Monte Carlo simulations of voltage-driven translocation of a signal sequence

Roman G. Efremov[a,*], Pavel E. Volynsky[a], Dmitry E. Nolde[a], Annemieke van Dalen[b], Ben de Kruijff[b], Alexander S. Arseniev[a]

[a] *M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Ul. Miklukho-Maklaya, 16/10, Moscow V-437, 117997 GSP, Russia*

[b] *Department of Biochemistry of Membranes, Centre for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*

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Abstract Transmembrane potentials play important but poorly understood roles in many biological processes, including signal sequence-mediated protein translocation across bacterial membranes. In this study we applied Monte Carlo techniques to simulate the way the potential acts on a signal sequence. The simulations demonstrate that in the absence of a potential the signal sequence prefers insertion in both helical hairpin and transmembrane α-helical conformations. However, in the presence of a potential only the transmembrane α-helical conformation is the state of lowest energy for the signal sequence. From these results it is concluded that the membrane potential stabilizes the transmembrane orientation of a signal sequence, explaining the membrane potential dependence of preprotein translocation. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Membrane potential; Monte Carlo simulation; Signal sequence conformation; Transmembrane insertion

1. Introduction

Large transmembrane (TM) potentials ($\Delta\psi$) are present across biological membranes. They feed and control many biological processes, ranging from energy conversion and channel gating to protein insertion and translocation. How the potential acts at the molecular level is a mystery because so far no experimental systems exist to directly measure the effect of $\Delta\psi$ on the structure and dynamics of the proteins involved. This calls for the development and application of computational methods. In this study we use Monte Carlo (MC) techniques to analyze voltage-dependent translocation of preproteins across bacterial membranes. Preproteins contain an N-terminal extension, the signal sequence, which is both necessary and sufficient to target the preprotein to the membrane and initiate its translocation. Typically signal sequences are around 20 amino acids long and they lack primary structure homology. They are characterized by a positively charged N-terminus, which anchors the signal sequence to the inside of the membrane, followed by a hydrophobic core region and the signal sequence processing site. Within the core region often a Gly or Pro residue is present which is expected to destabilize an α-helical conformation as has been observed in nuclear magnetic resonance (NMR) studies on micelle-associated signal peptides [1–3]. Fig. 1A illustrates these features for the signal sequence of the well-studied precursor of the *Escherichia coli* outer membrane protein PhoE.

Signal sequences interact with many components in the secretory pathway including the translocase components SecA and SecY and the membrane lipids. Translocation is stimulated by the proton motive force (pmf) which consists of both a $\Delta\psi$ and a ΔpH component. Mutagenesis experiments suggested an involvement of the pmf early in translocation and indicated an electrophoretic effect of $\Delta\psi$ on the signal sequence [4–6]. This suggestion was corroborated by the finding that $\Delta\psi$ could translocate the cleavage site of a prePhoE signal sequence elongated with seven amino acids of the mature sequence (SP+7, Fig. 1A) across a bilayer [7].

We have used the SP+7 in our MC simulations because (1) the results can be directly compared to experimental data, (2) SP+7 includes the N-terminus of the mature sequence which plays a role in the translocation process [5,6] and (3) SP+7 is small enough to be amenable to simulations. To assess the lowest energy states of SP+7 in a membrane environment we used the implicit two-phase solvation model [8,9]. This theoretical membrane model takes into account the hydrophobic membrane interior and the membrane interface and hydration. This model has been proven to well predict the insertion of α-helical and β-structural polypeptides in a membrane environment [8]. The results demonstrate a profound effect of the membrane potential which stabilizes the TM-inserted conformation of the signal sequence.

2. Materials and methods

2.1. The membrane model

The medium represents a continuum described by an effective solvation potential. The membrane is considered as a 'hydrophobic slab' defined by atomic solvation parameters (ASP) for gas–cyclohexane and gas–water transfer, which mimic the hydrophobic membrane core, lipid–water interface, and aqueous solution [8,9]. All-atom potential energy function for protein is taken as follows: $E_{\text{total}} = E_{\text{ECEPP}/2} + E_{\text{solv}} + E_{\Delta\psi}$. The term $E_{\text{ECEPP}/2}$ includes Lennard–Jones, tor-

*Corresponding author. Fax: (7)-095-335 50 33.
E-mail address: efremov@nmr.ru (R.G. Efremov).

Abbreviations: SP+7, the signal peptide of PhoE, elongated with the first seven amino acids of the mature part of PhoE; SP+7 G(−10)L, analog of SP+7 with mutation Gly→Leu at position −10; MC, Monte Carlo; TM, transmembrane; HP, hairpin

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sion, electrostatic, and hydrogen bonding contributions [10]. E_{solv} is the solvation energy:

$$E_{solv} = \sum_{i=1}^{N} \Delta\sigma_i \, ASA_i \quad (1)$$

where ASA_i and $\Delta\sigma_i$ are accessible surface area (ASA) and ASP of atom i, and N is the number of atoms. The values of ASPs are taken from our previous work [11]. Interaction of protein with both aqueous and membrane environment, is given by Eq. 2, where $\Delta\sigma_i$ depends on the z coordinate of atom i (axis z is normal to the membrane plane). It is assumed that apart from the water–membrane interfaces, the values of ASPs correspond to those either for bulk water or cyclohexane:

$$\Delta\sigma_i(z) = \begin{cases} \Delta\sigma_i^{mem} - 0.5\cdot(\Delta\sigma_i^{mem} - \Delta\sigma_i^{wat})\cdot e^{(|z|-z_0)/\lambda}, & \text{if } |z| < z_0 \\ \Delta\sigma_i^{wat} - 0.5\cdot(\Delta\sigma_i^{mem} - \Delta\sigma_i^{wat})\cdot e^{(|z|-z_0)/\lambda}, & \text{if } |z| \geq z_0 \end{cases} \quad (2)$$

where $\Delta\sigma_i^{mem}$ and $\Delta\sigma_i^{wat}$ are ASPs for an atom of type i in aqueous (wat) or hydrophobic (mem) environments, respectively; z_0 is a half-width of the membrane (i.e. the hydrophobic layer is restricted by the planes given by the equation $|z| = z_0$); $D = 2z_0$ is the membrane thickness (30 Å), λ is a characteristic half-width of the water–membrane interface (in this study $\lambda = 1.5$ Å). The effect of TM potential $\Delta\psi$ was modelled by the term [12]:

$$E_{\Delta\psi} = (F\Delta\psi/D) \sum_{i=1}^{N} q_i \, z_i \quad (3)$$

where q_i and z_i are partial charge and coordinate z of atom i, and F is Faraday's constant. For $|z_i| > D/2$, $\psi(z) = \text{constant}$.

2.2. MC simulations

Conformational space of peptides was explored via unrestrained variable-temperature MC search in torsion angles space using the modified FANTOM program [13]. At each MC step the structures were minimized via 50–120 conjugate gradient iterations. To explore during the simulations all possible orientations of SP+7 with respect to the membrane, 14 N-terminal dummy residues were attached with the first one placed in the center of the hydrophobic layer with coordinates (0,0,0). The dummy residues do not contribute to the energy of the system. All dihedral angles were sampled, except angles ω in 'real' residues. The step of variation of each dihedral was chosen randomly in the range −180°–180°. Non-bond interactions were truncated with spherical cutoff of 25 Å. Electrostatic interactions were treated with distance-dependent dielectric permeability $\varepsilon = 4 \times r$. The choice of the dielectric screening model was discussed in detail elsewhere [11]. Before MC simulations, the structures were subjected to 100 cycles of conjugate gradient minimization. Acceptance of the MC states was done according to the Metropolis criterion [14]. To cross the energy barriers between local minima, the adaptive-temperature schedule protocol [13] was employed. The peptides' termini, as well as all ionizable groups, were taken in their charged states. In the absence of $\Delta\psi$, several independent MC simulations were performed for SP+7 and its Leu mutant with the following starting structures arbitrary placed in aqueous phase: (1) random coil; (2) entire α-helix; (3) α-helical hairpin (HP) with the helix break at position −10 of the sequence. Other details of the computational protocols have been described elsewhere [8,9]. MC simulations with applied TM voltage (100 mV) were carried out for both peptides starting with the membrane-bound lowest energy TM and HP states found with $\Delta\psi$ = 0 mV, as well as with the α-helix arbitrarily placed in aqueous phase. To address the convergence problem in MC search, several independent simulations with different seed numbers were carried out for each peptide. It is important to note that the sets of low energy states obtained for each peptide in all the simulations are quite similar in total energy and its individual terms, structure, and mode of membrane binding. This provides strong reasons to believe that the essential sampling of the peptides' conformational space was reached in each MC simulation.

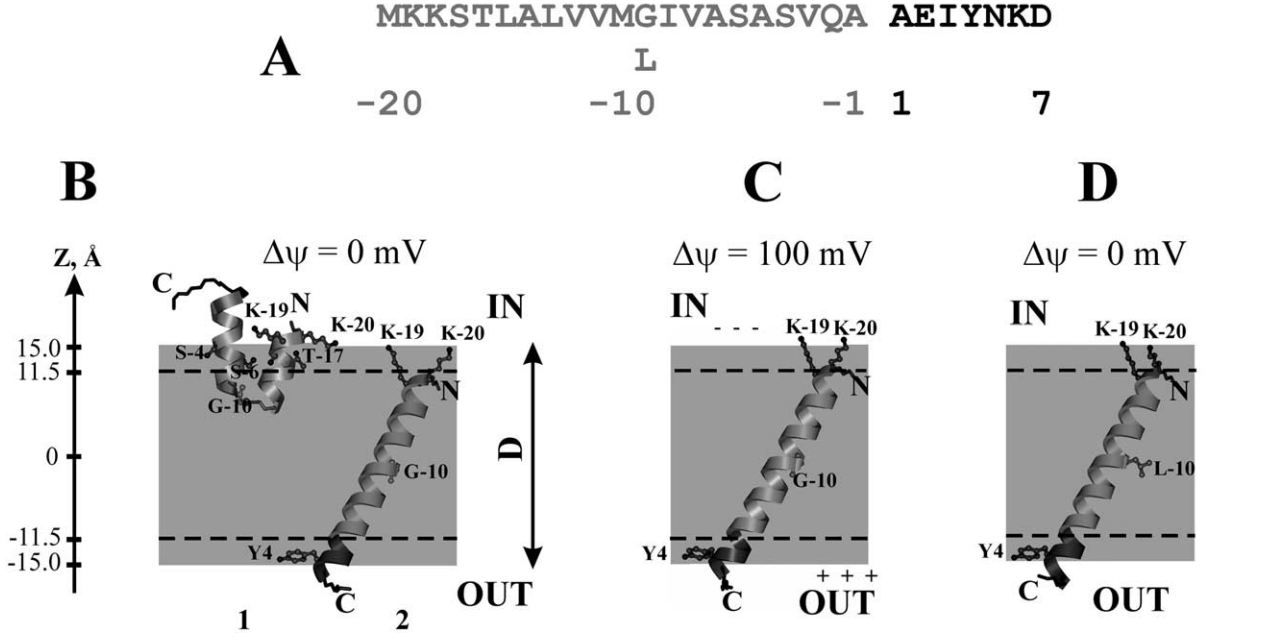

Fig. 1. Insertion of the signal sequence in a heterogeneous membrane model with and without a transmembrane potential. A: Sequence of SP+7, the signal peptide of PhoE elongated with the first seven amino acids of the mature protein. The replacement of Gly for a Leu at position −10 as in the SP+7 G(−10)L mutant is indicated. B–D: Results of MC conformational search for SP+7 and SP+7 G(−10)L mutant peptide. Only the lowest energy HP-like and/or TM states are drawn. The peptide backbone is given in ribbon drawing. Side chains of residues on the water–membrane interface and Gly −10 or Leu −10 are shown in ball-and-stick presentation. The seven amino acids of the mature sequence of PhoE are shown in bold. The membrane (with thickness $D = 30$ Å) is shown in gray. The z-axis shows the dimensions of the hydrophobic layer and the interfaces separated by dashed lines. Positively and negatively charged sides of the membrane are shown with symbols '+ + +' and '− − −', respectively. B,C: Results of MC conformational search for SP+7 without ($\Delta\psi$ = 0 mV) and with an applied transmembrane voltage ($\Delta\psi$ = 100 mV), respectively. D: Results of MC conformational search for the SP+7 G(−10)L mutant peptide in the absence of a transmembrane potential.

3. Results and discussion

3.1. MC simulations of SP+7 in the absence of $\Delta\psi$

In the absence of a TM voltage the simulation revealed two very different energetically most favored states of the peptide inserted into the membrane. These are illustrated in Fig. 1B and the corresponding energies and some structural parameters are summarized in Table 1. One represents an α-helical HP state (state 1) with the N-terminal lysines at position −20 and −19 and the C-terminal serines −4 and −6 anchoring the peptide at the same membrane interface denoted IN. The mature part is fully in the aqueous phase and is unstructured towards the C-terminus. The helical segments contain residues −19 to −11 and −8 to +2 and are inserted almost perpendicularly (74° and 86° respectively) into the membrane. The second low energy state is a TM α-helix with the positively charged side chains of lysines −20 and −19 'snorkeling' to the IN interface and the tyrosine +4 anchoring the peptide at the OUT side of the membrane (Fig. 1B, 2). In this orientation the processing site (between −1 and 1) is in the OUT interface where the active site of the leader peptidase, the enzyme that removes the signal sequence during or shortly after translocation, is located [15]. The helix is tilted with an angle of 58° to the plane of the membrane.

There is just a small energy difference between the HP and TM states with the helical HP conformation being only 0.4 kcal/mol more stable. It should be noted that the conformations shown are representative (with the lowest energy) of two large families of related structures. Both α-helical HP and TM states exist over wide energy ranges and corresponding structures might have minor differences in geometry of insertion, hydrogen bonding and so forth. Furthermore, the energy ranges characteristic for the two states overlap, suggesting that both structures could be present in equilibrium. Other states found via the MC search revealed a wide variety of secondary structures but all of them are at least 15 kcal/mol higher in energy. The occurrence of most favored HP and TM states was independent of whether the peptide was initially present in water or in the membrane or whether the starting conformation of the peptide was a random coil, a TM helix or an HP conformation (with a break around glycine −10).

The reason that the drastically different HP and TM states have similar total energies is the result of different counteracting effects. The TM α-helix is more stabilized due to a larger number of intra-helix hydrogen bonds, while the HP structure has additional side chain interactions between the two aligned α-helices, resulting in additional stabilizing van der Waals interactions and inter-helix hydrogen bonds (such as between Ser −18 and Ser −6, Fig. 1B, 1). Interactions of SP+7 with the membrane interface and hydrophobic layer are much more favored for the TM state, but are counterbalanced by the more favorable interactions with the aqueous phase of the HP states (data not shown).

3.2. Effect of $\Delta\psi$ on SP+7

Introducing the potential energy function describing $\Delta\psi$ into the simulations resulted in a dramatically different result. In the presence of a $\Delta\psi$ of 100 mV in the direction as it occurs in the *E. coli* inner membrane (positive out) leads to a substantial decrease in energy of the TM α-helix which now becomes the lowest energy state of SP+7 (Fig. 1C, Table 1). The helix is inserted very similar to the situation in the absence of the potential (compare Fig. 1B, 2 with 1C). Strikingly, the potential only causes a marginal decrease in the total energy of the HP conformations of the signal sequence such that the TM orientation is preferred by more than 6 kcal/mol over the lowest energy state HP conformation. It is important to note that the voltage-dependent energy term ($E_{\Delta\psi}$) is small with respect to the total energetics of the membrane association of SP+7 and therefore can act to trigger switching of the signal sequence from HP to TM states. The implication of this finding for protein translocation is that the positive TM potential across the inner membrane can switch the HP conformation of the signal sequence to a TM conformation, thereby initiating translocation of the N-terminus of the mature protein across the membrane. The action of $\Delta\psi$ on the signal is ascribed to the non-uniform distribution of charges along the sequence with the positively charged N-terminus (Lys −20, −19) and one positive (Lys +6) and two negative (Glu +2 and Asp +7) charged residues in its C-terminus. The direction of the potential (positive OUT) is such that it will favor the localization of the N-terminus of the mature sequence on the OUT side of the membrane. Interestingly, evidence is available that the negatively charged residues at the extreme N-terminus of mature proteins play a role in the potential dependence of preprotein translocation [6].

3.3. Simulations of the SP+7 G(−10)L mutant with and without $\Delta\psi$

In vivo and in vitro experiments have shown that mutating Gly −10 into α-helix promoting residues strongly reduced the membrane potential dependence of prePhoE translocation with the largest effects being observed for the Gly to Leu substitution [4]. We reasoned that this might be caused by an increased tendency to adopt the TM state, thereby bypassing the requirement of $\Delta\psi$ to stretch the signal sequence. MC calculations on the SP+7 G(−10)L mutant in the absence of the potential showed indeed that the lowest energy state cor-

Table 1
Characteristics of the lowest energy states of SP+7 and its Leu mutant as obtained from MC simulations in the absence and presence of a membrane potential

Peptide	$\Delta\psi$ = 0 mV				$\Delta\psi$ = 100 mV			
	State	Residues in α-helix	θ[a]	E_{total} (kcal/mol)	State	Residues in α-helix	θ	E_{total} (kcal/mol)
SP+7	TM	−20 to +5	58°	−294.1	TM	−20 to +5	60°	−302.6
	HP	−20 to −12	74°	−294.5	HP	−20 to −12	76°	−296.3
		−9 to +2	86°			−9 to +2	79°	
SP+7 G(−10)L	TM	−20 to +6	59°	−301.2	TM	−20 to +6	61°	−306.4
	HP	−20 to −12	74°	−298.5	HP	−20 to −12	72°	−300.2
		−9 to +6	58°			−9 to +6	57°	

[a] θ = angle between α-helical segment(s) and the membrane plane.

responded to a TM α-helix (Fig. 1D, Table 1). This helix was slightly more extended than that of the wild-type but otherwise had a very similar mode of insertion. The energetically closest HP state had a 2.7 kcal/mol higher energy. Statistical analysis of the outcome of several MC runs demonstrated that the number of low energy TM states of the Leu mutant was much higher than that of the HP states, the reverse being true for the wild-type peptide (data not shown). This strongly suggests that in the absence of a potential the signal sequence of the Leu mutant prefers to insert directly into a TM orientation, thereby initiating translocation of the mature part. In the presence of a potential of 100 mV the preference to acquire a TM orientation for the Leu mutant is even further increased (Table 1).

This study addresses the question why the membrane potential stimulates preprotein translocation across bacterial membranes. The MC computational approach demonstrates that the membrane potential exerts a direct effect on TM insertion of a signal sequence.

The results are in excellent agreement with the in vivo and in vitro data [4–6] and with studies in which the $\Delta\psi$ dependence of translocation of signal sequences was tested in a model system [7]. The MC results further explain the previously observed two states of insertion of signal sequences in lipids [16] and the conformational flexibility of the signal sequence as observed in NMR experiments [3].

The results lead to the following model for the initial stages of preprotein translocation. The signal sequence of the preprotein is delivered by SecA to the translocase and inserts there as a helical HP into the membrane possibly at an interface between the TM parts of SecY, SecE and the membrane lipids. The TM potential now stretches the signal sequence which drags the N-terminus of the mature protein into the aqueous channel of the translocase thereby initiating protein translocation.

The present study demonstrates the feasibility of studying by MC techniques the effect of a TM potential on membrane insertion of a polypeptide. It opens the way to get insight into the many effects that the potential has on different membrane processes and the membrane proteins involved.

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References

[1] Rizo, J., Blanco, F.J., Kobe, B., Bruch, M.D. and Gierasch, L.M. (1993) Biochemistry 32, 4881–4894.
[2] Wang, Z., Jones, J.D., Rizo, J. and Gierasch, L.M. (1993) Biochemistry 32, 13991–13999.
[3] Chupin, V., Killian, J.A., Breg, J., de Jongh, H.H., Boelens, R., Kaptein, R. and de Kruijff, B. (1995) Biochemistry 34, 11617–11624.
[4] Nouwen, N., de Kruijff, B. and Tommassen, J. (1996) Mol. Microbiol. 19, 1205–1214.
[5] Lu, H.M., Yamada, H. and Mizushima, S. (1991) J. Biol. Chem. 266, 9977–9982.
[6] Geller, B., Zhu, H.Y., Cheng, S., Kuhn, A. and Dalbey, R.E. (1993) J. Biol. Chem. 268, 9442–9447.
[7] van Dalen, A., Killian, J.A. and de Kruijff, B. (1999) J. Biol. Chem. 274, 19913–19918.
[8] Efremov, R.G., Nolde, D.E., Volynsky, P.E. and Arseniev, A.S. (2000) Mol. Simul. 24, 275–291.
[9] Efremov, R.G., Volynsky, P.E., Nolde, D.E., Dubovskii, P.V. and Arseniev, A.S. (2002) Biophys. J. 83, 144–153.
[10] Némethy, G., Pottle, M.S. and Scheraga, H.A. (1983) J. Phys. Chem. 87, 1883–1887.
[11] Efremov, R.G., Nolde, D.E., Vergoten, G. and Arseniev, A.S. (1999) Biophys. J. 76, 2448–2459.
[12] Gazit, E., Miller, I.R., Biggin, P.C., Sansom, M.S.P. and Shai, Y. (1996) J. Mol. Biol. 258, 860–870.
[13] von Freyberg, B. and Braun, W. (1991) J. Comp. Chem. 12, 1065–1076.
[14] Metropolis, N., Rosenbluth, A.W., Teller, A.H. and Teller, E. (1953) J. Chem. Phys. 21, 1087–1092.
[15] Dalbey, R.E. and Wickner, W. (1985) J. Biol. Chem. 260, 15925–15931.
[16] Batenburg, A.M., Demel, R.A., Verkleij, A.J. and de Kruijff, B. (1988) Biochemistry 27, 5678–5685.