

# Drug–protein interaction with Vpu from HIV-1: proposing binding sites for amiloride and one of its derivatives

C. G. Kim · V. Lemaitre · A. Watts · W. B. Fischer

Received: 10 June 2006 / Revised: 1 September 2006 / Accepted: 5 September 2006 / Published online: 3 November 2006  
© Springer-Verlag 2006

**Abstract** Vpu is an 81-amino-acid auxiliary protein of the genome of HIV-1. It is proposed that one of its roles is to enhance particle release by self-assembling to form water-filled channels enabling the flux of ions at the site of the plasma membrane of the infected cell. Hexamethylene amiloride has been shown to block Vpu channel activity when the protein is reconstituted into lipid bilayers. In a docking approach with monomeric, pentameric and hexameric bundle models of Vpu corresponding to the transmembrane part of the protein, a putative binding site of hexamethylene amiloride is proposed and is compared with the site for the nonpotent amiloride. The binding mode for both ligands is achieved by optimizing hydrogen bond interactions with serines. Binding energies and binding constants are the lowest for protonated hexamethylene amiloride in the pentameric bundle.

**Keywords** Viral ion channels · Vpu · HIV-1 · Docking simulations · Drug–protein interactions

## Introduction

It was recently discovered that derivatives of amiloride (Am), cyclohexamethylene amiloride (Hma) and, to a lesser extent, dimethyl amiloride inhibit the budding of virus-like particles from HeLa cells when they are expressing Gag and Vpu proteins from human immunodeficiency virus type-1 (HIV-1) [1]. Experiments with full-length Vpu and with a peptide corresponding to its N terminal region, reconstituted into lipid bilayers, indicate that the derivatives block channel activity, thus linking the blocking to Vpu [1]. Blocking of another viral channel protein, p7 from hepatitis C virus, by the amiloride derivative Hma has also been reported [2]. However, experimental binding constants have not yet been measured. Amiloride itself is known to block the epithelial Na<sup>+</sup> channel [3]. Based on these studies, it is believed that derivatives of amiloride may have therapeutic potential as antiviral compounds.

Virus protein “u” (Vpu) is an 81-amino-acid phosphorylated protein encoded by HIV-1 [4, 5]. It has two distinct domains: a helical transmembrane (TM) domain [6, 7] and a cytoplasmic domain [8, 9]. Its role is to amplify the release of progeny virus particles. This is achieved by inducing degradation of the receptor protein CD4 along the ubiquitin–proteasome pathway via interaction of the cytoplasmic domain of Vpu with CD4 in the endoplasmic reticulum. Vpu has been found in the Golgi membranes, where it is proposed to oligomerise and form ion channels. The latter function has been attributed to the TM domain by *in vitro* studies. Ion channel activity has been reported with full-length Vpu and peptides corresponding to the TM sequence of Vpu reconstituted into artificial lipid bilayers. Thus, in this study, only the TM sequence (Vpu<sub>2–33</sub>) is used to model the drug–protein interaction, since the channel activity of the peptide can also be blocked by the drug [1].

---

C. G. Kim · A. Watts  
Biomembrane Structure Unit, Department of Biochemistry,  
Oxford University,  
South Parks Road,  
Oxford OX1 3QU, UK

W. B. Fischer (✉)  
Bionanotechnology Interdisciplinary Research Collaboration,  
Clarendon Laboratory, Department of Physics, Oxford University,  
Parks Road,  
Oxford OX1 3PU, UK  
e-mail: wolfgang.fischer@bioch.ox.ac.uk

V. Lemaitre  
BioAnalytical Science Department, Nestec S.A.,  
Vers-Chez-Les-Blanc,  
1000 Lausanne 26, Switzerland

The aim of this case study is to assess the binding modes of Hma and Am, which should fill the gap until more biophysical data on the binding are available.

The putative binding site of the ligand at the protein and its conformation are assessed using a docking simulation method (AUTODOCK 3.0) which implements a Lamarckian genetic algorithm [10, 11]. During the search, the conformation of the ligand is allowed to change. The search protocol includes energy minimization steps for local optimization of the docked conformation. Free energy estimation allows binding constants to be predicted. The adaptation of the method to a binding site which is a hollow pore is a rather novel approach. However, the outcome of the docking simulations reveals extremely plausible sites for the ligand and so this approach can be used for further applications to ion channels.

## Materials and methods

### The model structures

All models of Vpu are taken from previous MD simulations performed using a fully-hydrated lipid bilayer with helices including the first 32 amino acids of Vpu (Vpu<sub>2–33</sub>) after a 1 ns simulation (see [12] for the bundles). The amino acid sequence used for the models was:

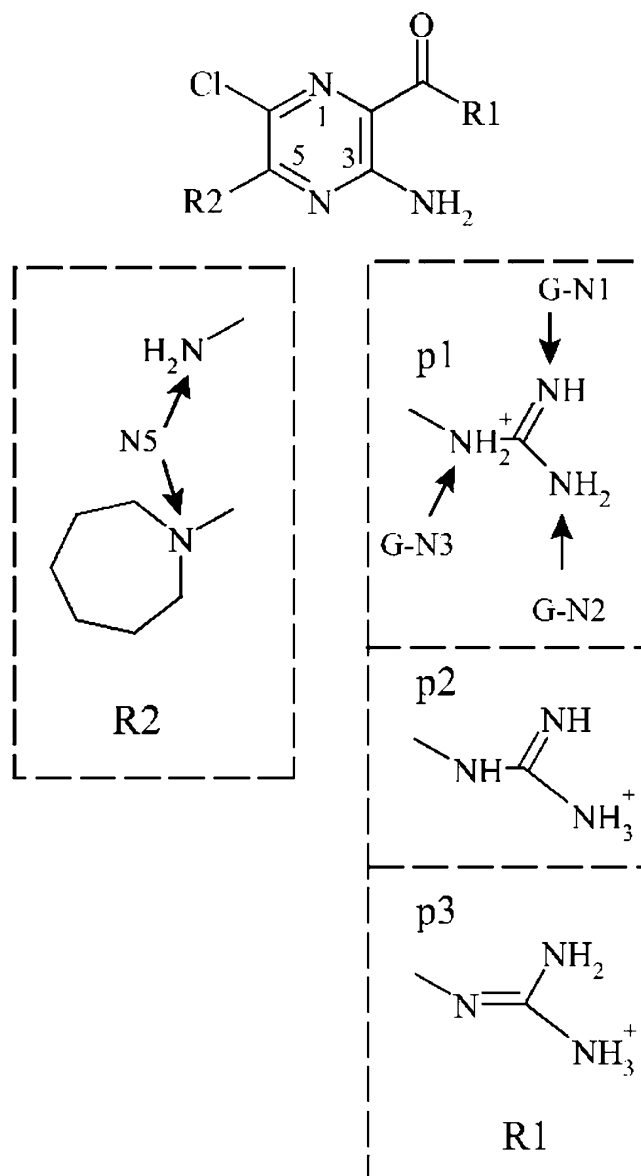
QPIPIVAIV<sup>10</sup> ALVVAIHAI<sup>20</sup> VVWSIVHIEY<sup>30</sup> RKI.

This sequence includes the TM sequence of the Vpu protein. The structure was removed from the lipid bilayer and proper polar hydrogen atoms were added to the protein molecules using the “protonate” program, which is part of the AUTODOCK 3.0 package [10, 11]. Partial protein charges were then added using Kollman united atom charges [13].

### Ligand docking

The cation transport inhibitor 3,5-diamino-6-chloro-*N*-(diaminomethylene)-pyrazinecarboxamide and its derivative, 5-(*N,N*-hexamethylene)amiloride, both used here in their unprotonated (AM, HMA) and protonated (AMp, HMAp) forms, were generated as pdb files using WebLab ViewerPro. In the docking simulation, the protonated forms of the ligands (shown in Fig. 1) were imported into AUTODOCK 3.0. Ligand docking to each protein molecule was performed permitting full torsional flexibility about their variable bonds. Docking simulations between ligands and proteins were performed for the free ligand starting from a distal “luminal” positional grid.

In the docking simulations, a population size of 50 with a maximum of 10,000,000 energy evaluations, up to 50,000



**Fig. 1** Scheme for the Am and Hma used for the docking simulations. *p1*, *p2* and *p3* refer to the type of protonation

generations, a gene mutation rate of 0.02, and a crossover of 0.8 were used for the global search algorithm. For the local search using Solis & Wets’ algorithm [14], the number of iterations was 3,000. Each docking simulation contained 50 runs to generate clusters of ultimate docking positions with a positional RMSD tolerance of 2 Å.

The partial charges of the ligands were calculated by WebLab ViewerPro using the AMBER force field. Ligands were initially placed in arbitrary positions restricted to the volume above the extracellular domain of the Vpu. The final ligand–protein complexes were visualized and analysed using PyMOL (<http://www.pymol.org>).

Simulations were performed using a Dell Precision 530 computer with two Intel Xeon 2.4 GHz processors

operating in parallel, and with 1 Gb RAM. A Linux cluster at the Oxford Supercomputer Centre was also used.

## Results

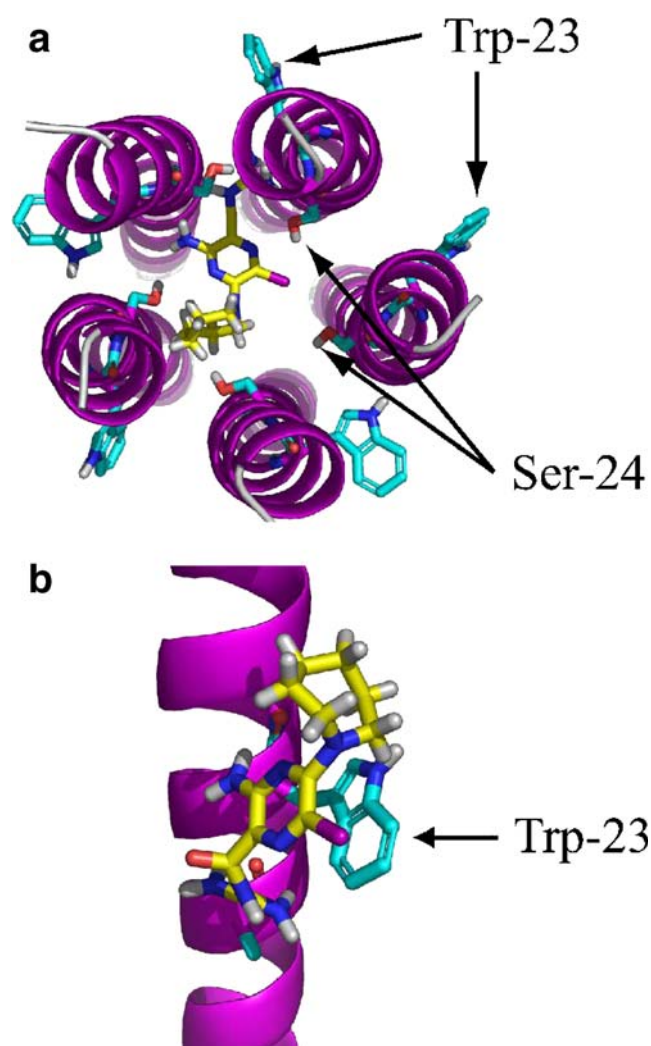
Am consists of pyrazine ring and a carbonyl–guanidine moiety with amino groups (N) at positions 3 (N3) and 5 (N5) on the pyrazine ring. Hma has the hydrogens on N5 substituted for a cyclic hexamethylene ring. The guanidine group consists of the three N atoms labelled here as G-N1, G-N2 and G-N3 (Fig. 1). The TM part of Vpu consists of a longer hydrophobic end (consisting of valines, leucines and isoleucines) towards the N-terminal end, and a hydrophilic part, including a serine and an arginine, towards the C terminal end, which both point into the pore of the model.

### Hydrogen bonds

In this study, the threshold for a hydrogen bond was a maximum A–H distance of 0.25 nm and a maximum A–H–D angle of 60°, where A is the acceptor and D the donor atom. Independent of the protonation state, all Am molecules are positioned towards the C terminus, and they interact with Ser-24 via hydrogen bonding (Fig. 2). G-N1 and G-N2 of AM are ~2.0–2.25 Å away from Ser-24. N3 also forms hydrogen bonds with the backbone carbonyl group of Ile-25. AMp2 adopts a position in which G-N1 forms hydrogen bonds with the O of Ser-24, whilst G-N2 is not involved in the contact. N3 is only weakly involved in hydrogen bonding, since its distance to Ser-24 is 2.91 Å. N5 satisfies hydrogen bonding with the backbone carbonyl group of Ile-25. For AMp3, both G-N1 and G-N2 form hydrogen bonds with Ser-24. AMp1 is the only ligand which is located around Tyr-30, Arg-31 and Ile-33, where G-N3 satisfies its hydrogen bonding via the backbone carbonyl of Ile-33. Steric hindrance prevents an energetically favourable interaction at the site of Ser-24. Thus, a driving force for the interaction of Am with the single strand is to maximise the hydrogen bonding with the most exposed polar residue, which is in this case Ser-24.

In a similar way, Hma orients itself so that, with G-N1 fully protonated (HMAp2 and HMAp3), a maximum number of hydrogens are formed with Ser-24 (Fig. 2a). In these orientations, the cyclohexamethylene ring is close to Val-26 and Ile-27 to optimize van der Waals interactions. HMA and HMAp1 adopt a reversed orientation, with the cyclohexamethylene ring pointing between the Trp-23 and Ser-24 region and the guanidinium moiety towards the C terminus.

In some cases, the pyrazine ring comes close to assuming a staggered  $\pi$ – $\pi$ -based interaction with Trp-23 in the single-strand models (Fig. 2b). However, the



**Fig. 2** **a** Pentameric bundle of Vpu<sub>2–33</sub> with Trp-23 and Ser-24 in stick modus. The lowest energy conformation of HMA<sup>+</sup> (CPK colour code in grey scale) is shown in the centre of the bundle. The view is from the C- down to the N-terminal end. The pictures of the models were generated using PyMOL (<http://www.pymol.org>). **b** Close-up view of the lowest energy conformation of HMA<sup>+</sup> with the monomeric Vpu<sub>2–33</sub>. Trp-23 highlighted in stick modus

distances between the two ring systems are too large (>0.4 nm) to allow for optimized packing.

Am and Hma both share the lowest energy position at the level of the serines in the pentameric bundle. Hydrogen bonding of all ligands is again maximised so that the guanidine moiety in particular is involved in hydrogen bond formation. Whenever sterically possible, the other amines of the pyrazine ring form hydrogen bonds with either serines from other helices or with the carbonyl backbones of other amino acids in reach.

For the hexameric bundles, the positions of the ligands follow the same pattern as mentioned previously for the pentamer in terms of hydrogen bonding. However, due to the space available in the pore, Am ligands orient in such a way that the plane of the pyrazine ring orients perpendic-

ular to the plane of a lipid bilayer. The cyclohexamethylene ring in Hma has no tendency to orient towards either end of the bundle in all the docking simulations, and this also true for the pentameric bundle.

#### Docking energy and binding constants

The binding energies for all of the ligands are 15–40 kcal/mol (Table 1), with the values for Hma slightly lower than those for Am, independent of the protonation state in the protein model used. Independent of the ligand and its protonation state, the binding energy decreases in the order monomer>hexamer>pentamer. Amp1 and HMAp1 do have the highest binding energies:  $-14.11 > -23.45 > -20.68$  kJ/mol and  $-16.75 > -27.80 > -24.87$  kJ/mol, respectively. The lowest values were found for AMP2, AMP3 and HMAp2, HMAp3, with  $-34.38$  kJ/mol and  $-38.44$  kJ/mol obtained for AMP2 and HMAp3, respectively, in the pentameric bundle.

Similar to the energy values, the binding constant was the lowest for the pentameric bundle and the largest for the monomer for all the ligands. HMA and HMAp2 give the lowest binding constants: 0.83 and 0.58  $\mu$ M, respectively. For the pentameric and hexameric bundles, the binding constants for the Am ligands are larger than those for the Hma ligands. For the monomer, the binding constants are similar but slightly larger for Hma. Thus the binding energies and binding constants favour the interaction of Hma with the pentameric model.

#### Discussion

The reason for using AUTODOCK is the idea that the binding of a ligand into a pore parallels the binding of a small flexible ligand into the confined geometry of a

binding site in an enzyme. AUTODOCK has proven to be successful for the latter case based on a more physical approach that proposes the global orientation of the ligand at the site of the protein by combining local (simulated annealing) and global (genetic algorithms based on Lamarckian genetics) search strategies. In addition, its potential to deliver an estimated free energy ( $\Delta G$ ) for ligand binding allows the data to be compared with those derived from experiments.

Current investigations are based on models of the TM part of Vpu as pentameric and hexameric assemblies as well as a monomeric unit for comparison. All Vpu models have been taken from MD simulations. The models have undergone a short energy minimization. All models are plausible considering current theories on ion channels, where hydrophilic residues point into a pore (here the Ser-24) and tryptophans (here Trp-23) are in a position where they can anchor the bundle within the phospholipid headgroup region [15–21]. Experimental data using Vpu<sub>1–32</sub> (with the first residue a methionine) indicate that channel activity is blocked in the presence of Hma [1, 22].

The most important residue for ligand binding with Vpu is Ser-24. Amiloride is a prototypic inhibitor of the epithelial Na<sup>+</sup> channel (ENaC) [3] and binding of amiloride with ENaC is proposed to also involve regions within the pore [23–26] with a serine residue as part of a binding site [26]. Thus, the proposed binding site obtained with the Vpu models agrees with these findings for the ENaC.

The optimum drug–protein interaction is driven by the need to maximise hydrogen bond formation, which is best achieved with the smaller pore formed by the highly hydrophobic amino acids containing the TM part of Vpu. Increasing the pore size decreases the binding efficiency. As an additional effect, binding is enhanced if van der Waals interactions also contribute, like they do with the cyclohexamethylene ring. Finally, with the bulky ring, Hma occludes the pore much more than Am does.

#### Conclusion

The main behaviour of the drug investigated is that it forms hydrogen bonds with the hydrophilic pore lining residue Ser-24. Based on the models and the software used, it is suggested that the binding site of Am and the blocker Hma lies within the pore. The best binding conditions are achieved with the pentameric TM Vpu bundle.

Regarding future drug design, potential ligands should have the ability to form hydrogen bonds, and a spacious hydrophobic tail is also required.

**Table 1** Final docking energy  $\Delta G$  (in kJ/mol) and estimated inhibition constants  $K_i$  (in  $\mu$ M) for the ligands

		$\Delta G$ [kJ/mol]			$K_i$ [ $\mu$ M]		
		M	P	H	M	P	H
Am	AM	-20.18	-32.78	-28.72	673	2.89	22.0
	AMp1	-14.11	-23.45	-20.68	844	31.0	96.6
	AMp2	-20.77	-34.38	-29.81	627	2.23	14.1
	AMp3	-20.98	-34.33	-28.97	478	2.34	20.2
Hma	HMA	-21.19	-37.10	-31.99	612	0.83	6.86
	HMAp1	-16.75	-27.80	-24.87	1235	11.3	37.2
	HMAp2	-20.98	-38.44	-30.15	665	0.58	14.0
	HMAp3	-21.10	-36.22	-31.49	571	1.01	8.65

M stands for monomer and P and H for pentameric and hexameric bundles of Vpu<sub>1–32</sub>, respectively

**Acknowledgement** C.G. Kim acknowledges the CJ Corporation for funding. V.L. thanks BBSRC for an Industrial CASE scholarship. W. B.F. and A.W. thank the Bionanotechnology IRC, MRC and BBSRC for grant support in this work. We thank the Oxford Supercomputer Centre for use of their computer facilities and their service, and Peter Judge (Oxford) for valuable discussions.

## References

1. Ewart GD, Mills K, Cox GB, Gage PW (2002) *Eur Biophys J* 31:26–35
2. Premkumar A, Wilson L, Ewart GD, Gage PW (2004) *FEBS Lett* 557:99–103
3. Kleyman TR, Cragoe EJ Jr (1990) *Methods Enzymol* 191:739–755
4. Strebel K, Klimkait T, Martin MA (1988) *Science* 241:1221–1223
5. Cohen EA, Terwilliger EF, Sodroski JG, Haseltine WA (1988) *Nature* 334:532–534
6. Wray V, Kinder R, Federau T, Henklein P, Bechinger B, Schubert U (1999) *Biochemistry* 38:5272–5282
7. Ma C, Marassi FM, Jones DH, Straus SK, Bour S, Strebel K, Schubert U, Oblatt-Montal M, Montal M, Opella SJ (2002) *Protein Sci* 11:546–557
8. Wray V, Federau T, Henklein P, Klabunde S, Kunert O, Schomburg D, Schubert U (1995) *Int J Peptide Protein Res* 45:35–43
9. Willbold D, Hoffmann S, Rösch P (1997) *Eur J Biochem* 245:581–588
10. Morris GM, Goodsell DS, Huey R, Olson AJ (1996) *J Comput Aid Mol Des* 10:293–304
11. Goodsell DS, Morris GM, Olson AJ (1996) *J Mol Recognit* 9:1–5
12. Cordes FS, Tustian A, Sansom MSP, Watts A, Fischer WB (2002) *Biochemistry* 41:7359–7365
13. Weiner SJ, Kollman PA, Case DA, Singh UC, Ghio C, Alagona G, Profeta S, Weiner P (1984) *J Am Chem Soc* 106:765–784
14. Solis FJ, Wets RJ (1981) *Math Oper Res* 6:19–30
15. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M (2000) *Science* 289:739–745
16. Leonard RJ, Labarca CG, Charnet P, Davidson N, Lester HA (1988) *Science* 242:1578–1581
17. Revah F, Galzi JL, Giraudat J, Haumont PY, Lederer F, Changeux JP (1990) *Proc Natl Acad Sci USA* 87:4675–4679
18. Pebay-Peyroula E, Rummerl G, Rosenbusch JP, Landau EM (1997) *Science* 277:1676–1681
19. Sass HJ, Büldt G, Gessenich R, Hehn D, Neff D, Schlesinger R, Berendzen J, Ormos P (2000) *Nature* 406:649–653
20. Pautsch A, Schulz GE (2000) *J Mol Biol* 298:273–282
21. Miyazawa A, Fujiyoshi Y, Unwin N (2003) *Nature* 423:949–955
22. Römer W, Lam YH, Fischer D, Watts A, Fischer WB, Göring P, Wehrspohn RB, Gösele U, Steinem C (2004) *J Am Chem Soc* 126:16267–16274
23. Schild L, Schneeberger E, Gautschi I, Firsov D (1997) *J Gen Physiol* 109:15–26
24. Ismailov II, Kieber-Emmos T, Lin C, Berdiev BK, Shlyonsky VG, Patton HK, Fuller CM, Worrell R, Zuckerman JB, Sun W, Eaton DC, Benos DJ, Kleyman TR (1997) *J Biol Chem* 272:21075–21083
25. Fuller CM, Berdiev BK, Shlyonsky VG, Ismailov II, Benos DJ (1997) *Biophys J* 72:1622–1632
26. Waldmann R, Champigny G, Lazdunski M (1995) *J Biol Chem* 270:11735–11737