-5ALA, which indicates that the molecules

might pass through this region at slower rates. MM displayed the lowest energy barrier of the oxime derivatives, in the same range as the barrier for 5ALA, whereas HH, which displayed the largest gain in free energy when moving into the lipids, also had the largest energy barrier in the center of the membrane. MM, together with HM, was found to have the lowest computed permeability across the membrane. As the permeability is calculated from the free energy, both the magnitude of the decrease in free energy when moving into the membrane and the height of the free energy barrier in the center of the bilayer contribute to this. The highest permeability of the 5ALA oxime derivatives was found for MH and HH, which both displayed enhanced permeability compared to 5ALA. 5ALA is known to be able to pass through the plasma membrane as the compound has been successfully used in PDT treatment, requiring that the molecule is converted to PpIX within the cell, and HH and MH might from this perspective constitute possible alternatives to be applied in PDT. However, none of the 5ALA oxime derivatives showed enhanced permeability over Me-5ALA.

Acknowledgments The University of Gothenburg and the Swedish Science Research Council (VR) are gratefully acknowledged for financial support. The authors wish to acknowledge the Swedish National Infrastructure Committee (SNIC) and the C3SE supercomputing facility for the provision of computational facilities and support.

References

1. Van Hillegersberg R, Van den Berg JW, Kort WJ, Terpstra OT, Wilson JH (1992) Gastroenterology 103:647
2. Szeimies RM, Karrer S, Radakovic-Fijan S, Tanew A, Calzavara-Pinton PG, Zane C, Sidoroff A, Hempel M, Ulrich J, Proebstle T, Meffert H, Mulder M, Salomon D, Dittmar HC, Bauer JW, Kernland K, Braathen L (2002) J Am Acad Dermatol 47:258
3. Pariser DM, Lowe NJ, Stewart DM, Jarratt MT, Lucky AW, Pariser RJ, Yamauchi PS (2003) J Am Acad Dermatol 48:227
4. Freeman M, Vinciullo C, Francis D, Spelman L, Nguyen R, Fergin P, Thai KE, Murrell D, Weightman W, Anderson C, Reid C, Watson A, Foley P (2003) J Dermatolog Treat 14:99
5. Soler AM, Warloe T, Berner A, Giercksky KE (2001) Br J Dermatol 145:467
6. Horn M, Wolf P, Wulf HC, Warloe T, Fritsch C, Rhodes LE, Kaufmann R, De Rie M, Legat FJ, Stender IM, Soler AM, Wennberg AM, Wong GA, Larko O (2003) Br J Dermatol 149:1242
7. Rhodes LE, de Rie M, Enstrom Y, Groves R, Morken T, Goulden V, Wong GA, Grob JJ, Varma S, Wolf P (2004) Arch Dermatol 140:17
8. Vinciullo C, Elliott T, Francis D, Gebauer K, Spelman L, Nguyen R, Weightman W, Sheridan A, Reid C, Czarniecki D, Murrell D (2005) Br J Dermatol 152:765
9. Lange N, Jichlinski P, Zellweger M, Forrer M, Marti A, Guillou L, Kucera P, Wagnieres G, van den Bergh H (1999) Br J Cancer 80:185
10. Jichlinski P, Guillou L, Karlsen SJ, Malmstrom PU, Jocham D, Brennhovd B, Johansson E, Gartner T, Lange N, van den Bergh H, Leisinger HJ (2003) J Urol 170:226

11. Schmidbauer J, Witjes F, Schmeller N, Donat R, Susani M, Marberger M (2004) *J Urol* 171:135
12. Klok J, Akkermans W, van Henegouwen GMB (1998) *Photochem Photobiol* 67:150
13. Erdtman E, dos Santos DJVA, Löfgren L, Eriksson LA (2008) *Chem Phys Lett* 463:178
14. Erdtman E, dos Santos DJVA, Löfgren L, Eriksson LA (2009) *Chem Phys Lett* 470:369
15. Eriksson, L. A.; Löfgren, L. WO 2010/024775; Austr patent 2009286182
16. Kalla J, Raines RT (2008) *Angew Chemie Int Ed Engl* 47:7523
17. dos Santos DJVA, Eriksson LA (2006) *Biophys J* 91:2464
18. Eriksson ESE, dos Santos DJVA, Guedes RC, Eriksson LA (2009) *J Chem Theory Comput* 5:3139
19. Eriksson ESE, Eriksson LA (2011) *J Chem Theory Comput* 7:560
20. Hess B, Kutzner C, van der Spoel D, Lindahl EJ (2008) *Chem Theor Comput* 4:435
21. Marrink SJ, Berger O, Tieleman P Jahnig, F. *Biophys. J.* 1998, 74:931; Biocomputing at the University of Calgary, structures and topologies. http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies. (Accessed 1 May 2009), File: dppc64.pdb
22. Gaussian 09, Revision B.01, Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA Jr, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas Ö, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ. Gaussian, Inc., Wallingford CT, 2009
23. Schüttelkopf AW, van Aalten DM (2004) *Acta Crystallogr D Biol Crystallogr* 60:1355
24. Biocomputing at the University of Calgary, structures and topologies. http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies (Accessed 1 May 2009), Files: lipid.itp and dppc.itp
25. Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J. Interaction models in water in relation to protein hydration. In: Pullman B, editor. *Intermolecular forces*. Reidel Dordrecht: Publishing Company, 1981, pp 331–342
26. Nose S (1984) *Mol Phys* 52:255
27. Hoover WG (1985) *Phys Rev* 31:1695
28. Parrinello M, Rahman A (1981) *J Appl Phys* 52:7182
29. Nose S, Klein ML (1983) *Phys Rev Lett* 50:1207
30. Darden T, York D, Pedersen L (1993) *J Chem Phys* 98:10089
31. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG (1995) *J Chem Phys* 103:8577
32. Hess B, Bekker H, Berendsen HJC, Fraaije J (1997) *J Comput Chem* 18:1463
33. Ryckaert JP, Ciccotti G, Berendsen HJC (1977) *J Comput Phys* 23:327
34. Marrink SJ, Berendsen HJC (1994) *J Phys Chem* 98:4155
35. Allen MP, Tildesley DJ (1990) *Computer simulation of liquids*. Oxford University Press, Oxford
36. Müller-Plathe F, Rogers SC, van Gunsteren WF (1992) *Chem Phys Lett* 199:237
37. Bemporad D, Essex JW, Luttmann C (2004) *J Phys Chem B* 108:4875