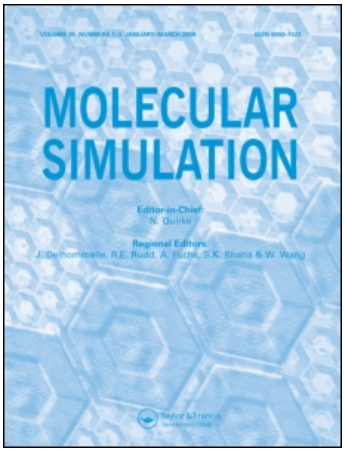

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Yuri A. Kosinsky[a]; Peter V. Dubovskii[a]; Dmitry E. Nolde[a]; Alexander S. Arseniev[a]; Roman G. Efremov[a]
[a] M. M. Shemyakin & Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

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FUSION PEPTIDE INTERACTION WITH LIPID BILAYER: MODELING WITH MONTE CARLO SIMULATION AND CONTINUUM ELECTROSTATICS CALCULATION

YURI A. KOSINSKY*, PETER V. DUBOVSKII,
DMITRY E. NOLDE, ALEXANDER S. ARSENIEV
and ROMAN G. EFREMOV

M. M. Shemyakin & Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Ul. Miklukho-Maklaya, 16/10, Moscow, Russia

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Conformation of 20-residue peptide E5, an analog of the fusion peptide of influenza virus hemagglutinin, was explored by Monte-Carlo technique starting with the fully buried in the membrane ideal α-helix. The lipid bilayer (of 30 Å width) together with surrounding water were modeled by the atomic solvation parameters. During the simulation, residues 2–18 of the peptide retained α-helical conformation, and the peptide was found to be partially immersed into the bilayer. In the resulting low-energy conformers, the N-terminus was buried inside the membrane, its position with respect to the bilayer surface (Z_{NT}) being varied from 2.5 to 7.5 Å, and the orientation of the helical axis relative to the membrane plane (Θ) – from 10 to 35°. The low-energy conformers (below −200 kcal/mol) were clustered in the space (Z_{NT}, Θ) into 4 groups. To select low-energy states of the peptide compatible with NMR data, we calculated pK_a values of E5 ionizable groups and compared them with the experimental values. It was shown that the best correlation coefficient (0.87) and rmsd (0.68 in pH units) were obtained for the group of states which is characterized by $\Theta = 15–19°$ and $Z_{NT} = 3.5–4.5$ Å.

Keywords: Fusion peptides; lipid bilayers; continuum electrostatics

INTRODUCTION

Membrane-fusion activities of the conservative fragments of viral membrane proteins, so called "fusion peptides", consisting of about 20 amino

*Corresponding author. e-mail: ykos@nmr.ru

acid residues (White, 1992), are widely investigated because of their ability to perturb phospholipid bilayer in a way which is strongly dependent upon the peptide sequence. One example is provided by the peptide E5 – a 20-residue water-soluble analog of the fusion peptide of influenza virus hemagglutinin (Murata *et al.*, 1992). It was studied by CD, IR-ATR, and 1H-NMR spectroscopy techniques in complex with DPC micelles pH (Dubovskii *et al.*). At pH $< \sim 6$ the peptide adopts an α-helical conformation. pK_a's of all 5 Glu residues were determined by analysis of the proton chemical shifts of the micelle-bound peptide at different pH (Dubovskii *et al.*).

Understanding of the mechanisms of protein insertion into lipid membrane as well as its stabilization in the membrane-bound state is of a fundamental importance. Because of the difficulties in obtaining experimental structural information on membrane proteins, computer simulations became of a special interest (*e.g.*, Jones and Taylor, 1998; Sansom, 1998). The aim of this study is to assess possible orientations of E5 relative to the lipid bilayer using molecular modeling methods. Recently (Nolde *et al.*, 1997; Efremov *et al.*, 1999; Volynsky *et al.*, 1999) we proposed an implicit solvation model which mimics heterogeneous nature of a membrane. The model was further employed in Monte Carlo (MC) and molecular dynamics (MD) simulations of a number of transmembrane (TM) and membrane-active peptides. The results were compared with the experimental data, and it was shown that the model reproduces fairly well principal trends of peptides' behavior in membrane-like media. Among the crucial questions which could be addressed by the computational methods, we would like to outline the following: what are the possible conformations and energetic characteristics of peptides in the presence of a membrane? Answers to this question will provide an insight into physical mechanism of integral membrane protein folding and will be helpful for design of peptides with predetermined mode of action (*e.g.*, altered fusion or channel-forming activity, *etc.*).

METHODS

MC Simulations

To mimic heterogeneous nature of a membrane, in this work we apply a model which is based on combined employment of atomic solvation parameters (ASP) for water (hydrated headgroups of lipids) and hydrocarbon (acyl chains of lipids) (Nolde *et al.*, 1997; Volynsky *et al.*, 1999).

Potential energy function was taken in the form: $E_{total} = E_{conf} + E_{solv}$. The term E_{conf} includes van der Waals, torsion, electrostatic and H-bonding contributions (Nemethy *et al.*, 1983). E_{solv} is a solvation energy, which is determined by ASP's and related accessible surface area. All-atom protein presentation was used. Recently we have demonstrated (Nolde *et al.*, 1997; Efremov *et al.*, 1999), that interaction of a protein with membrane interior could be adequately modelled using this approximation. ASPs sets imitating both nonpolar hydrocarbon core of a membrane and aqueous solution, were taken from (Nolde *et al.*, 1997). Other details of the membrane model could be found in (Volynsky *et al.*, 1999).

The 20-residue peptide E5 (sequence: GLFEAIAEFIEGGWEGLIEG) (Takahashi, 1990) was built in an α-helical conformation. To change during MC simulation orientation of the peptide with respect to the bilayer, fragment of 12 dummy residues was attached to the N-terminus. First atom of the N-terminal dummy residue was always placed in the center of the bilayer with coordinates (0, 0, 0). MC runs were performed starting from intra-bilayer position of the helix (Fig. 1). Conformational space of the peptide was explored in non-restrained MC simulations in torsion angles space using the FANTOM program (von Freyberg and Braun, 1991). The omega angles were fixed (except N-terminal dummy residues), spherical cutoff 20 Å for nonbonded interactions was used. The simulation length was 5000 MC cycles. At each step, five randomly selected dihedrals were

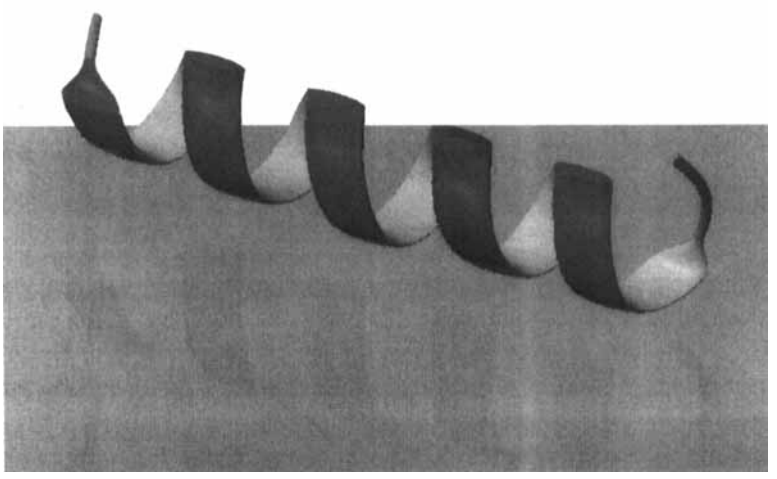

FIGURE 1 Ribbon representation of starting conformer used in Monte Carlo conformational search with membrane-mimetic solvation model. The position of lipid bilayer is shown by light green.

sampled, and the current structure was minimized *via* 100 conjugate gradient iterations. To cross the energy barriers between local minima, adaptive-temperature schedule protocol (von Freyberg and Braun, 1991) was employed. Other details of the simulations could be found in (Volynsky *et al.*, 1999).

Secondary structure, solvent accessible surface area and H-bonding patterns were analyzed using the MOLMOL program (Koradi *et al.*, 1996). Helical angles (Θ) and depths in the membrane (Z_{NT}) were calculated using auxiliary programs specially written for this purpose. Ribbon diagrams of the molecules were produced with the MOLMOL program.

pK_a Calculation

pK_a's were calculated using the MULTIMEAD (Version 1.1.8) program (You and Bashford, 1995). Partial atomic charges and van der Waals radia were taken from the CHARMM (Brooks III *et al.*, 1983) library. The calculations were undertaken with the parameters imitating the conditions used for the NMR experiments: temperature 303 K and zero ionic strength. Dielectric constants (epsilon) for protein or lipid bilayer interior, and aqueous solution were set to 4 and 80, respectively. Numerical solutions of the Poisson-Boltzmann equation were estimated first on external grid (81 points, grid spacing 1.00 Å) with origin in the geometrical center of the peptide and zero electrostatic potential assigned to all boundary points, and next – on internal grid (81 points, grid spacing 0.25 Å) centered on the ionizable group under investigation. pK_a's of Glu residues of E5 were determined by analysis of the proton chemical shifts of the micelle-bound peptide at pH range 4–6 (Dubovskii *et al.*, in preparation) (Tab. IV).

RESULTS AND DISCUSSION

According to the NMR data, the main conformation of E5 represents an α-helix partially buried in the bilayer. To access in more detail this principal state of the peptide (conformations of residues, orientation with respect to the membrane, *etc.*) we have performed non-restrained MC conformational search in torsion angles space.

Resulting low-energy states of E5 could be subdivided into two sets (Tab. I). First of them is characterized by an entire α-helix (Fig. 2a) with N-terminus in the lipid bilayer (3–6 Å from the surface), angle Θ = 10–35°. For the second one, the helix is broken on residue Ile10 (Fig. 2b),

TABLE I Distribution of the α-helical content in E5 peptide

Residue	*% of α-helical conformation (from the 263 conformers)*
2	69.32
3	98.86
4	98.86
5–8	100.00
9	83.33
10	81.82
11–17	100.00
18	98.11
19	26.89

and the N-terminal part is buried in the membrane 4–7.5 Å down from the surface. Most of the low-energy conformers (~82%) belong to the first group. As follows from our previous testing of the solvation model (Efremov *et al.*, 1999; Volynsky *et al.*, 1999), it is reasonable to expect that the major conformational state observed in the experiment, has also low total energy. Therefore, in subsequent analysis we were focused on the states with energies (E) less than -200 kcal/mol (in total 90 of 263 structures). Interestingly, all states from the set-2 (broken α-helix) have $E > -195$ kcal/mol and therefore, were rejected.

The N-terminal depth (Z_{NT}) and angle Θ of the low-energy states are shown in Figure 3. Statistical analysis of these data has revealed four groups of peptide orientations relative to the membrane plane (Tab. IV). To select the native-like orientation from these 4 sets, pK_a values of ionizable groups of the conformers were estimated using a continuum electrostatic model. We propose that a good agreement between measured and calculated pK_a's could serve as a criterion to select the native-like states of E5. Another assumption made is that the pK_a's measured by NMR are determined mainly by the peptide orientation with respect to the membrane, whereas the effects of side-chains flexibility are significant only for Glu11: its accessible surface area anticorrelates well (correlation coefficient, $r = -0.77$) with intrinsic pK_a value (pK_a value calculated without taking into account of other ionizable groups). pK_a value of the C-terminal carboxyl was not measured, but ionic state of this group affects pK_a's of Glu15 and Glu19.

The electrostatic calculations showed the strongly-coupled pairs of ionizable groups in E5 (Tab. II). The interaction between N-terminal amino group and Glu4 carboxylate was excluded because the N-terminus was always neutral due to its buried position within lipid bilayer. Along with the significant electrostatic interaction between neighboring Glu residues the strong coupling was detected between carboxyl groups of Glu15 residue

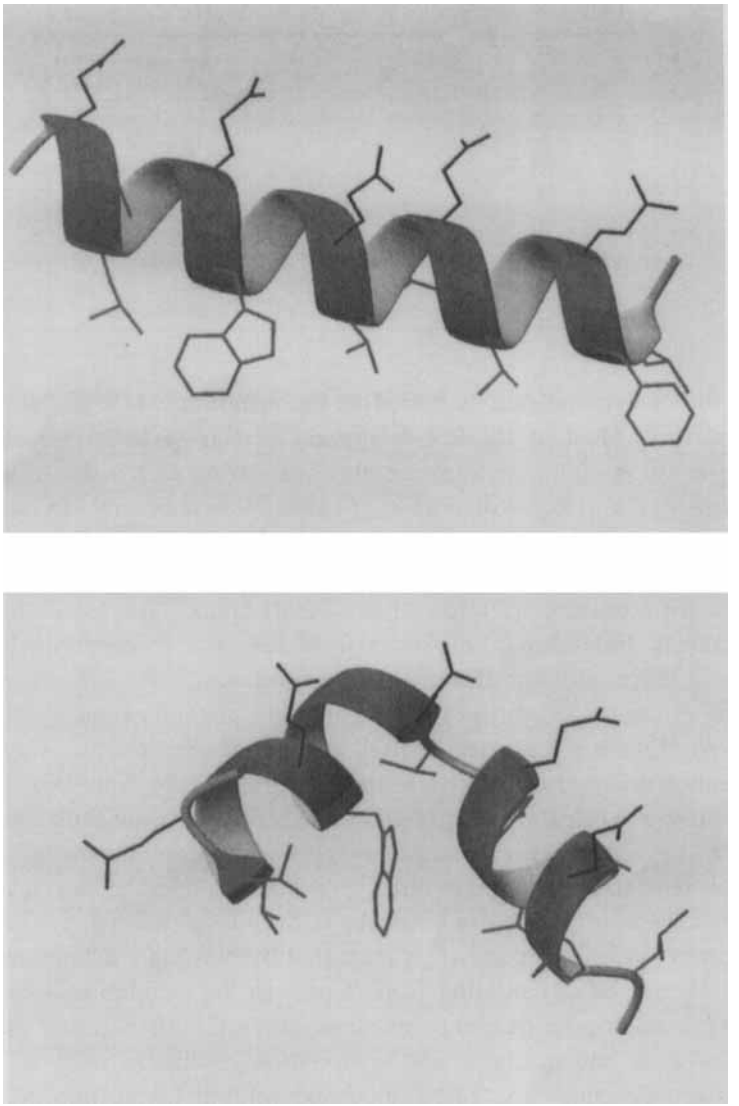

FIGURE 2 Two sets of the conformers observed in MC-search. (See Color Plate XI).

and C-terminus (Tab. II). The high mobility of the C-terminus resulted in the relatively broad range of pK_a values of its carboxyl group. This induced significant varyation in the pK_a values of Glu15 carboxylate. Table III shows that pK_a's of Glu8, 15, 19 have relatively low variation in the set of E5 structures. In addition, pK_a's of Glu11 and 19 are significantly higher then those measured by NMR. The significant anti-correlation

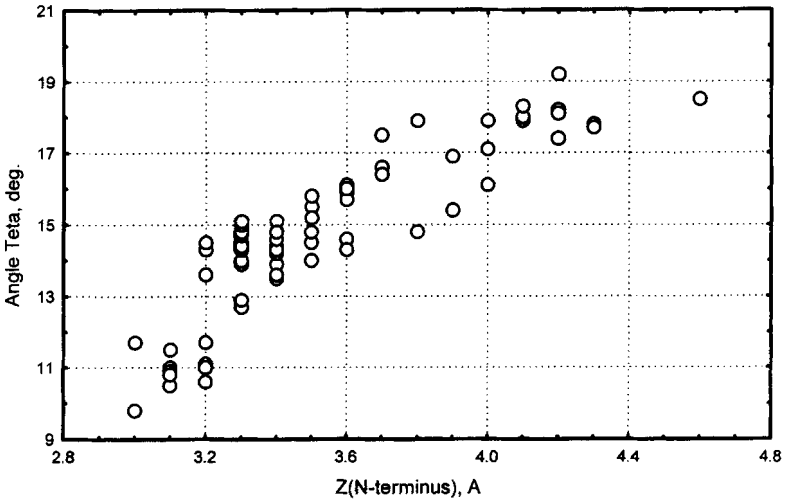

FIGURE 3 Distribution of helix orientation among 90 low-energy conformers.

TABLE II Strongly-coupled pairs of E5 ionizable groups (with the interaction energy exceeding 2.0 (conventional units))

Pairs of ionizable groups	$\langle E \rangle$[1]	σ_E[2]
NT-E4	8.24	7.61
E4-E8	2.74	0.37
E8-E11	2.52	0.50
E11-E15	2.68	0.31
E15-CT20	2.75	1.01
E19-CT20	2.23	0.47

[1] Mean value of E, c. u.
[2] Standard deviation of E, c. u.

TABLE III pK_a values of ionizable groups of E5[1]

Residue	*Intrinsic* pK_a[2]	dpK_a[3]	pK_a	*Experiment*[4]
N-Terminus	-4.689 ± 1.802	0.000 ± 0.000	-4.689 ± 1.802	–
E4	4.542 ± 0.474	0.587 ± 1.482	5.129 ± 0.741	4.2
E8	4.452 ± 0.022	0.805 ± 0.309	5.257 ± 0.300	5.2
E11	5.205 ± 0.603	1.809 ± 0.350	7.014 ± 0.912	5.6
E15	4.561 ± 0.035	1.028 ± 0.374	5.588 ± 0.372	~ 5.2
E19	4.845 ± 0.135	0.793 ± 0.372	5.638 ± 0.395	4.7
C-Terminus	5.744 ± 1.027	1.482 ± 0.599	7.226 ± 1.402	–

[1] Data for 90 low-energy conformations.
[2] pK_a value calculated without taking into account other ionizable groups.
[3] pK_a shifts corrected on site–site interactions.
[4] detected from NMR data (Dubovskii *et al.*, in preparation).

TABLE IV Conformational and electrostatic parameters of E5 ionizable groups (among 90 low-energy states)

Group of states[1]	Z_{NT}[2]	Θ[3]	*E*	pK_a *rmsd*[4]	pK_a *r*[5]
1 (16)	4.1 ± 0.2	17.9 ± 0.5	− 201.8 ± 0.5	0.68 ± 0.20	0.87 ± 0.02
2 (11)	3.7 ± 0.2	16.0 ± 0.5	− 202.7 ± 2.2	0.75 ± 0.38	0.81 ± 0.18
3 (48)	3.4 ± 0.1	14.4 ± 0.4	− 204.6 ± 2.3	1.19 ± 0.26	0.36 ± 0.36
4 (15)	3.2 ± 0.1	11.3 ± 0.9	− 202.1 ± 2.1	0.96 ± 0.45	0.56 ± 0.17

[1] In brackets – number of states;
[2] Z_{NT} – depth of the N-terminus insertion into the membrane (mean value ± standard deviation);
[3] Θ – angle within helical axis and the membrane plane;
[4] rmsd and
[5] *r* – root-mean-square deviation and correlation coefficient, respectively, between calculated and measured pK_a values of five Glu residues of E5.

between *z*-coordinates of C-atoms of carboxyl groups and of their calculated pK_a's allowed us to find out the "main" orientation of E5 helix in the membrane.

As seen in Table IV, the best agreement between the calculated and experimental pK_a values is observed for the groups 1 and 2 of the low-energy states.

To the limitations of the method proposed belong: first, the peptide orientation obtained in the MC-search varies depending on the ionization state of the peptide. In the present work, all ionizable groups of E5, except Glu11, were charged. This corresponds to the experimental conditions employed in the NMR study (Dubovskii *et al.*). Second, we should also note that the spherical shape of micelles was not taken into account in the simulations. In the current model the membrane is planar, and the N-terminus fully buried in the membrane is neutral. This affects the calculated pK_a value of Glu4 carboxylate. In the spherical micelles the E5 N-terminus part might be solvated by water, and therefore, be positively charged.

The principal result of this study implies that the native-like orientation of the E5 helix relative to the membrane plane is characterized by the angle $\Theta = 15-19°$ and by the depth of the N-terminus $Z_{NT} = 3.5-4.5$ Å. This state was selected from numerous possible candidates by the combination of MC simulations with continuum electrostatic calculations.

Acknowledgments

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