# The gramicidin A channel

## Role of the ethanolamine end chain on the energy profile for single occupancy by $Na^+$

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Received 8 March 1984

The inclusion of the presence and flexibility of the $CH_2CH_2OH$ end chain in the computation of the energy profile for single occupancy by $Na^+$ of the gramicidin A channel modifies substantially the profile obtained without that chain. The binding site (deepest minimum) in the profile is situated at 10.5 Å from the center of the channel, in satisfactory agreement with the conclusions based on $^{13}C$-NMR studies. The existence of an external minimum at the mouth is confirmed.

*Gramicidin A* *Ethanolamine end* *Energy profile* *Sodium* *Single occupancy* *Theoretical computation*

## 1. INTRODUCTION

In the first paper of this series [1], we have reported the energy profile computed for $Na^+$ in the channel formed by the gramicidin A (GA) helical backbone assumed to be in Urry's head-to-head dimeric structure [2–4], taking into account all the terms in the theory of intermolecular interactions. In this first study, the model adopted for the channel was the entire polypeptide structure of GA including the formyl heads but without the side chains. The end $CH_2OH$ group of the ethanolamine chain, the conformation of which was not known, was not considered in the computations either. Here, we investigate the possible effect of this terminal chain on the energy profile for $Na^+$, allowing complete conformational freedom to the $CH_2CH_2OH$ chain.

## 2. STANDPOINT AND METHOD

In the absence of precise structural information, it was first necessary to determine by computation the most stable conformation of the $CH_2CH_2OH$ end with respect to the GA backbone (kept rigid) in the absence of the cation. Then an $Na^+$ cation was introduced in successive planes perpendicular to the channel axis and regularly spaced, and in each plane the cation was allowed to find its most favorable position while, at the same time, the $CH_2CH_2OH$ end was allowed to reoptimize its conformation.

The computational procedure utilized is the same as in [1] and consists [5] of the evaluation of the binding energies as a sum of terms: electrostatic, polarization, repulsion, dispersion and charge transfer, parametrized on accurate ab initio calculations, themselves tested on experimental energy measurements on small systems. The same technique was recently extended in our laboratory [6] to the evaluation of the variations in energy brought about by rotations around single bonds. For this purpose the molecule is divided into appropriate subunits, whose wave functions can be easily computed [7].

Here, each monomer of GA was con-

*Published by Elsevier Science Publishers B.V.*
00145793/84/$3.00 

structed from 7 dipeptide subunits, -CONHCH$_2$CONHCH$_2$-, the eighth one being -CONHCH$_2$CONHCH-, with the bond lengths, angles and dihedral angles previously used [1] and given in [8]. The CH$_2$CH$_2$OH terminal group is built out of 3 fragments, two -CH$_2$-, and one -OH, with standard bond lengths and angles. As in [1] the side chains have been omitted and the conformation of the molecule was maintained rigid except for that of the CH$_2$CH$_2$OH group. Complete flexibility of this tail was allowed by performing rotations about the single bonds NC, CC, and CO using the three torsion angles, $\phi_1$, $\phi_2$, $\phi_3$ defined in fig.1.

In order to evaluate the effect of the CH$_2$CH$_2$OH tail on the energy profile for $Na^+$, we proceeded as follows:

(i) The most stable conformation of the ethanolamine end with respect to the backbone was determined in the absence of the ion.

(ii) The ion was introduced as said above in planes from 16 to 0 Å (in steps of 0.5 Å, between 16 and 10 Å, and in steps of 1 Å between 9 and 0 Å), from the center of the channel and allowed to reach its optimal position in every plane while simultaneously reoptimizing the conformation of the tail with respect to the cation and the GA backbone.

In each of the optimal configurations we get the global interaction energy $E_g$ for the system backbone–tail–$Na^+$. It comprises the interaction energy of $Na^+$ with the backbone, *NaB*, the interaction energy of $Na^+$ with the tail in the corresponding conformation, *NaT*, and the energy of interaction, *TB*, of the tail in that conformation with the polypeptide backbone. This can be written:

$$E_g = NaB + NaT + TB = E_{Na} + TB \quad (1)$$

If we call $T_oB$ the interaction energy of the tail with the backbone in its most stable conformation in the empty channel, the quantity:

$$E = E_g - T_oB = E_{Na} + \delta(TB) \quad (2)$$

represents the energy of the system at each step, including the variation in energy $\delta(TB)$ brought about by the change in the conformation of the tail. Within the approximations adopted, $E$ may be considered as the energy profile accompanying the progression of the ion, when the tail is allowed conformational freedom.

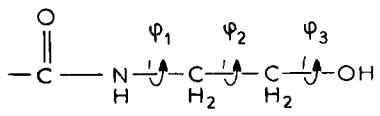


Fig.1. Torsion angles defining the changes in the conformation of the ethanolamine end chain.

## 3. RESULTS AND DISCUSSION

The most stable conformation found for the ethanolamine end with respect to the backbone in the absence of the cation corresponds to the set of torsion angles $\phi_1 = 80°$, $\phi_2 = 65°$, $\phi_3 = 200°$. Examination of the corresponding distances and angles indicates that this structure is stabilized by two hydrogen bonds, one, noted 1, involving the hydrogen of the hydroxyl group and the carbonyl oxygen of Trp11, the other, noted 2, involving the oxygen of the hydroxyl group and the hydrogen of the Trp11 N-H group. The distances and the angles defining these two hydrogen bonds are given in table 1 and the corresponding structure is illustrated in fig.2 where the hydrogen bonds are indicated by dotted lines (only a part of the molecule is represented).

When the cation is present, at the entrance or in the channel, and allowed to reach its preferred position while it progresses along it, the ethanolamine end changes its conformation in order to interact at best with the cation through its hydroxyl oxygen. Different conformations of the tail are observed at each step of the progression.

Table 1

Distances and angles for hydrogen bonds 1 and 2 in the most stable conformation of the ethanolamine end without the cation

| Distance (Å) | | Angle (degrees) | |
|---|---|---|---|
| $d_1$ | 2.04 | O-H....O | 151.63 |
| $d_2$ | 2.01 | O....H-N | 172.50 |

$d_1$ and $d_2$ are the distances H-O in $H_{hydroxyl}$....O=C (Trp11) and O-H in $O_{hydroxyl}$....H-N (Trp11), respectively

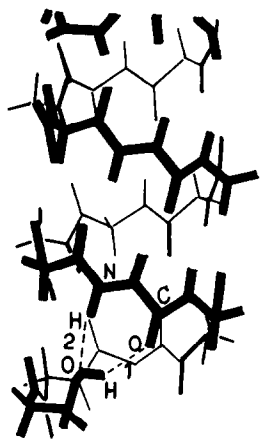


Fig.2. Diagrammatic representation of the most favourable conformation of the ethanolamine end chain in the absence of the cation.

The evolution of these conformations can be followed by the variations in the values of the optimal dihedral angles $\phi_1$, $\phi_2$, $\phi_3$ (table 2) at every step, or by considering the fate of the hydrogen bonds 1 and 2 as indicated for instance by the distances $d_1$ between the hydroxyl hydrogen and the oxygen of the carbonyl group of Trp11, and $d_2$ between the oxygen of the hydroxyl group and the hydrogen of the NH bond of Trp11, respectively (fig.3, curves 1 and 2): this shows that when the cation is in the neighborhood of the entrance of the channel, the two hydrogen bonds 1 and 2 are disrupted, but when the cation is inside the channel and advances along it, progressive re-formation of the two hydrogen bonds is observed. Fig.3 shows that hydrogen bond 1 is formed first when the ion is at about 14 Å from the center of the channel, the second hydrogen bond is only observed when the cation is about 12.5 Å from the center. From that point until 9 Å, hydrogen bond 1 is lengthened but remains present. From 9 Å to 0 Å, the two hydrogen bonds are simultaneously observed, the tail having recovered its intrinsic preferred conformation as in the absence of the cation. The different values of the torsion angles $\phi_1$, $\phi_2$, $\phi_3$, given in table 2 show clearly that only small changes in the conformation of the tail are required during the progression of the cation in zone I except when the cation is before the entrance, namely between 16 and 15 Å.

Table 2

Values of the torsion angles $\phi_1$, $\phi_2$, $\phi_3$ for each position of the cation in the first zone ($Z$ is the distance to the center of the channel)

| $Z$ | $\phi_1$ | $\phi_2$ | $\phi_3$ |
|---|---|---|---|
| 16.0 | 50.9 | −110.6 | −78.7 |
| 15.5 | 78.3 | −68.8 | 149.3 |
| 15.0 | 90.3 | −61.0 | 148.0 |
| 14.5 | 102.0 | −62.0 | 155.6 |
| 14.0 | 113.1 | −61.8 | 162.6 |
| 13.5 | 114.5 | −60.6 | 176.8 |
| 13.0 | 119.5 | −60.2 | −174.9 |
| 12.5 | 122.2 | −57.7 | −167.3 |
| 12.0 | 133.4 | −63.8 | −140.9 |
| 11.5 | 134.1 | −61.6 | −140.3 |
| 11.0 | 134.0 | −60.0 | −121.6 |
| 10.5 | 126.9 | −43.8 | −120.0 |
| 10.0 | 125.9 | −37.7 | −116.1 |
| 9.0 | 78.1 | 62.4 | 195.2 |
| 8.0 | 78.2 | 58.9 | 191.0 |

The variations in energy accompanying the ion progression are represented in fig.4 where the two curves A and B represent, respectively, the energy profile $E$ defined by eq.2 above, and the sole sodium interaction energy $E_{Na}$.

Curves A and B present two different zones I and II marked on the figure, the first one extending from 16 to about 9 Å, the second one from

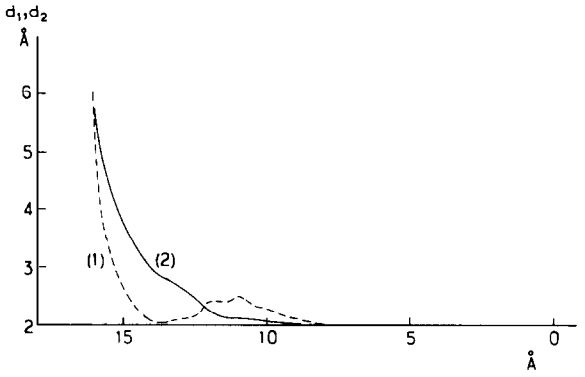


Fig.3. Representation of the two hydrogen bonds 1 and 2 between the tail and the atoms of the gramicidin polypeptide backbone. Curve 1, distance between the hydroxyl hydrogen and the Trp11 carbonyl oxygen, curve 2, distance between the hydroxyl oxygen and the hydrogen of the Trp11 N-H group, for each position of the cation along the channel.

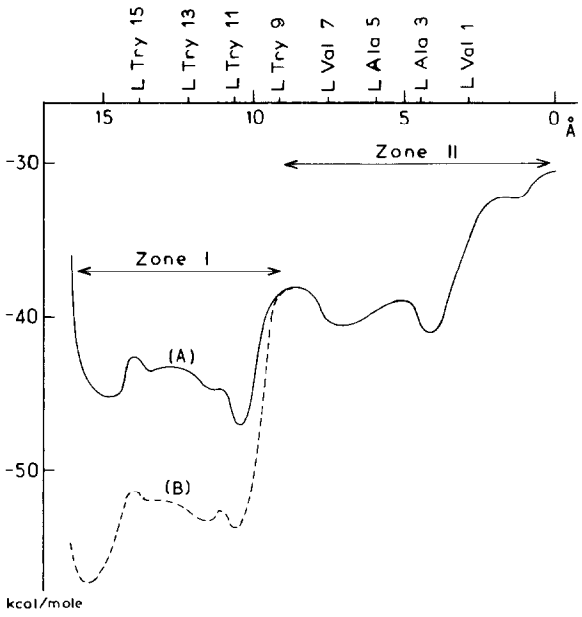


Fig.4. Curve A, energy profile $E$ from 16 Å to the center of the channel for single occupancy of the gramicidin channel. Curve B, interaction energy of $Na^+$ with the tail and backbone, $E_{Na}$ (see text for definitions). Energies in kcal/mol, distances in angstroms. The location of the carbonyl oxygens for the L residues is indicated for convenience.

9 Å to the center of the channel. In the first zone, curves A and B are clearly distinct, while they become practically coincident in zone II. This evolution reflects the effect of the variations in the conformation of the tail described above which become practically negligible when the ion reaches 9 Å and beyond. The energy profile $E$ is obviously strongly affected by the presence of the $CH_2CH_2OH$ end at the early stage of the ion progression.

The second zone is characterized by an energy profile less favourable than the previous one. This fact is essentially due to the loss of the interaction of $Na^+$ with the hydroxyl oxygen. At 9 Å, although the hydroxyl group is relatively far from the cation, its effect is not entirely negligible as can be seen by comparison with the corresponding profile obtained in [1] but this effect fades slowly towards the center, the two profiles practically coinciding from 4 Å up to the central barrier.

The comparison of the results obtained here and which include the contribution of the presence and flexibility of the whole ethanolamine end with those obtained previously in which the effect of this end was neglected, demonstrates the very significant influence of the present extension. Thus the comparison of curve A of fig.4 with curve B of fig.1 in [1] shows an appreciable difference in the evolution of the energy profile. If we divide the trajectory of the cation into three portions, entrance (16–14 Å), penetration (14–9 Å), and progression (9–0 Å), one observes a strong deepening of the energy profile in the first two sections, to the point that these sections are now lower in energy than the third one, in contrast to the situation observed in the absence of the effect of the $CH_2CH_2OH$ end.

The minimum observed at the mouth of the channel is now 14 kcal/mol deeper than previously. A particularly significant result is, furthermore, the appearance of the minimum minimorum at about 10.5 Å from the center. A small barrier separates this principal site from the secondary one at the entrance.

It may be noted that the present results correlate very satisfactorily with a number of recent experimental observations. Thus the location of the binding site at 10.5 Å from the center, which in [1] was within the binding zone found for double occupancy but rather far from that found for single occupancy, corresponds very closely to the location deduced in [9] from ion-induced $^{13}C$-chemical shifts of the carbonyl carbons. Furthermore, the existence of an external site (first minimum) for $Na^+$ is confirmed and the relative depths of this external site and of the inner site for $Na^+$ are in agreement with the conclusions reached in [10].

## ACKNOWLEDGEMENT

The authors wish to thank N. Gresh for letting them use his SIBFA program before publication.

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