# The gramicidin A channel: the energy profile for single and double occupancy in a head-to-head $\beta^{6,3}_{3,3}$-helical dimer backbone

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The energy profile for $Na^+$ in the channel formed by the gramicidin A $\beta$-helical dimer backbone was computed introducing all the terms in the theory of intermolecular interactions. The effect of allowing the ion to reach its successive optimal positions shows the presence of a series of energy minima associated with different carbonyls. The presence of a second ion lowers the central barrier for the first one and facilitates its progression and exit. The energy profile for double occupancy indicates the presence of two symmetrical minima at about 13 Å from the center.

*Gramicidin A* *Energy profile* *Sodium* *Channel* *Theoretical computation*

## 1. INTRODUCTION

Gramicidin A is the best characterized prototype of an ion-transmembrane channel [1–3], providing an ideal model for understanding, at the microscopic level, the mechanisms involved in channel transport. A fundamental problem in this field is the determination of the energy profile felt by ion(s) inside the channel. Previous theoretical calculations of this quantity have considered essentially the Coulomb interaction [4–9] of the ion(s) with an array of dipolar ligand groups, arranged [7,8] so as to mimic the disposition of the peptide carbonyls of gramicidin A along the $\beta^{6,3}_{3,3}$-helical dimeric structure [3,10] which seems the most likely one for the channel in membranes and phospholipids [11].

We try to go beyond such simplified models by using: (i) a more precise geometry of the channel backbone, generated from the structural data proposed in [3]; (ii) a significantly more refined method for calculating ion-channel intermolecular interactions [12,13]. We present here the results of such computations for single and double occupancy by $Na^+$ of a head-to-head $\beta$-helical dimer.

## 2. METHODS

The procedure, described in [12], and applied successfully to the study of the complexing selectivity of valinomycin [14], nonactin [15], and calcimycin [16], computes the binding energy as a sum of terms: electrostatic, polarization, repulsion, dispersion and charge transfer, the parameters of which have been chosen so as to reproduce the results of accurate ab initio calculations on small systems, themselves tested on experimental energy measurements. The macromolecule is built [17] from appropriate subunits whose wave functions can be easily computed. Thus each monomer of gramicidin was constructed from 8 dipeptide subunits, -$HCONHCH_2CONHCH_2$-, with alternate L and D conformations at the $C^\alpha$ carbons using the $\phi,\psi$ dihedral angles of reference [3], with standard bond length and angles. The orientation of the second monomer was obtained so as to form 6 ap-

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propriate CO...HN intermolecular hydrogen bonds and minimizing geometrically the repulsion between the two formyl hydrogens. In this exploratory study, the side chains have not been introduced explicitly, nor the terminal $CH_2OH$, the conformation of the dimer was maintained rigid and the role of water has not yet been considered.

The energy profile for a sodium ion in the channel was computed in two steps:

(i) Constraining the ion to remain on the channel axis, computing the ion-channel interaction energy at an array of successive points in steps of 0.5 Å;

(ii) Allowing $Na^+$ to optimize its position in a plane perpendicular to the axis at every previous point. The resulting energy profiles are given as curves A and B of fig.1.

Double occupancy by two $Na^+$ ions was considered in two ways. First the effect on the energy of the first ion entered, $I_1$, of a second ion, $I_2$, placed at various spots along the axis was computed. The corresponding profiles for 8 locations of $I_2$ are given in fig.2. Then, in view of the evaluation of the energy profile for double occupancy the global interaction energy, $\Delta E$, was computed in the system gramicidin A plus two ions placed in symmetrical positions on the axis. From this value the interaction energy $E$ of one of the ions with the channel in the presence of the other is obtained as $(\Delta E - I_1I_2)/2$ where $I_1I_2$ is the ion–ion repulsion, and the total energy felt by each ion is $E + I_1I_2$. This quantity plotted as a function of the position of the ions gives the energy profile of fig.3. In these computations, the two ions have been constrained to remain on the axis.

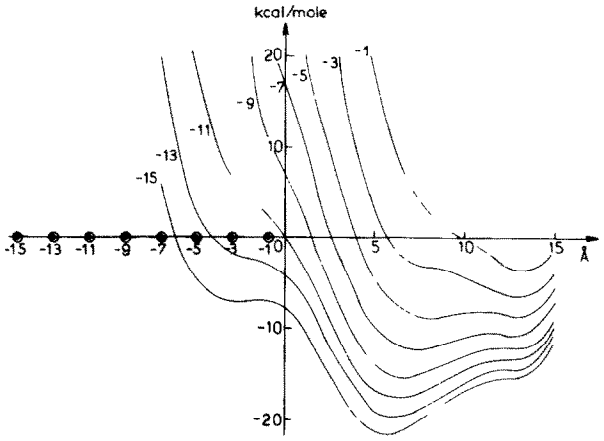


Fig.2. The energy of $I_1$ moving along the axis under the influence of a second ion, $I_2$, at a fixed position. Each curve is labeled according to the position of $I_2$ marked by a correspondingly labeled dot. Units as in fig.1.

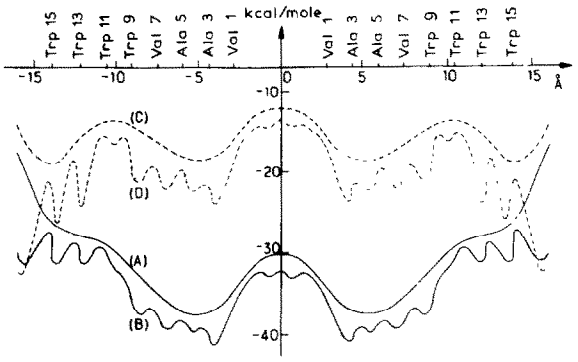


Fig.1. Energy profile for single occupancy by $Na^+$ of the gramicidin A channel. (A) Ion constrained to remain on the channel axis; (B) ion allowed to reach its preferred position at each point (see text); (C) pure electrostatic component corresponding to curve A; (D) pure electrostatic component corresponding to curve B. Energies in kcal/mol, distances in angstroms. The location of the carbonyl oxygens for the L residues is indicated for convenience.

## 3. RESULTS AND DISCUSSION

### 3.1. *Single occupancy*

Curve A of fig.1 indicates that the energy profile for one ion constrained to remain on the axis presents two symmetrical minima with a central relatively small barrier, two higher barriers at the exterior of the channel near its mouths, and two inflexion points inside the channel close to the ends.

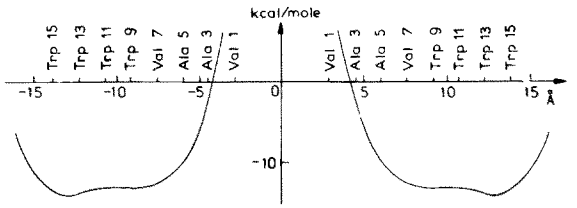


Fig.3. The energy profile for symmetrical double occupancy by $Na^+$ ions constrained to the channel axis. Units as in fig.1.

When the ion is allowed to find its preferred position at each step while progressing along the channel, local minima and maxima appear in the energy profile, bringing about an appreciable overall lowering in energy near the extremities. The first minimum (−31.4 kcal/mol) is external to the channel, with $Na^+$ bound to the carbonyl oxygen of $Trp_{15}$ at 2.02 Å from it, slightly towards the axis. This value, which corresponds to single binding of the ion to the most external carbonyl (in the presence of the whole molecule) is 5 kcal/mol more negative than the corresponding energy of single binding of $Na^+$ to water, computed by the same method. The location of a minimum just outside the channel entrance and of an inner deeper one agrees with the conclusions reached in [3] in the framework of their 3B4S model.

Worth noting is the fact that the profile obtained upon introducing all terms in the energy calculation differs appreciably from that deduced on the basis of the Coulomb component alone (curve C,D, fig.1). A more detailed analysis of the curves and their interrelations will be given elsewhere.

### 3.2. *Double occupancy*

The curves of fig.2 indicate the effect of the presence of a second ion on the energy profile of the first one: the most striking feature of these curves is the disappearance of the central energy barrier, a disappearance already practically achieved when $I_2$ is at −15 Å from the center, close to the entrance. For this location of $I_2$ an appreciable (although less deep than for $I_1$ alone) minimum remains at first on the right side of the center. When $I_2$ progresses, the minimum for $I_1$ becomes less and less negative and glides towards the exit while the barrier to exit becomes gradually smaller. Thus, upon progression of the second ion, the first one will have a tendency to move along the channel to reach its successive optimal positions and finally to leave. One notes that when $I_2$ reaches about −6 Å from the center, the position of $I_1$ becomes quasi-indifferent between +7 and +14 Å.

As for the energy profile for double occupancy (fig.3) it is characterized by the presence of two symmetrical energy wells separated by a high central barrier. The absolute minimum is at ±13 Å from the center and a quite flat region extends between ±13 and ±8 Å. It is satisfying that the location of these minima is in good agreement with the indications deduced from ion-induced $^{13}C$ chemical shifts of the carbonyl carbons in the gramicidin channel, which point to the involvement of the Trp 9, 11 and 13 carbonyls in the binding of $Na^+$ [18]. A more precise location will be obtained by allowing the ion to depart from the channel axis.

## 4. CONCLUDING REMARKS

The present exploratory study gives, for the first time, the energy profile computed for a sodium ion in a model of the gramicidin channel built with the entire structure of the backbone as in [3], and without neglecting any of the components of the intermolecular interactions. The smooth profile obtained when constraining the ion to remain on the channel axis is modified when the constraint is released, so as to present successive local minima clearly associated with binding to successive carbonyls. This preference of sodium for departing from a central position in order to reach a more favorable one has already been found in valinomycin [14]. It remains to be seen whether and how the suggested libration of the carbonyls [10] can help the passage over the intermediate barriers observed.

The location of the minima obtained in the energy profile for double occupancy is quite gratifying in view of the simplifications made here. It seems to indicate that the main features of the field in the channel are dominated by the effect of the backbone. We are presently engaged in an examination of the effect of the other elements present, particularly of water.

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