J Comput Aided Mol Des (2007) 21:675–679
DOI 10.1007/s10822-007-9141-z

An atomistic model of passive membrane permeability: application to a series of FDA approved drugs

Chakrapani Kalyanaraman · Matthew P. Jacobson

Received: 1 March 2007 / Accepted: 15 October 2007 / Published online: 8 November 2007

Abstract We apply an atomistic model of passive membrane permeability to a series of weakly basic drugs. The computational model uses conformational sampling in combination with an all-atom force field and implicit solvent model to estimate relative passive membrane permeabilities. The model does not require the use of training data for rank-ordering compounds, and as such represents a different approach from the more commonly employed QSPR models. We compare the computational results to previously published experimental PAMPA and Caco-2 permeabilities.

Keywords Membrane permeability · PAMPA · Molecular mechanics · Implicit solvent

In earlier studies, we and others have demonstrated the utility of molecular mechanics models, in conjunction with appropriate sampling methods, for predicting the relative passive membrane permeabilities of series of compounds. A central theme in several prior studies of this type has been the importance of conformational flexibility and internal hydrogen bonding in facilitating passive membrane permeability [1–10]. Recently, we developed an atomistic computational model of passive membrane permeability based on torsion-angle conformational sampling and a molecular mechanics/implicit solvent energy function, and tested predictions that this model made for the relative permeabilities of 11 cyclic peptides in a parallel artificial membrane permeability assay (PAMPA) [2]. The experimental results, which were obtained after the computational results, showed excellent correlation with the computational predictions, with $R^2 = 0.96$. This study also examined a series of fluoroquinolone compounds using the same method, demonstrating its utility in predicting membrane permeability for drug-like small molecules as well ($R^2 = 0.83$ for correlation with PAMPA intrinsic permeability and $R^2 = 0.81$ for correlation with rat in situ absorption).

Our approach differs from most QSPR (quantitative structure-permeability relationships) models (e.g. Refs. [4, 11–19]) in several respects. The approach we describe is based on a simple physical model and a physically reasonable energy function. The molecular mechanics energy function contains numerous parameters, but they are not adjusted to specifically reproduce the permeability data that we are attempting to model. As discussed further below, the only adjustable parameters in this work are those from a linear regression between experimental and computational results. The model is atomistic and can be used to interpret the physical basis of differing permeabilities in series of compounds, which opens the possibility of rationally modifying compounds for improved permeability.

Other conceptually similar approaches have been published. For example, nearly a decade ago, Artursson et al. [20] used molecular mechanics methods to generate multiple conformations of six structurally similar FDA-approved drugs and calculated van der Waals and water accessible surface areas for the low energy conformers. They reported an excellent correlation between the "dynamic" (i.e., averaged over several low energy conformers) polar surface area and the cell permeabilities in Caco-2 cells and rat ileum.

Here we apply our model to a more chemically diverse set of compounds than we have considered in previous

C. Kalyanaraman · M. P. Jacobson (✉)
Department of Pharmaceutical Chemistry, UCSF,
San Francisco, CA 94158-2517, USA
e-mail: matt.jacobson@ucsf.edu

work, specifically a series of 11 weakly basic FDA-approved drugs, for which permeability values have been determined at multiple pHs in a double-sink PAMPA assay [21]. Our model of membrane permeability and the computational methods are described in greater detail in Ref. [2]. In brief, for any given compound, we attempt to identify the conformation that will minimize the free energy cost of transferring the compound from water (high dielectric) to the interior of the membrane (low dielectric). In a practical sense, we identify this conformation by searching for the lowest energy conformation using a molecular mechanics (MM) energy function consisting of the OPLS all-atom force field [22, 23] and a GB/SA (generalized Born/surface area) implicit solvent model [24] with parameters tuned to represent chloroform [25] as a mimic of the hydrophobic interior of the membrane [26]. The transfer free energy of this conformation between water and chloroform is then used as a measure of its relative passive membrane permeability.

In this work, the 3D structures of the drugs in their neutral form were obtained using LigPrep v.2.0106 (Schrödinger Inc). Energy parameters for the compounds are generated automatically using the utility software script *hetgrp_ffgen* (Schrödinger Inc), which generates OPLS-2001 or OPLS-2005 parameters for each ligand using a pre-defined rule based procedure. Multiple conformations for each compound were generated using MacroModel v.9.1106 (Schrödinger Inc). A 1,000 step torsional sampling was performed and the conformers that met the following two criteria were retained: (a) the minimized energy of the conformer is within 50 kJ/mol of the global minimum, and (b) identical atoms in any pair of conformations must differ by more than 0.25 Å. In total, anywhere from 200 to nearly 900 conformations were saved for the compounds studied.

Each of these conformations is then energy minimized in a low dielectric medium (chloroform) to identify the conformation with the lowest free energy (referred to as the "low dielectric conformation" (LDC) in our previous work [2]). We then compute the energy of the LDC in the high dielectric medium (water), and the free energy of transfer of the LDC between the high and low dielectric (ΔG_I) is used to rank compounds according to predicted passive membrane permeability. This approach implicitly assumes

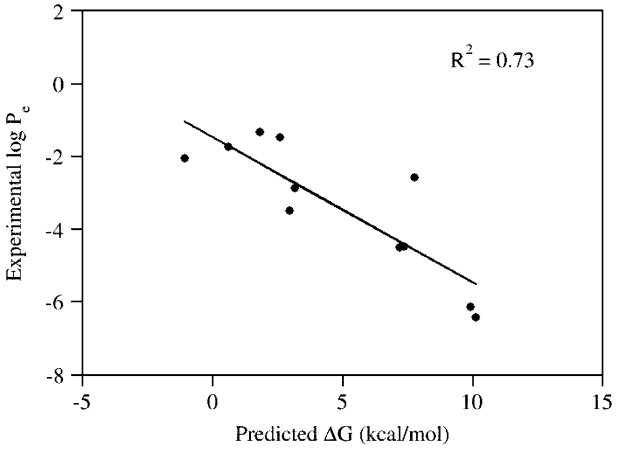

Fig. 2 Predicted ΔG_I is plotted against the log(intrinsic permeability) from a Caco-2 cell based assay. The units of P_0 are cm/s

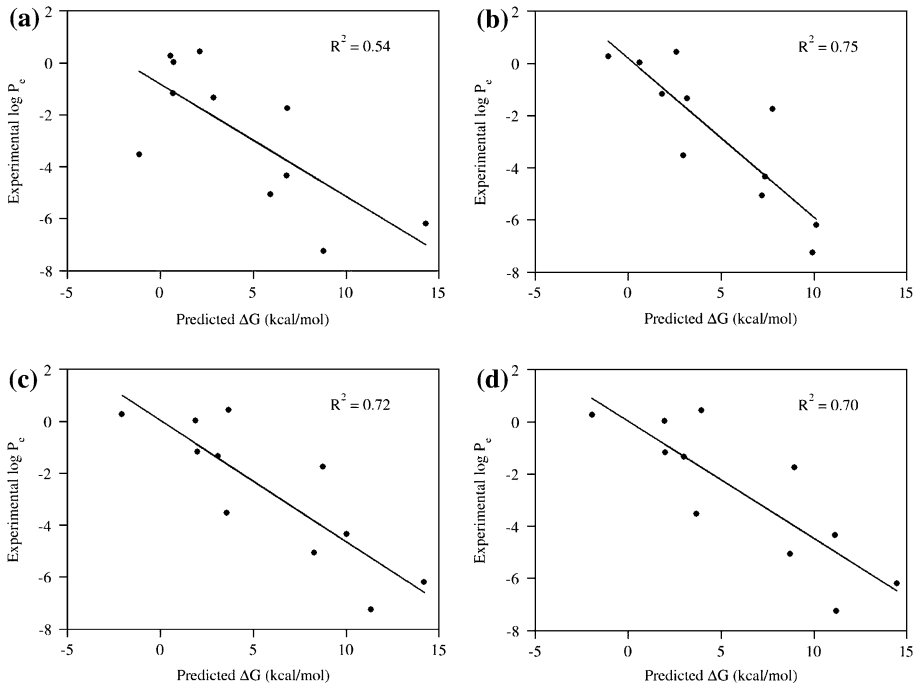

Fig. 1 (**a**) Predicted free energy of exchange from high dielectric medium to the low dielectric medium is plotted against the log(intrinsic permeability) from PAMPA assay, using OPLS-2001 parameters; (**b**) Same as (a), but using OPLS-2005 force field parameters. (**c**) Same as (b), but only energy minimization of a single low-energy conformation. (**d**) Same as (c), but without performing energy minimization. The units of P_e are cm/s

that the LDC is populated significantly in water, possibly among many other conformations.

We initially used OPLS-2001 parameters for the ligands. The correlation between the log of the intrinsic PAMPA permeability (P_e) and ΔG_I is plotted for the 11 FDA approved drugs in Fig. 1a. The linear correlation ($R^2 = 0.54$, slope $= -0.43$, calculated using the linear regression method available with the graphics package XMGrace v5.1.14) between the two is reasonable. The energy values calculated using the molecular mechanics energy function are highly sensitive to the parameter sets used and these parameters are frequently revised and improved. We decided to try the most recent OPLS-2005 force field parameter set for all the ligands. The results, in Fig. 1b,show a significantly improved linear correlation ($R^2 = 0.75$, slope $= -0.61$). The correlation with Caco-2 cell-based intrinsic permeability is shown in Fig. 2 with the same OPLS-2005 parameters, and is similar ($R^2 = 0.73$) to the correlation seen for the PAMPA intrinsic permeability.

In order to examine the role of conformational sampling, we have recomputed the ΔG_I values without performing the conformational expansion using Macromodel. In this test we used the conformations generated by Ligprep directly, which are low-energy and geometrically reasonable. In Fig. 1d, the ΔG_I are computed using the conformations generated by Ligprep directly ($R^2 = 0.70$) and in Fig. 1c,

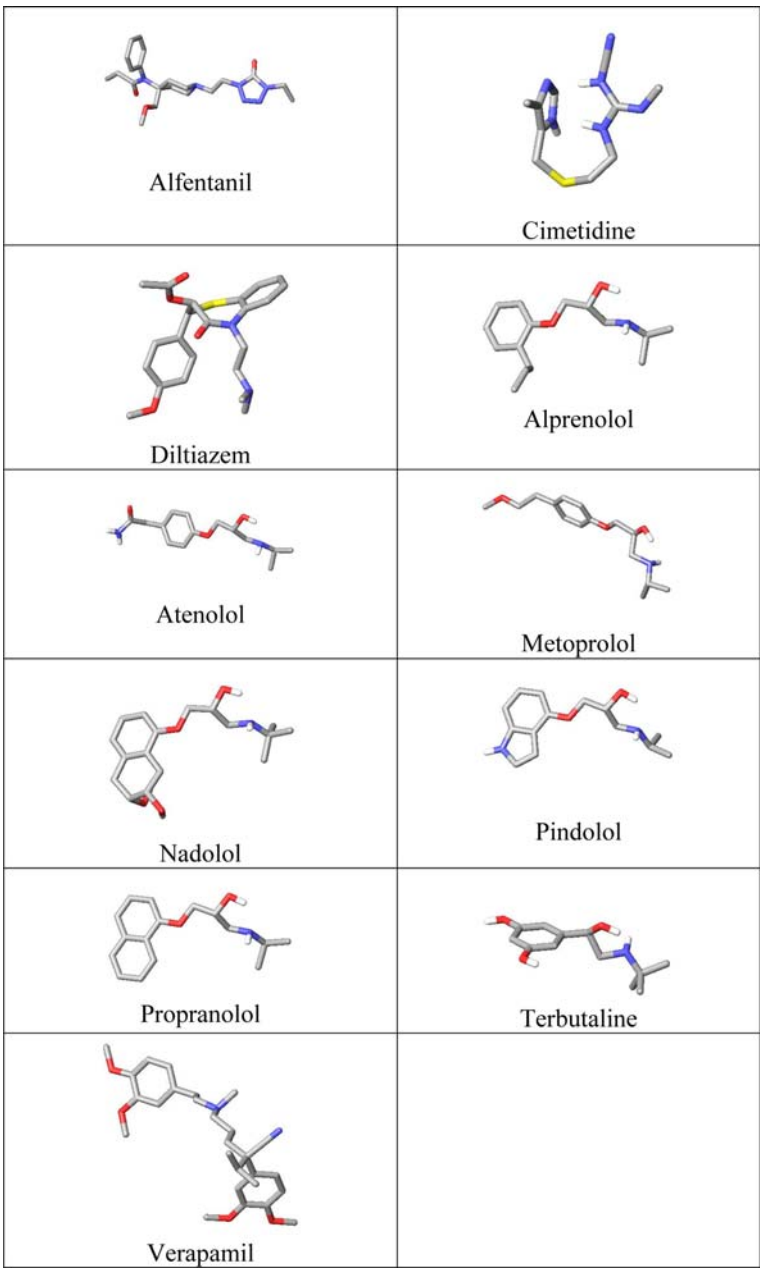

Fig. 3 Predicted low dielectric conformations of the drugs studied in the present work

Table 1 The free energy difference for the LDC between high and low dielectric (ΔG_I), and the energy difference between this conformation in water and the lowest energy conformation found in water (ΔG_W)

No	Compound	ΔG_I (kcal/mol)	ΔG_W (kcal/mol)	Log(Permeability^{PAMPA}) (cm/s) [20]	Log(Permeability^{Caco2}) (cm/s) [20]
1	Alfentanil	2.9	0.2	−3.53	−3.49
2	Cimetidine	10.1	0.1	−6.20	−6.44
3	Diltiazem	3.2	0.2	−1.33	−2.88
4	Alprenolol	0.6	1.1	0.017	−1.75
5	Atenolol	7.2	0.0	−5.07	−4.50
6	Metoprolol	1.8	0.0	−1.71	−1.35
7	Nadolol	7.4	0.1	−4.34	−4.49
8	Pindolol	7.8	0.0	−1.75	−2.58
9	Propranolol	2.6	0.0	0.43	−1.48
10	Terbutaline	9.9	0.3	−7.25	−6.15
11	Verapamil	-1.1	0.5	0.26	−2.07

these conformations are energy minimized in low dielectric ($R^2 = 0.72$).

Although the correlation coefficient does not change significantly with varying the extent of conformational sampling, the slope of the correlation does. Our model of passive membrane permeability is based on a simple thermodynamic computation: the predicted free energy of transfer of a particular conformation of a compound from high to low dielectric. This model can be considered a simplification of the classical solubility-diffusion model of passive membrane transport [27, 28], i.e., $P_e = K_p D/d$, where P_e is the permeabililty (cm/s), K_p is the unitless partition coefficient of the compound into the membrane, D is the diffusion coefficient in the membrane (cm^2/s), and d is the membrane thickness (cm). The key simplification is that we assume that differences in the rate of intramembrane diffusion are unimportant for the relatively similar (in terms of size and shape) compounds we consider here. This assumption may of course limit the accuracy of the predictions, but the rates of diffusion in the membrane are difficult to compute.

Given this assumption, the relationship between our computed transfer free energy and the permeability would be log P_e ($\propto -\Delta G_I/2.3RT$, predicting a slope for the correlations of −0.71, using energy units of kcal/mol. The actual slope in Fig. 1b is −0.61, in reasonable agreement. The deviation may be due to neglect of entropic losses upon entering the membrane, neglect of differences in the rate of diffusion, incorrect assumptions about the effective dielectric constant in the membrane, neglect of electronic polarization (the atomic partial charges are assumed to be the same in water and in the membrane), or other limitations of our simple model. The slopes in Fig. 1c, d, in which we did not perform a conformational search, are −0.47 and −0.45 respectively. That is, although performing the conformational search only slightly improves the correlation coefficient, it significantly improves the slope.

The predicted low dielectric conformations of all of the drugs that we studied are shown in Fig. 3. All compounds that contain both hydrogen bond donors and acceptors form internal hydrogen bonds, the most common motif being a hydroxyl group donating a hydrogen bond to a nearby amine. Higher energy conformations in the low dielectric medium contain either strained hydrogen bonds or none at all (data not shown).

One assumption of our model is that the LDC, i.e., the lowest energy conformation in chloroform which we use to compute the transfer free energies, can be at least transiently populated in water. This assumption is testable computationally by performing the conformational search in water as well as chloroform. Table 1 lists the energy difference between the LDC in water and the lowest energy conformation in water (ΔG_W). If one conformation of the molecule is the lowest in energy in both low and high dielectric, then this value is zero, as it is for three of the compounds. That is, for these compounds, we predict no significant conformational change between low and high dielectric. For most of the other compounds, the energy difference is small but nonzero, indicating that the conformation that we predict the compound to adopt in the membrane should at least be transiently populated in water as well.

In summary, although this is clearly a small scale study, we have provided additional evidence that it is possible to predict passive membrane permeability based on a simple atomistic model using a molecular mechanics energy function. The only fitted parameters are those of the linear regression. In combination with our previous work [2], these results suggest that the model can be applied both to series of chemically similar compounds and to more diverse compounds. The results highlight the need for reasonable partial atomic charges in order to obtain good results, and the importance of performing a semi-exhaustive 3D conformational search. The results also provide

additional evidence for the utility of implicit solvent models in predicting passive membrane permeability. Although our focus is on further developing a physics-based model (including entropic losses, for example), we suspect that an implicit solvent model would also be a valuable descriptor in the context of QSPR models. That is, the implicit solvent model captures solvation free energy is a more precise and physically detailed way than polar surface area or similar measures. Efforts to further validate our approach on diverse ligands are underway.

Acknowledgments This work was supported by NIH grant AI035707. MPJ is a consultant to Schrödinger, Inc.

References

1. Bemporad D, Luttmann C, Essex JW (2005) Biochim Biophys Acta 1718:1
2. Rezai T, Bock JE, Zhou MV, Kalyanaraman C, Lokey RS, Jacobson MP (2006) J Am Chem Soc 128:14073
3. Rezai T, Yu B, Millhauser GL, Jacobson MP, Lokey RS (2006) J Am Chem Soc 128:2510
4. Fujikawa M, Ano R, Nakao K, Shimizu R, Akamatsu M (2005) Bioorg Med Chem 13:4721
5. Bemporad D, Luttmann C, Essex JW (2004) Biophys J 87:1
6. Conradi RA, Hilgers AR, Ho NFH, Burton PS (1991) Pharm Res 8:1453
7. Huque FT, Box K, Platts JA, Comer J (2004) Eur J Pharm Sci 23:223
8. Ano R, Kimura Y, Shima M, Matsuno R, Ueno T, Akamatsu M (2004) Bioorg Med Chem 12:257
9. Winiwarter S, Ax F, Lennernas H, Hallberg A, Pettersson C, Karlen A (2003) J Mol Graph Model 21:273
10. Di Fenza A, Alagona G, Ghio C, Leonardi R, Giolitti A, Madami A (2007) J Comput Aided Mol Des
11. Ekins S, Rose J (2002) J Mol Graph Model 20:305
12. Refsgaard HH, Jensen BF, Brockhoff PB, Padkjaer SB, Guldbrandt M, Christensen MS (2005) J Med Chem 48:805
13. Krarup LH, Christensen IT, Hovgaard L, Frokjaer S (1998) Pharm Res 15:972
14. Ekins S, Waller CL, Swaan PW, Cruciani G, Wrighton SA, Wikel JH (2000) J Pharmacol Toxicol Methods 44:251
15. Ekins S, Durst GL, Stratford RE, Thorner DA, Lewis R, Loncharich RJ, Wikel JH (2001) J Chem Inf Comput Sci 41:1578
16. Yamashita F, Wanchana S, Hashida M (2002) J Pharm Sci 91:2230
17. Malkia A, Murtomaki L, Urtti A, Kontturi K (2004) Eur J Pharm Sci 23:13
18. Fujiwara S, Yamashita F, Hashida M (2002) Int J Pharm 237:95
19. Stouch TR, Kenyon JR, Johnson SR, Chen XQ, Doweyko A, Li Y (2003) J Comput Aided Mol Des 17:83
20. Palm K, Luthman K, Ungell A-L, Strandlund G, Artrusson P (1996) J Pharm Sci 85:32
21. Avdeef A, Artursson P, Neuhoff S, Lazorova L, Grasjo J, Tavelin S (2005) Eur J Pharm Sci 24:333
22. Jorgensen WL, Tiradorives J (1988) J Am Chem Soc 110:1666
23. Kaminski GA, Friesner RA, Tirado-Rives J, Jorgensen WL (2001) J Phys Chem B 105:6474
24. Ghosh A, Rapp CS, Friesner RA (1998) J Phys Chem B 102:10983
25. Luo R, Head MS, Given JA, Gilson MK (1999) Biophys Chem 78:183
26. Goodwin JT, Mao B, Vidmar TJ, Conradi RA, Burton PS (1999) J Pept Res 53:355
27. Pohorille A, New MH, Schweighofer K, Wilson MA (1999) Curr Topics Membr 48:49
28. Walter A, Gutknecht J (1986) J Membr Biol 90:207