The gramicidin A channel

The energy profile calculated for Na^+ in the presence of water with inclusion of the flexibility of the ethanolamine tail

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The effect of the conformational freedom of the ethanolamine tail of gramicidin A on the energy profile for the transfer of Na^+, computed in the presence of water, shows an appreciable lowering of the minimum at 10.5 Å, and a splitting of the entrance barrier. The deep energy region at the channel mouth remains, however, the deepest one and contains a site of strong interaction of the ion with the Trp 11 carbonyl, at about 12 Å from the center.

Gramicidin A *Energy profile* *Ethanolamine end* *Water* *Na^+* *Theoretical computation*

1. INTRODUCTION

We undertook, a few years ago, theoretical calculations of the energy profile felt by cations in the gramicidin A channel [1–5], proceeding by successive introduction of the essential structural elements of the channel (polypeptide backbone, ethanolamine tails and amino acid side chains) in view of a delineation of their respective roles.

These studies, carried out initially in vacuo, with extremely suggestive results, have recently been extended by the explicit inclusion of water into the system [6]. In this last treatment the ethanolamine tails of the gramicidin A skeleton were, however, maintained rigid in their most stable conformation. In view of significant effects found in vacuo by allowing for the flexibility of the tail [2], we have presently included this flexibility in the calculation of the energy profile of the gramicidin-cation system in water.

The principle of our approach is the same as that adopted in our previous papers [1–6] and consists of the computation of the variations of the optimal energy of the system along the progression of the ion. In the present study, as in [6], the total system comprises the gramicidin A dimer (GA), the sodium ion and 22 molecules of water.

The dimer is assumed, as before, to be in Urry's head-to-head structure [7,9], with the conformations of the side chains as described in [10] and the ethanolamine tail $NHCH_2CH_2OH$, attached to C_α (Trp 15), initially fixed in its intrinsically preferred conformation [2] (fig.1). The flexibility of the tail is obtained by varying the torsion angles ϕ_1, ϕ_2, ϕ_3, around the single bonds NC, CO and OH, respectively ($\phi_1 = C_\alpha NC_1C_2$, $\phi_2 = NC_1C_2O$, $\phi_3 = C_1C_2OH$). The most stable conformation of the tail computed in vacuo [2] corresponds to $\phi_1 = 80°$, $\phi_2 = 65°$, $\phi_3 = 200°$ and is stabilized by two hydrogen bonds, one between the hydroxyl oxygen and the NH hydrogen of the Trp 11 residue and one between the hydroxyl hydrogen and the carbonyl oxygen of the same residue.

For the study of its progression, the cation is placed in successive planes perpendicular to the channel axis and its position in the plane as well as the positions of all the water molecules and the conformation of the tails are optimized

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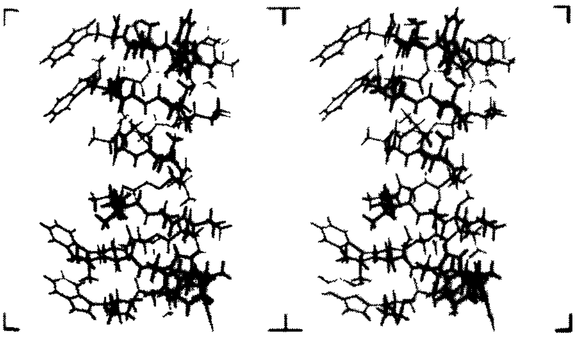

Fig.1. The left-handed helical head-to-head dimer of GA (stereo view). The ethanolamine tails are shown in their most stable conformation [2] (the side chains are in the conformation of [10]).

simultaneously. The energy formulation being described in details elsewhere [11] we only recall here that it involves the calculation of intra- and intermolecular energies as a sum of electrostatic, Lennard-Jones, hydrogen-bonding, torsion and polarization components. The process of energy optimization is performed, as in [6], with the help of the 'BFGS conjugate gradient' minimizer requiring the analytical energy derivatives (see [11] for details).

It is useful to partition the intermolecular energy of the system GA-water-Na^+ into interactions involving its different parts, namely GA with the water molecules, $E_{GA\text{-}w}$, the water molecules among themselves, $E_{W\text{-}W}$, the water molecules with the ion, $E_{W\text{-}Na^+}$, and GA with the ion, $E_{GA\text{-}Na^+}$. The conformational energy is defined as the difference Δ between the intramolecular energy E_1 obtained for the different conformations adopted by the tail during the progression of the cation and the corresponding energy E_2 obtained with the tail fixed. With these conventions, the energy of a given configuration of the system (GA-Na^+-22 water molecules) is written as:

$$E_{tot} = E_{GA\text{-}Na^+} + E_{GA\text{-}W} + E_{W\text{-}W} + E_{W\text{-}Na^+} + E_{pol} + \Delta$$

It is the variation of this energy that will be followed upon the progression of the ion. Owing to the considerable cost of the calculations, we have limited our investigations to the region extending from the mouth of the channel to the distance of 9 Å from the center, studies in vacuo [3] having shown that the conformational flexibility of the tail does not affect appreciably the energies beyond this limit due to its inability to follow the ion further.

2. RESULTS AND DISCUSSION

We present in fig.2, the variation of E_{tot} upon the progression of the ion (curve A), in comparison to the corresponding variation of the energy of the same system obtained with the tail fixed (curve B). As explained above, curve A rejoins curve B after 9 Å.

Concerning the effect on the energy profile of the labilization of the ethanolamine tail in water,

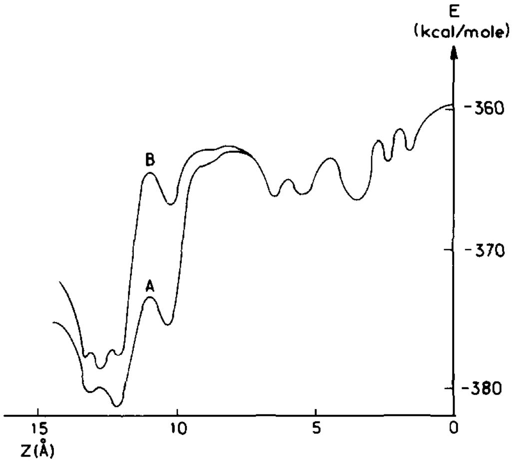

Fig.2. The total interaction energy (kcal/mol) of the system GA-Na^+-water: curve A, computed with the flexible tail; curve B, with the tail fixed in the conformation of fig.1. Distances in Å from the center. Only half of the profile is given.

Table 1

Components of the total energy for different equilibrium positions of the cation along the channel

Z	$E_{GA\text{-}W}$		$E_{W\text{-}W}$		$E_{W\text{-}Na^+}$		$E_{GA\text{-}Na^+}$		E_{INTER}		Δ	ϕ_1	ϕ_2	ϕ_3	r, θ^a OH...O=C Trp 11		r, θ^a HO...HN Trp 11	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)								
14.0	−112.6	−114.0	−85.1	−83.0	−137.0	−144.0	−31.0	−14.9	−385.0	−374.0	9.6	118	−61	184	2.1	46	2.7	11.2
13.2	−114.5	−115.0	−87.5	−84.7	−135.3	−141.2	−32.2	−18.4	−388.5	−377.8	8.5	126	−52	215	2.2	62	2.2	6
12.0	−123.1	−120.8	−94.7	−95.5	−126.7	−127.9	−19.8	−13.0	−386.0	−377.5	4.5	132	−48	−85	2.6	83	2.0	0
11.1	−125.0	−124.0	−100.0	−101.9	−102.0	−101.0	−30.1	−13.8	−379.0	−364.0	6.1	128	−51	−88	2.7	71	2.2	5
10.3	−118.0	−117.0	−119.0	−121.0	−83.0	−80.0	−34.0	−24.0	−378.3	−366.7	3.1	129	−42	−80	2.6	64	2.0	4
9.1	−117.0	−115.0	−126.0	−126.0	−70.0	−71.0	−25.0	−23.0	−365.1	−363.0	1.2	75	64	178	2.1	49	2.1	7

[a] θ is the angle (XH, XY) in the XH...Y hydrogen bond and r is the distance H...Y

Distances (Z) in Å. Energies for mobile (A) or fixed (B) tails in water are given in kcal/mol. Angles in degrees, the most stable conformation has $\phi_1 = 80°$, $\phi_2 = 65°$, $\phi_3 = 200°$

which is the main subject of this note, the comparison of the two curves leads to the following conclusions: (i) as in vacuo, the labilization produces a general lowering of the energy profile; (ii) the region of deepest energy remains located between 13 and 12 Å, with some qualitative differences, in particular the modification of the order of the heights of the local minima inside it; (iii) the minimum observed in curve B at about 10.5 Å from the center is still present at the same distance and is appreciably lowered, remaining, however, higher in energy than the region 13–12 Å; (iv) consecutive to this lowering, the high and steep energy barrier of 15 kcal/mol observed in curve B is not split into two, the first part amounting only to 8 kcal/mol.

The origin of the modifications observed in the energy profile can be clarified by considering the components of the energy together with the corresponding values of the three dihedral angles defining the conformations of the tail in case A (table 1). Also given are the two distances which join, at each step, the hydroxyl proton with the carbonyl oxygen of Trp 11 and the hydroxyl oxygen with the corresponding NH proton. The polarization energy has been omitted from table 1, because no difference between cases A and B has been observed. (This term regularly increases upon the progression of the cation in the channel in the two cases.)

The energy components fall into two groups: on the one hand, $E_{\text{GA-W}}$ and $E_{\text{W-W}}$, very similar in cases A and B, indicating that the introduction of the flexibility of the tail has little effect on the global interactions of the water molecules among themselves or with the channel. On the other hand, the lability of the tail has an appreciable effect on the interactions of the ion with GA and with water: in particular $E_{\text{GA-Na}^+}$ shows a gain of 10–20 kcal/mol whereas $E_{\text{W-Na}^+}$ decreases, although by a smaller quantity and only at the beginning of the path. As a result, the total interaction energy is improved throughout the curve. At the same time, the energy loss, Δ, which accompanies the departure of the tail from its preferred conformation, is the largest at the entrance and decreases upon the progression of the cation.

Both the gain in interaction energy and the (smaller) energy loss Δ are associated with the movements of the tail: the values of the ϕ angles indicate that the tail has left its preferred conformation and recovers it only at about 9.5 Å. The successive changes are accompanied by deformations of its hydrogen bonds with GA (see table 1 for distances and angles) until about 9.5 Å where the initial bonds are practically reformed. These movements occur so as to enable the best possible interaction of the hydroxyl oxygen with the ion, as can be seen in table 2 by comparing the corresponding O-Na$^+$ distances at the optimal position in each plane for cases A and B: the labilization of the tail allows a closer approach to the OH oxygen at each step, thereby improving $E_{\text{GA-Na}^+}$. Until about 12 Å, this is obtained at the expense of the interaction between Na$^+$ and water; in other words the mobile tail prevents the water molecules from interacting optimally with the cation. On the other hand, water decreases the amplitude of the movement of the tail as can be inferred from a comparison of the present data with those obtained in vacuo [2].

The decomposition of the steep desolvation barrier observed in curve B [6] into two parts in curve A is due to the interplay of the Na$^+$-water/hydroxyl interactions, the first part corresponding essentially to the loss of water molecules, the second to the loss of the hydroxyl influence and of the residual water interactions, until only two water molecules interact directly with the ion (see [6] for a detailed discussion).

Table 2

Distances (Å) between the cation and the hydroxyl oxygen at optimal positions in different planes perpendicular to the channel axis

Z	(A)	(B)
14.0	3.9	4.6
13.5	4.0	4.7
13.2	4.0	4.6
12.7	3.8	4.1
12.3	3.0	3.3
12.0	2.9	3.1
11.5	2.2	3.3
11.1	2.5	3.4
10.3	2.2	2.7
9.9	2.2	3.0
9.5	4.0	4.0
9.1	4.2	4.2

(A) Tail mobile in water; (B) tail fixed in water. *Z* is the distance of the plane from the channel center

A final important result of the present study concerns the location and depths of the minima observed in curve A, as well as the evolution of the distances of the ion to the individual carbonyl oxygens along its progression (table 3): a close interaction is observed with O(Trp 13) from 14 to 12.7 Å, then with O(Trp 11) down to 11.1 Å, finally with O(Trp 9) until 9 Å. The deepest energy minimum about 12 Å corresponds to the interaction with Trp 11, the next one to the interaction with Trp 13 and the next one (at 10.5 Å) to the interaction with Trp 9. These results can be correlated with those of the NMR measurements of ^{13}C chemical shifts by Urry et al. [12], indicating a large ^{13}C shift for the carbonyl of Trp 11, a less important shift for that of Trp 13 and a still smaller shift for that of Trp 9. The relative depths of the local minima indicate the same order of decreasing interaction.

3. CONCLUSIONS

Altogether, the effects brought about in the energy profile in water by the labilization of the ethanolamine tail of GA are qualitatively similar to those seen in vacuo [3–5], and consist essentially of an appreciable lowering of the 10.5 Å minimum due to an improved interaction of the ion with the hydroxyl group. This minimum was the deepest one in vacuo. The explicit introduction of water in the calculations [6] has allowed the detection of a region of deep energy at 12–13Å, preceding a steep desolvation barrier which was, of course, impossible to locate in vacuo. The present calculations show that this region remains largely the deepest one upon labilization of the tail which, however, modifies the order of the local inner wells and splits the 'entrance' barrier into two. The analysis of the relative location and depths of the final minima, together with that of the location and steepness of the respective in- and out-barriers seems to indicate that the 'site' of the ion inferred on the basis of ^{13}C NMR measurements [12], which appeared identifiable with the 10.5 deepest minimum computed in vacuo, corresponds rather to the minimum minimorum observed at 12 Å from the center. The overall shape of the profile is largely governed by the presence of water and the desolvation process.

Table 3

Distances (R in Å) between the cation and the nearest carbonyl oxygens of GA at optimal positions in different planes perpendicular to the channel axis

Z	R	Residue
14.0	2.4	L Trp 13
13.5	2.0	L Trp 13
13.2	2.2	L Trp 13
12.7	2.3	L Trp 13
12.3	2.5	L Trp 11
12.0	2.3	L Trp 11
11.5	2.1	L Trp 11
11.1	2.1	L Trp 11
10.3	2.3	L Trp 9
9.9	2.2	L Trp 9
9.5	2.2	L Trp 9
9.1	2.2	L Trp 9

Z (Å) is the distance of the plane from the channel center

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