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Molecular modelling of amphipathic basic model peptides: Interactions in aqueous solutions and in the presence of lipids*

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Abstract. Molecular modelling calculations based on experimental data obtained in solution and in small unilamellar vesicles are used to study interactions between amphiphilic basic peptides and membranes. The behaviour of such peptides during the initial and final stages of the adsorption process is our primary interest. Primary sequences of 20 amino acid residues were designed with equal numbers of basic lysines and hydrophobic leucines in order to get an amphipathic α helix. First, in solution, aggregates with an increasing number (up to nine) of helical monomers were built up and the hydrophobic solvent accessible surface per monomer was analysed on energy minimised structures. This showed that aggregates with 5–8 of monomers should be equally probable, in reasonable accordance with experimental data. In addition, models of membranes with 21 dimyristoylphosphatidylcholine lipids were constructed; amphiphilic peptides were merged into these assemblies with their axes parallel to the monolayer surface and the whole lipid/peptide complex was submitted to a few steps of simulated annealing and further energy minimisation techniques in order to equilibrate alkyl chains in the vicinity of the peptide. These simulations yield an estimation of the penetration depth for the peptide in the monolayer of $\sim$3.2 Å, whereas experimental approaches to this question were not productive. The modification in the peptide net electrical charge by interchanging Leu in Lys residues in such systems is also examined: for low-charged peptides the penetration depth increases.

Key words: Model peptides – Monolayers – Penetration depth – Modelling

1 Introduction

The study of model amphipathic helicogen peptides is a field of interest for understanding molecular interactions between membrane lipids and peptides. Among many various biological processes involving these interactions, we can mention membrane fusion present in endo/exocytose, viral infection, membrane alteration such as pore induction by peptides leading to membrane cytolysis, signal transduction through the membranes, insertion of peptide segments from proteins into membranes. These peptides are generally not more than 20 or 30 amino acids long and may contain, besides hydrophobic residues, positive and negative charged residues.

Thus, a model peptide, entirely amphipathic, with ten leucine residues and ten lysine residues was designed: LKKLLKKLLKLLKKLLKKLK. A thorough experimental study including NMR, circular dichroism spectroscopy, fluorescence spectroscopy of four derivatives containing tryptophan residues in place of leucine, monolayers and sedimentation techniques has already been published [1, 2]. The main results of that experimental work are the basis of the modelling study presented in this paper. They can be summarised as follows:

1. In solution, the peptides tend to form aggregates at very low concentrations (ca 10^{-6} M); the monomers are structured as α helices and the aggregates comprise a various number of monomers in equilibrium [3].
2. In the presence of lipids (large or small unilamellar vesicles, SUV), the binding process is very rapid and insertion takes place with peptides oriented parallel to the surface model membrane and structured as single α helices.
3. The tryptophan residue of peptide derivatives lies in a rather hydrophobic environment. The lipid molecules get organised around the peptide α helix as "wheat grains around an ear" and thus experimental measurement of fluorescence quenching by brominated probes fails to determine the depth of penetration of the peptide into the monolayers.

X-ray crystallography and proton NMR, on account of signal enlargement, are also not very well suited to accurately revealing the peptide helix location in lipid bilayers. Consequently, we turn to modelling, and in this

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paper we concentrate on three particular points: the possible structures of peptide aggregates in solution, the penetration depth of entirely amphipathic peptides into model monolayers, and the influence of the peptide charge on this parameter.

2 Methods

2.1 General specifications

For the modelling of the starting structures, Sybyl 6.3 software (Tripos Inc, St. Louis, Mo., USA) running on a Silicon Graphics R4000 workstation was used. All the energy calculations were carried out with the following specifications: Tripos force field [4], Pullman charges [5], distance dependent dielectric constant in order to mimic the solvent screening effect [6], and the Powell method was used for the energy minimizations. In addition, in order to explore a larger conformational space and to enable high energy barriers to be crossed and the system to escape from local minima, simulated annealing procedures were applied: for several cycles, a rapid heating phase during which the "temperature" of the system is increased to a high value (700 K) then a slow cooling phase with exponential temperature decrease to a low value (200 K) followed by further minimization steps. Finally, surface accessibilities to solvent molecules were measured by the "savol" algorithm included in Sybyl: a rigid sphere of radius 1.4 Å simulating a water molecule was allowed to roll on the van der Waals surface of selected atoms.

2.2 Protocol for the penetration depth determinations

The lipid membrane model consisted of an assembly of 21 dimyristoyl-phosphatidylcholine (DMPC) lipid molecules arranged as a monolayer. This assembly was built up progressively with intermediate energy minimizations, then submitted to a final global minimisation. The resulting structure was in a gel-like phase.

The modelling protocol for the determination of the peptide penetration depth was the following. First, the peptide was merged in the lipid assembly with its helical axis parallel to the monolayer surface [2, 7]. Due to its size, it was not possible to merge it without disrupting the lipid assembly, which rather was separated into two parts on each side of the peptide. Second, constraints were imposed between the ten lysine amino groups and the phosphate groups of the nearest lipids, and the total system was energy minimized. Then 12 lipids located on the assembly periphery were kept fixed and a simulated annealing procedure was used in order to allow the flexible lipid chains to overcome the low energy *all-trans* conformation and to match the helical shape of the peptide; only the structures where the peptide was still in an α helix conformation were kept. Finally, the global assembly with the lowest potential energy was submitted to a new energy minimization. The position of the peptide relative to the model membrane was defined as the height d of the peptide axis above/below a reference plane containing the phosphate groups of the lipids free from direct interaction with the helical peptide (Fig. 1). Different peptide initial positions d_i were examined and new minimizations resulted in final structures where the above-defined peptide relative position was denoted d_f.

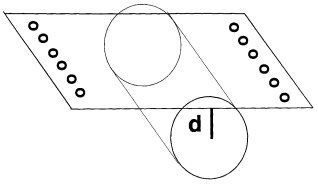

Fig. 1. Definition of d in the peptide/lipid system. The peptide is represented by a *cylinder*, the *circles* are the phosphate groups of the external lipids used to define a reference plane, and d is the distance between the peptide axis and this plane

3 Results and discussion

3.1 Peptide aggregation in solution

Using experimental results starting, compact structures of n monomeric peptide units in standard α helix conformations, with either parallel or antiparallel N-C directions, were built up. Such configurations indeed meet the requirement of minimal hydrophobic residue exposition to the solvent. Then these starting structures were energy minimized. Due to the cohesion of such a system, maintained by intra-helix NH···OC hydrogen bonds and intermolecular hydrophobic driving forces, it can be assumed that no further exploration of the conformational space is necessary.

The calculation results show that compact aggregates with close hydrophobic core and mean energy values per monomer $\sim$−23 kcal mol^{-1} are possible (Fig. 2). The relative accessibility, expressed as the ratio r between leucine surface accessibility in the aggregate and the

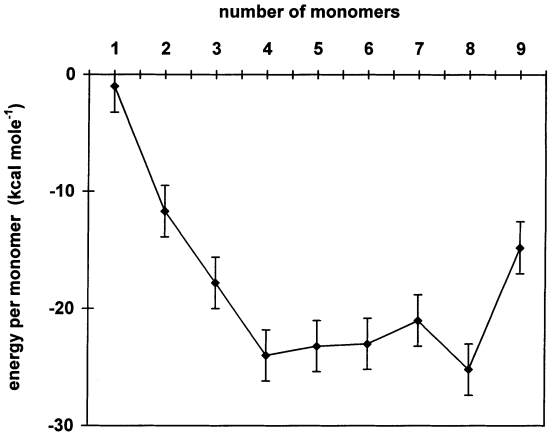

Fig. 2. Peptide aggregates in solution: energy per monomer versus number of monomeric units in the aggregate n

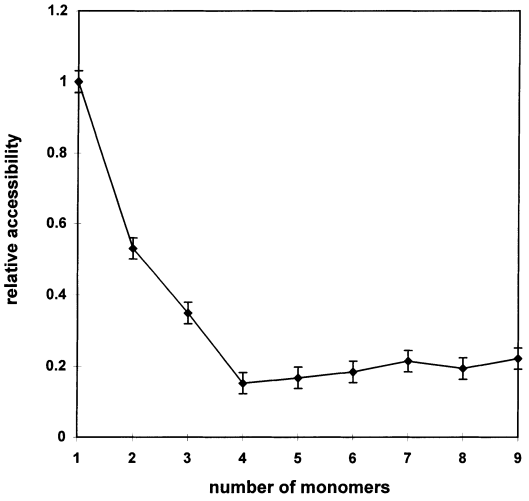

Fig. 3. Relative accessibility of leucine versus the number of monomers present in the aggregate n

product of n times the leucine surface accessibility for the monomer, is displayed in Fig. 3. First we see decrease in r for n below 4, then a broad valley with nearly constant values for n ranging from 4 to 8, then a slight increase at higher n. Moreover a parity effect is superimposed on this general trend. This effect can be interpreted in terms of both electrostatic and steric interactions. For even n, all the helices can be antiparallel resulting in a helix dipole stabilization [8] and high interdigitation of leucine residues belonging to two antiparallel monomers in contact, whereas such a disposition is less compact in the case of parallel α helices. In fact, because of $-60°$ standard values for the first side chain torsion angle in an α helix, two neighbouring antiparallel helices look like two herring bones in which lateral bones find an optimal interpenetration. This leads to a favourable conformational energy term for even values of n ($\sim$20% lower), a lower overall cross-section for the aggregate and thus a lower hydrophobic surface accessibility. From $n = 1$–4, the decrease of the relative accessibility is due to the possibility of a larger screening of hydrophobic residues to the solvent by accommodating an increasing number of monomers in the aggregate; the tetramer corresponds to the maximal compact structure without a central hydrophobic channel. When $n > 4$, compact aggregates are still possible: each new helix coming into the vicinity of a given aggregate has to screen the leucine still accessible to the solvent in the superficial aggregate crevices. Four supplementary helices can thus be accommodated as an external ring around the initial

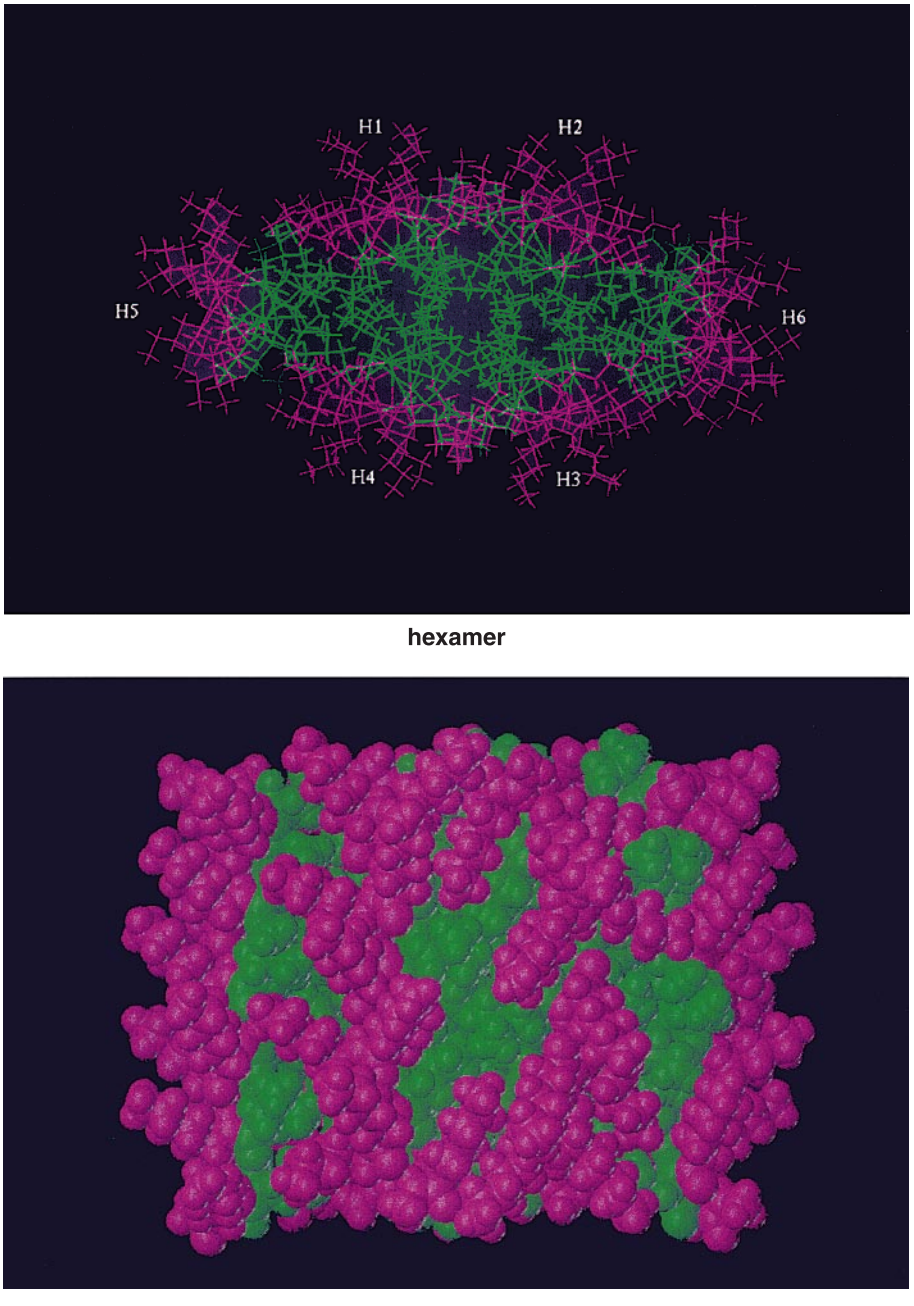

Fig. 4. Hexameric aggregate model with hydrophobic residues coloured in green and basic residues in magenta H_i refer to the helix locations in the aggregate

tetramer aggregates. The model hexamer aggregate is shown in Fig. 4. For $n = 8$, the area of the hydrophobic residue exposed to the solvent is not sufficient to allow the addition of supplementary monomers on a second external ring. For the nonamer, the total energy per monomer rises and existence of higher n-mers becomes less probable.

The model is in agreement with osmometry measurements which gave a mean value of n close to 7, while sedimentation measurements showed a statistical repartition of aggregates with various numbers of monomers (n mainly in the range of 5–10 at 10^{-6} M) [3]. The tendency to form aggregates seems to be governed by the amphipathic nature of these model peptides and hydrophobic interactions and could be related to the unusual temperature dependence of such peptides, which get structured as the temperature rises [3].

3.2. Peptide in a lipidic environment

The ten lysine amphipathic peptide penetration depth has been estimated at $D = 3.2$ Å [2]. In fact, when varying the initial distance d_i (Fig. 1), the peptide initially located in the polar head area deepens into the external leaflet, whereas the peptide initially located deep in the hydrophobic chain area moves towards the head region. For a specific relative distance, the difference between initial d_i and final d_f values goes through 0, and simultaneously the total energy of the system goes through a minimum (c.f. Fig. 7, $Z = 10$ below). Consequently, the penetration depth D of the peptide into the model monolayer is defined as this specific d_f distance.

Peptide global charge Z was modified, either by replacing in the initial models with $Z = 10$, lysine residues by leucine residues, or conversaly. These substitutions were made symmetrically relative to Lys 10 (Fig. 5), in order to maintain the orientation of the hydrophobic peptide momentum [9] as close as possible to the initial one, that is, perpendicular to the fictitious plane separating the ten leucine area from the ten lysine area. Moreover, the range $3 \leq Z \leq 13$ was chosen in order to be sure that the peptide is still capable of insertion into SUV anchored by electrostatic interaction to the phosphate groups and, that the conformation remains helicoidal.

For this range of Z, and starting from the same initial peptide position relative to the surface membrane d_i, the energy variations and the corresponding d_f are displayed in Fig. 6. It is important to note that these d_f are not the penetration depths D defined above, but are indicative of the influence of the charge Z alone, for a fixed value of d_i. The general trend observed is that the more hydrophobic the peptide is, the deeper its insertion into the hydrophobic region of the lipid phase.

In order to get penetration depth estimations, $Z = 5$, 7 and 13 charged peptides were submitted to the same procedure as used for the ten lysine peptide. Figure 7 shows the $d_f - d_i$ difference versus d_f curves. For highly charged lipids $Z = 13$ and 10, the penetration depth is more or less constant, 3 and 3.2 Å, respectively. However, the slope of the curve $d_f - d_i$ for $d_f = D$ exhibits a noticeable decrease: from >10 for $Z = 13$ to ~ 2.2 for $Z = 10$. For the more hydrophobic peptides with $Z = 7$ and 5, the D increases: $D = 3.9$ Å, that is, the peptide dips into the lipid chain region. Moreover, the slope of the curve near D is lower, ~ 1.6 and 1.3, respectively. The physical significance of the slope behaviour could be described in the following terms. Any

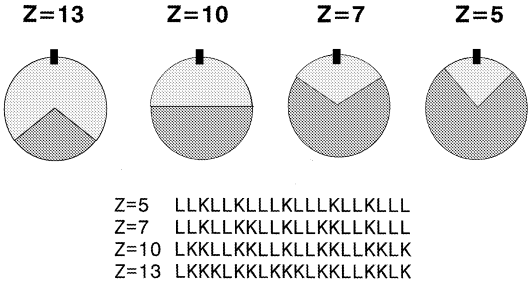

Fig. 5. Sketch of the four charged peptides ($Z = 5$, $Z = 7$, $Z = 10$ and $Z = 13$) with their primary sequences; in dark grey hydrophobic areas, the *vertical bar* position of the lysine 10 conserved residue

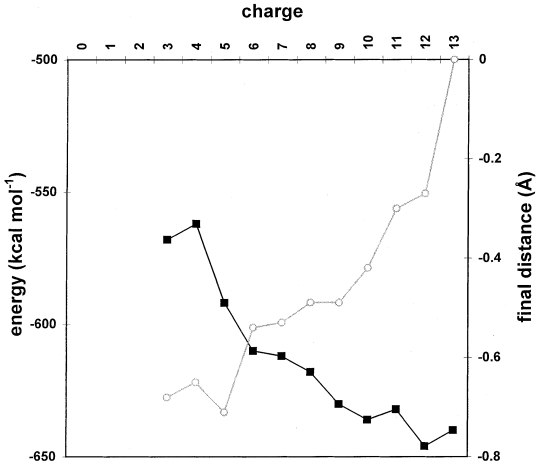

Fig. 6. Energy (■) and final distance d_f (○) versus the peptide charge Z for the same initial relative locations of the peptide d_i. The value $Z = 0$ is only used as a limit, because for entirely hydrophobic peptides, the peptide orientation should be no longer parallel to the monolayer surface

Table 1. Electrostatic contribution to the total energy for peptides with charge Z [energy units kcal mol^{-1}]

Z	$-E_{elec}$	$-E_{total}$	E_{elec}/E_{total}
0	251	533	0.47
3	300	568	0.53
4	309	571	0.54
5	332	592	0.56
6	345	610	0.57
7	353	612	0.58
8	361	618	0.58
9	375	630	0.59
10	383	636	0.60
11	385	632	0.61
12	393	646	0.61
13	397	640	0.62

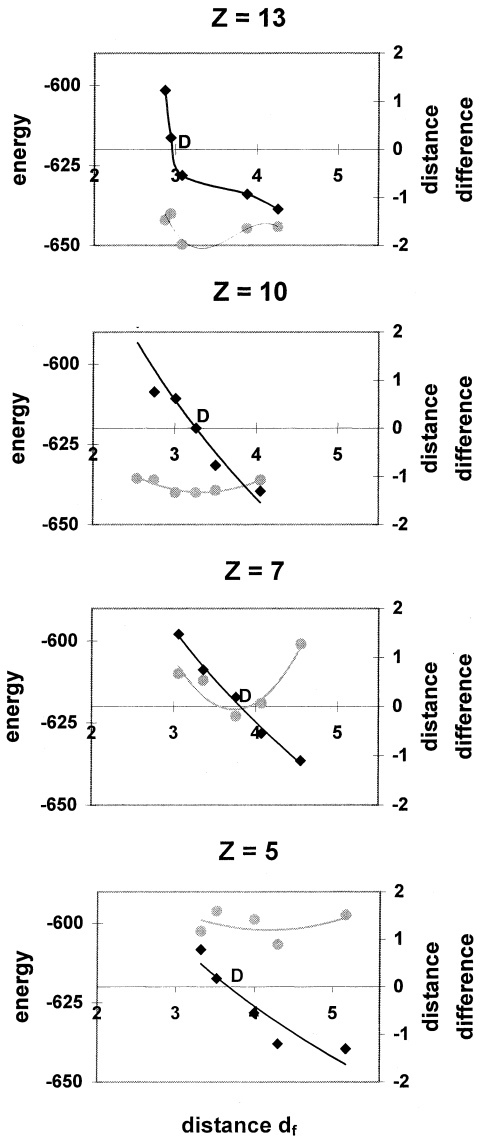

Fig. 7. Energy and distance difference plots versus d_f for $Z = 5$, 7, 10 and 13 peptides: energy in *grey circles*, distance difference in *black diamonds*

disturbance of the lipid organisation would provide various possibilities of penetration into the membrane outer leaflet; whereas highly charged peptides would find an equilibrium location characterised by a mean value of D without much significant deviation, low charged peptide locations would be more widely distributed. This can be related to the more frequent gauche-trans isomerisations near the chain methyl terminals [10].

In order to follow the reequilibration of electrostatic contributions relative to the van der Waals energy for the different charged peptides, the partial electrostatic energy terms together with the total energy are listed in Table 1. It should be noted that the main contribution in the total electrostatic term comes from the interaction between lipid negative phosphate groups and lysine positive amino groups of the peptide: more than 50% for the ten lysine peptide. The fraction of the electrostatic interaction increases with Z.

At the $Z = 0$ limit, the peptide $(Leu)_{20}$ is still inserted in the membrane but no anchoring can justify an orientation parallel to the membrane. Therefore, a complex with the peptide axis parallel to the membrane would always be possible, the total energy being -533 kcal mol^{-1}. For the sake of comparison, another complex with the peptide in a perpendicular orientation was built up; this all-leucine peptide was shortened by half in order to match the monolayer thickness. The energy of such a system is ~ -500 kcal mol^{-1} and should be doubled for a bilayer complex with the 20 amino acid peptide. Consequently, the perpendicular orientation of a single $(Leu)_{20}$ peptide would be energetically favoured [12].

4 Conclusion

Models of amphipathic peptide aggregates in solution were constructed on the basis of the following hypothesis: that the accessibility to the solvent of leucine hydrophobic residues is minimum. In model of monolayers, the amphipathic peptide, oriented parallel to the lipid layer surface, is capable of migrating towards the alkyl chain hydrophobic region. Depending on its charge, the higher charged peptide remains closer to the hydrophilic polar head layer. This shows that the electrostatic anchoring interaction plays a key role in the insertion process; moreover, when this direct electrostatic interaction becomes zero for neutral peptides, the perpendicular orientation should be favoured significantly, by ~ 20 kcal mol^{-1} per residue. Penetration depths were estimated at 3, 3.2, 3.9 and 3.9 Å for $Z = 13$, 10, 7, 5 peptides, respectively. Such estimates have not been possible by fluorescence studies of the quenching by brominated lipid probes because the relative positions of the lipid in close contact with the peptide are conserved because of electrostatic anchoring. This is in accordance with molecular dynamics results on different peptides [12, 13]. We expect that our predicted penetration depth values can be validated by further experimental studies, for example solid phase NMR.

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