The gramicidin A channel: comparison of the energy profiles of Na^+, K^+ and Cs^+

Influence of the flexibility of the ethanolamine end chain on the profiles

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The energy profiles for single occupancy by Cs^+, K^+ and Na^+ in the gramicidin A channel assumed to be in a head-to-head $\beta^{6.3}_{3.3}$ helical dimeric structure, were computed: (A) allowing complete conformational freedom to the ethanolamine end, (B) constraining it to stay in its intrinsically preferred conformation. Whatever the constraint, both the entrance barrier and the central barrier appear in the order $Cs^+ < K^+ < Na^+$. Introducing the flexibility of the tail modifies appreciably the profiles and the location of the extrema along it.

Energy profile *Gramicidin A* *Ethanolamine end* *Caesium* *Potassium* *Sodium* *Energy barrier* *Theoretical computation*

1. INTRODUCTION

In [1,2] we reported computations of the energy profile for Na^+ in the channel formed by the gramicidin A (GA) helical backbone assumed to be in the head-to-head dimeric structure proposed in [3–5]. In [1], the energy profile for single and double occupancy by Na^+ was computed in the model made of the entire polypeptide chain including the formyl heads, but omitting the side chains and the terminal CH_2OH group of the ethanolamine end, the conformation of which was not ascertained. In a second step [2], owing to recent developments in the methodology [6], we were able to study the role of the ethanolamine chain on the energy profile for single occupancy by Na^+. Introducing explicitly the CH_2CH_2OH end in the model, we optimized its conformation with respect to the rest of the GA backbone, then computed the energy profile following the progression of Na^+, allowing the tail to adopt its preferred conformation with respect to the GA backbone and to the cation. The inclusion of the presence and flexibility of the CH_2CH_2OH end chain modified substantially the profile obtained without that chain.

Here, we report an extension of our computations to two other representative alkali metal ions, K^+ and Cs^+, and compare the results to those obtained for Na^+. The energy profile for each cation was computed in two ways: (A) allowing complete conformational freedom to the CH_2 chain as in [2]; (B) constraining the tail to remain in its intrinsically preferred conformation with respect to the empty GA backbone.

The computational procedure was described in detail in [6,7] and summarized in [2]. The parameters used for Na^+, K^+, Cs^+ were given in [8]. Each cation was introduced in successive planes perpendicular to the channel axis, from 16.5 Å to the center of the channel (in steps of 0.5 Å), and allowed to reach its optimal position in the plane. In calculations (A), complete flexibility of the tail was allowed as in [2] by rotations around the single bonds, NC, CC, CO, using the 3 single

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torsion angles ϕ_1, ϕ_2, ϕ_3, around these bonds and a reoptimization of the conformation of the tail with respect to the cation and the GA backbone was done at each step. In calculations (B) the angles were fixed at the optimal values (80, 65, 200°) found for the empty channel and only the position of the cation was optimized at every step. In (A), the energy profile is defined as:

$$E = E_{ion} + \delta(TB)$$

comprising the interaction energy E_{ion} of the ion with the (backbone + rearranged tail) channel, and the loss of energy δ(TB) accompanying the rearrangement of the tail. In calculations (B) only the interaction energy of the ion with the (backbone + fixed tail) channel intervenes.

2. RESULTS AND DISCUSSION

To compare the influence of the nature of the cation on the results, we have reported in the same figures and table the outcome of the calculations for the 3 cations.

2.1. *Flexible tail*

The conformational changes of the tail along the progression of the ion can be followed by the evolution of the dihedral angles ϕ_1, ϕ_2, ϕ_3 and by the transformations undergone by the two hydrogen bonds (O hydroxyl - - - HN Trp 11) and H hydroxyl - - - O = C Trp 11) denoted 1 and 2, respectively, in [2] and which characterize the most favorable conformation of the tail in the empty channel. It is observed that for the 3 cations, the ethanolamine end changes its conformation to interact most favorably with the ion through its hydroxyl oxygen. At every step, the 3 angles for Cs^+ and K^+ (to be published) are in the same range as those obtained for Na^+ (see [2]). As observed in the case of Na^+, the two hydrogen bonds 1 and 2 are disrupted when the cation is at the entrance of the channel and progressively reformed when the cation progresses (fig.1). Hydrogen bond 1 is formed first when the ion is about 15–14 Å from the center of the channel, the second hydrogen bond 2 being formed only when the cation is between 13 and 12.5 Å, in the 3 cases. From this point onwards, hydrogen bond 1 is lengthened but is still present and from 9 to 0 Å the two hydrogen bonds are simultaneously present, the tail being in its intrinsically preferred conformation. Comparison of fig.1a,b and fig.2 of [2] indicates small differences according to the cation but a very striking global behavior.

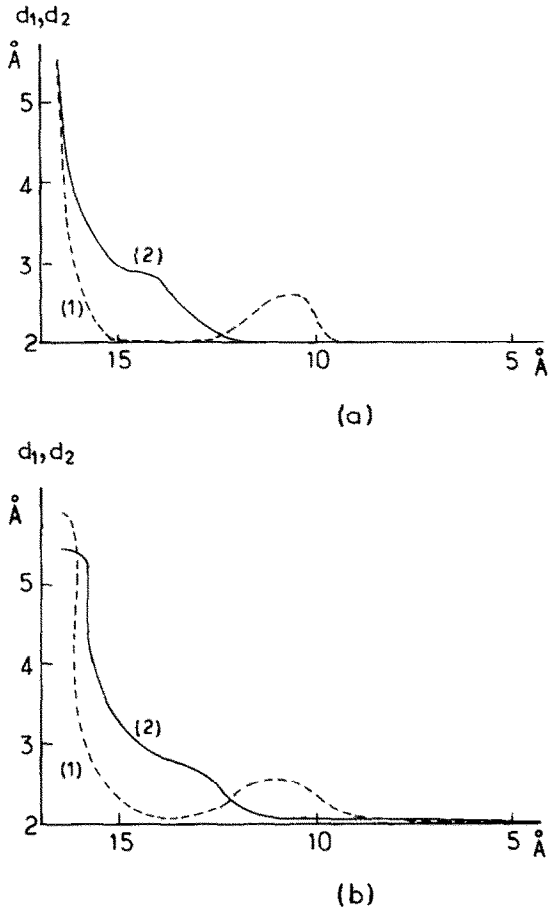

Fig.1. Evolution of the two hydrogen bonds 1 and 2 between the tail and the atoms of gramicidin backbone: (curve 1) distance between the hydroxyl hydrogen and the carbonyl oxygen of Trp 11; (curve 2) distance between the hydroxyl oxygen and the N-H nitrogen of Trp 11, for each position of the cation along the channel. (a) Cs^+, (b) K^+.

The corresponding energy profiles are given in fig.2 where we distinguish as previously two zones, marked I and II (from 16.5 to 9.5 Å and from 9.5 Å to the center, respectively).

In zone I the 3 curves are very different: the deepest one (Na^+) presents two conspicuous minima at 15 and 10.5 Å, the latter being somewhat deeper. The highest curve is that for Cs^+ which descends rapidly towards zone II with only

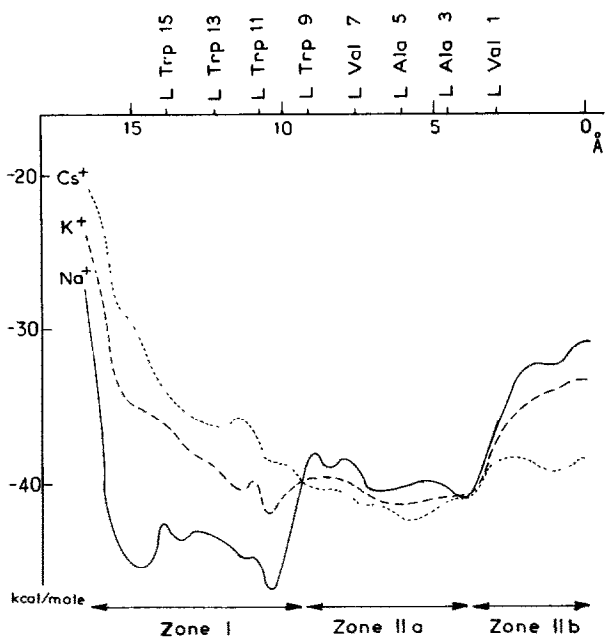

Fig.2. Energy profile for Cs^+ (···), K^+ (– – –), Na^+ (——) for single occupancy of the gramicidin channel when the tail is flexible. Energies in kcal/mol, distances in Å. The location of the carbonyl oxygens for the residues is indicated for convenience.

two very shallow minima separated by a very small barrier. The corresponding profile for K^+ is intermediate between the other two curves with the local minimum at 10.5 Å being more pronounced than for Cs^+. Clearly the interaction with the backbone and its tail is favored for the smallest cation in zone I and is the least favorable for the largest one. The order of the interaction energies is in relation to the size of the cation. Nevertheless, the profiles for K^+ and Cs^+ are similar, quite parallel, and distinct from that of Na^+. In all of zone I, the cations interact with the different carbonyl oxygens of the L residues and the hydroxyl oxygen of the tail as can be seen by the evolution of the distances between the cation and the hydroxyl oxygen given in fig. 3 and of the distances between the cation of the different carbonyl oxygens (to be published).

In zone II, the tail has practically returned to its intrinsically preferred conformation. The most striking feature of zone II is that the energy profile of Cs^+ has now become the deepest in energy with the minimum at 6 Å from the center, corresponding to the interaction of Cs^+ with the L Ala 5 and L Ala 3 carbonyl oxygens. The profile for Na^+ is now the highest in energy. This corresponds to the loss of the interaction with the hydroxyl oxygen (see fig.3) at 9 Å; throughout its progression in this zone, Na^+ interacts strongly essentially only with one carbonyl oxygen. Although Cs^+ also loses the interaction with the hydroxyl oxygen, its distance to this atom increases less rapidly than for Na^+, and furthermore, owing to its size and to the diameter of the channel, it can interact with more than one carbonyl oxygen at a time. This explains why zone II is more favorable for Cs^+ than zone I whereas the reverse occurs for Na^+. For K^+, the profile is intermediate between the profiles for Cs^+ and Na^+, with no abrupt change in the energy between the two zones but rather a smooth local ascent.

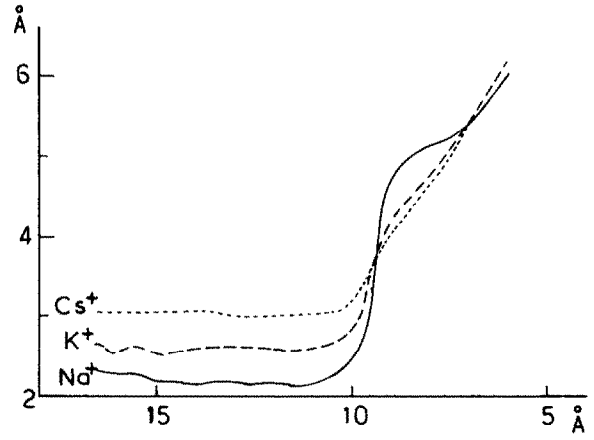

Fig.3. Distances between Cs^+ (···), K^+ (– – –) and Na^+ (——) and the hydroxyl oxygen between 16.5 and 7 Å.

Apart from this general behavior, the evolution of the 3 profiles in zone II indicates a subdivision into two regions, IIa from 9.5 to 4 Å, and IIb from 4 Å to the center. At 9.5 Å the 3 curves intersect and at 4 Å they touch, but without recrossing. Zone IIb is the zone of the central barrier following a local minimum. Interestingly, this central barrier is quite appreciable for Na^+ and K^+ and much lower for Cs^+: at the center of the channel, the profile for Cs^+ is 8 kcal/mol deeper than that of Na^+, a difference which appears to be due to the large dispersion term arising from Cs^+ interacting with more atoms than Na^+. It is notable that this behavior of the 3 cations at the center of the channel agrees with the conclusions in [9] indicating

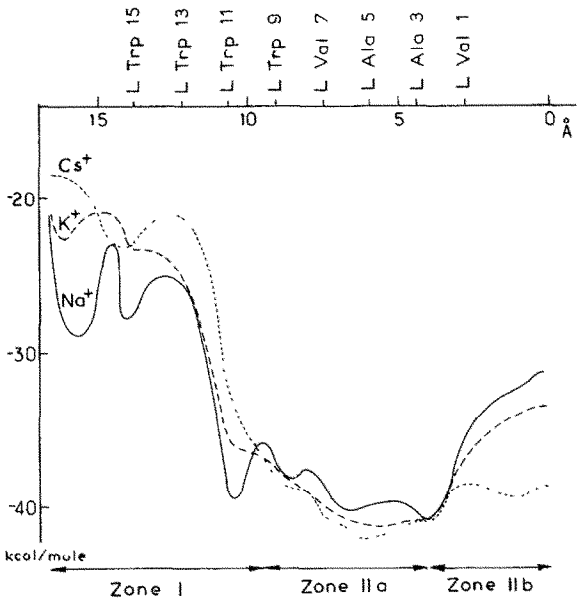

Fig.4. Energy profile for Cs^+ (···), K^+ (– – –), Na^+ (——), for single occupancy of the gramicidin channel when the tail is blocked. Energies in kcal/mol, distances in Å.

that the central barrier for Cs^+ is smaller than that for K^+, itself smaller than that from Na^+.

2.2. *Fixed tail*

The energy profiles for the 3 cations computed when the tail is fixed in its intrinsically preferred conformation are given in fig.4. At first glance the results appear very different from those of fig.2. A closer examination indicates that the most conspicuous differences appear in zone I where the 3 energy profiles are appreciably higher in energy when the tail is blocked, particularly during the early progress of the ions. This is clearly due to the loss of the favorable interaction with the hydroxyl oxygen of the tail. The smallest cation, Na^+, recovers this favorable interaction more easily than the two others as indicated by the minimum observed at 10.5 Å. In zone I, the energy profile for Na^+ is still deeper than those for K^+ and Cs^+ with local minima at 15.5 and 14 Å and two local maxima at 14.5 and 12.5 Å. For K^+, only one minimum at the entrance of the channel and a small barrier of 2 kcal/mol are observed. Between 15 and 14 Å, this disfavors K^+ with respect to Cs^+ which presents a local minimum between 14.5 and 14 Å, followed by a local barrier at 12.5 Å. From 14 Å on, thus conventionally inside the channel, the energy order of the 3 cations is the same as that observed when the ethanolamine end is flexible. The local barriers in the 3 energy profiles of zone I seem to be due to the presence of the hydroxyl hydrogen, which prevents the cations from interacting ideally with the different carbonyl oxygens.

In zone II, the energy profiles for the 3 cations are very similar to those observed in fig.2 and the characteristics noted above are the same, namely the inversion in the energy order of the 3 ions and the order of the central barriers. Note that for Na^+ the barrier separating the two zones has considerably decreased and that, owing to the weakening of zone I, the local minimum at 4 Å from the center is now somewhat deeper than that found at 10.5 Å. The energy profiles for K^+ and Cs^+ both present their minimum at 6 Å from the center of the channel. From 7 Å to the center of the channel the 3 curves are identical to those of fig.2.

It must be stressed at this point that the preceding discussion on the energy profiles refers to a naked cation without taking into account the energy necessary to dehydrate the ion (totally or partially). To obtain an idea of the relative order of the energy barriers at the entrance of the channel, this desolvation energy should be added to our computed energy values E. Although the real situation is clearly more complicated, due to the probable presence of water inside the channel [10–13] and to the fact that dehydration is likely to occur gradually, we have tentatively proceeded here as done successfully earlier to account for the

Table 1

Values of the partial energy balances δ in calculations (A) and (B) for 5 points able to represent the entrance of the channel[a] (E and δ in kcal/mol)

d (Å)	Tail mobile (A)			Tail fixed (B)		
	Na^+	K^+	Cs^+	Na^+	K^+	Cs^+
16.5	79.0	62.4	51.6	84.9	65.4	53.5
16	69.7	58.4	49.4	77.9	63.5	53.3
15.5	61.9	52.4	43.7	77.3	64.5	52.7
15	61.0	51.2	42.7	80.5	65.4	51.2
14.5	60.9	50.8	40.1	83.0	65.2	49.2

[a] $\delta = E + \Delta H$, with ΔH = 106, 86 and 72 kcal/mol for Na^+, K^+ and Cs^+, respectively [15]

specificity of valinomycin [8], nonactin [13] and calcimycin [14], using as desolvation energies the experimental values of the enthalpies of dehydration of the ions [15]. Since the channel entrance is not well defined, we have considered 5 points between 16.5 and 14.5 Å. The computed energy values for these points in computations A and B are reported in table 1. The values of the energy balance δ are all positive indicating that an energy barrier exists at the entrance of the channel, without, of course, allowing a quantitative evaluation. However, one may expect that the qualitative ordering is significant [16]. The most interesting result is that the ordering of δ is $Cs^+ < K^+ < Na^+$, indicative of a larger entrance barrier for Na^+ than for K^+, itself larger than for Cs^+, a result in agreement with the conclusions in [9] and with the observed selectivity of GA [9,17].

3. CONCLUDING REMARKS

This study has put into evidence the analogies and differences in the intrinsic energy profiles of 3 characteristic alkali metal ions assumed to move along the channel formed by Urry's GA dimer. It is clear that the results obtained must be considered within the constraints imposed by the model and that, in particular, we have not taken into account the modifications of the length and width of the channel that the crystallographic data [18] on the doubly occupied K^+ and Cs^+ complexes seem to suggest. Our computations indicate that, in fact, one large ion can interact favorably and pass through the channel without requiring its deformation. An examination of the effect of the suggested deformations, particularly for double occupancy, may lead to interesting differences, although it was suggested [19] that the above-mentioned crystals do not represent the channel form of GA.

We have also demonstrated that allowing for the flexibility of the ethanolamine end modifies notably the energy profiles for the 3 cations considered. The results obtained for Na^+ seem to point to the significance of this effect, at least for this ion: the presence of two nearly equivalent minima and the location of the lowest one at 4 Å for Na^+ when the tail is blocked appears in disagreement with the ^{13}C NMR observations indicating only one binding site situated in the vicinity of the L Trp 11 residue [4]. In contrast, in the corresponding profile which includes the flexibility of the tail, only one deep inner binding site is observed which corresponds very closely to the experimentally deduced location. We intend, in this connection, to examine in detail the fate of the GA analogs where the terminal group has been modified [20] or suppressed [21].

Although the flexibility of the tail may be influenced by the presence of the side-chains and by the structure of the dimple in the phospholipid at the channel mouth, the present study points to its possible role on the energy profiles and more generally to the influence of conformational changes on the relative location and height of the energy minima and maxima.

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