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Energy profiles in the acetylcholine receptor (AChR) channel

The MII-helix model and the role of the remaining helices

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It is demonstrated by theoretical computations that no favorable energy profile for cation transfer can be obtained in a model of the AChR channel constructed with the sole five MII helices of the inner wall. A favorable profile is obtained upon including the effect of the remaining helices of the five subunits. The decisive role, for the exit of the ion, of the charged residues situated at the N-terminal of the MII segments, established before, is underlined further. The role of the other elements of the channel wall (peptide carbonyl oxygens, hydrocarbon residues and polar side chains) is analyzed.

Acetylcholine receptor channel; MII-helix model; Helix dipole, α-; Subunit dipole; Na^+ energy profile

1. INTRODUCTION

In the currently prevalent model of the AChR channel [1], the five pentagonally disposed subunits ($\alpha, \beta, \alpha, \gamma, \delta$) participate in the transmembrane structure by four hydrophobic helices MI–MIV and contribute at least one helix, MII, to the inner wall of the channel. Labelling experiments [2–5] with non-competitive blockers (NCB), identifying the labelled residues as homologous serines in the five MII helices, suggested [6,7] that these residues face the interior of the pore, thereby fixing the orientation of the MIIs with respect to the central axis.

In a recent theoretical study [8], we have shown that, in order to satisfy the suggestion [3] that the high-affinity site for the NCB chlorpromazine lies at or near the level of the labelled serines on the axis of quasi-symmetry of the receptor and at minimum distances from all five chains, consecutive MII helices must be laterally in contact at this level rather than separated by another helix. Satisfying this condition with five parallel MII helices, we demonstrated further that the lower half of the model could accommodate sterically and with a favorable energy the largest permeant ion, dimethyldiethanolammonium (DMDEA). Furthermore, the calculation of the energy profile for DMDEA brought into evidence the decisive effect for the exit of the ion of the negatively charged glutamates at the N-terminal extremity of the MII helices (fig.1), a finding strikingly confirmed since by site-directed mutation experiments [9]. Extending our exploration to the whole length of the pore [10], we evaluated the minimal dimensions compatible both with the results of the labelling experiments and with the assumption that the large permeants or NCBs must diffuse through the upper part of the channel. We showed that the presence of bulky internal side chains in this upper part of the inner wall imposes a tilt of the MII helices calculated to be 7° with respect to the central vertical axis of the pore which thus becomes conical. The model leaves small gaps between adjacent MIIs in the upper part of the channel wall,

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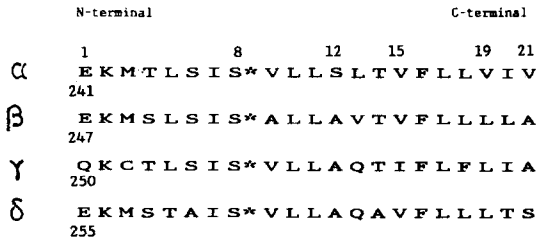

Fig.1. Aligned sequences (standard numbering indicated on first residue) of the MII segments of *Torpedo marmorata* AChR [18] within the limits discussed in [10]; (*) homologous serines labelled by [^{3}H]CPZ and [^{3}H]TPMP. For practical reasons a simplified numbering scheme is indicated on the top line.

which can, however, be easily closed by contact with another helix of each subunit. The model can sterically accommodate DMDEA even in its narrowest part, while blocking the NCBs in the neighborhood of the labelled serines.

Here, we wish to investigate the 'energy profile' generated by this refined model channel on a crossing Na^+ and to introduce, moreover, the effect of the remaining helices of the subunits.

2. METHODOLOGY

Calculation of the profile was performed as in [11–13] by optimization of the energy of interaction [14] of the ion with the whole channel in successive planes perpendicular to the central axis, regularly and closely spaced. The energy profile is given as a plot of this interaction energy as a function of the progression of the ion. In this exploratory study, the channel was maintained rigid. The five MII helices were placed in the regular conical disposition detailed in [10] with the homologous residues at the same level, their N-terminal being on the cytoplasmic side. Assuming the Cα atoms of the labelled serines to face the center of the pore, the 'helix wheels' request [8] that the Cα situated on the interior wall are those of residues 1, 4, 8, 12, 15, 19 (in the simplified notation of fig.1). According to our previous discussion [8,10], the upper limits of the MIIs are set 4 residues below Pro 265 in α and homologs in β, γ, δ. Two lower limits are considered: (i) the standard one starting with the sulfur residues (243 in α), (ii) the other commencing two residues earlier. In that case Glu 1 and Lys 2 form a salt bridge in α, β, δ; Gln 1 and Lys 2 in γ are H-bonded (see [10]).

The conformation of the internal serine side chains was fixed at the values optimized upon passage of an Na^+ in a bundle of α-helices enclosing a pore lined by serines [13]. Thr 4 in α, γ is in the most populated conformation found in α-helices of soluble proteins [15]. The conformations of the other side chains were those determined in [10].

3. RESULTS AND DISCUSSION

Fig.2 shows the profiles E_A and E_B calculated using the two limits (i) and (ii), respectively. The curves correspond to the profile felt by the ion when it is really inside the pore (3 residues away from the extremes on both sides).

Comparison of the two curves indicates the striking effect of inclusion of the charged residues on the lower end of the profile (right-hand side in fig.2) where the energy values change from positive (unfavorable) in the absence of these residues to negative (favorable) in their presence. This stems from the fact that, when included in the α-helices, the negative E residue (Q in MIIβ) is among the residues facing the interior of the pore while the positive K residue faces the outside of MII [8] and is thus further from the ion path. An essential result is, however, that in both cases the energies are globally less and less favorable from top to bottom of the channel (left to right in fig.2), a situation disadvantageous for the passage of the cation.

The reason for this feature appears upon analysis of the components of the global energy (fig.3a,b): it is observed that, in both cases, variations in the total interaction energy of Na^+ with the bundle are governed by variations in the electrostatic component of this energy, the polarization term varying only to a small extent (for clarity of the figures, the repulsion + dispersion Lennard-Jones component, which remains small everywhere, is not given). The dominant electrostatic component E_{el} of E becomes globally less and less favorable along the path of the cation. This reflects the fact that, due to the large dipole moment (about 74 Debye units for the large limit) of each MII α-helix, the conical bundle made of five nearly parallel MIIs presents a very large dipole moment with the negative pole oriented towards the synaptic side and the positive pole towards the cytoplasmic side, a situation unfavorable for the crossing of a cation. Although as seen above the negatively charged residues situated at the N-terminal end of the channel have an unquestionable favoring effect on the passage of a cation, this effect is not sufficient to invert that of the cumulating dipoles of the five nearly parallel MII helices (contrary to a recent suggestion [16]).

As a matter of fact, a profile calculated for a bundle of the sole five MII helices cannot be con-

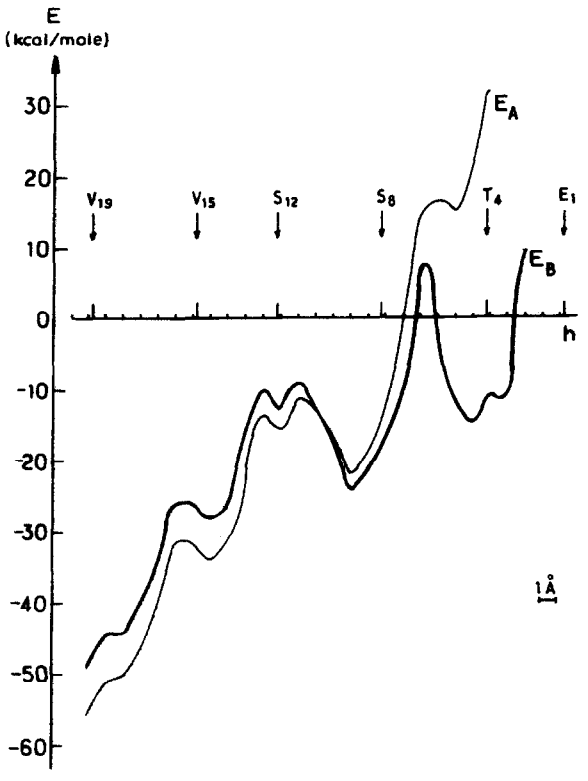

Fig.2. Energy profiles of Na^+ in the five MII-helix conical bundle. E, interaction energy of Na^+ with the five helices; E_A, limiting the helices to residues 3–21; E_B, including residues 1 and 2 in the helices. The scale h indicates the height of Na^+ within the channel (path from synaptic side, left, to cytoplasmic side, right). The arrows indicate the height of the C_α atoms of the residues facing the interior of the pore in MIIα (see fig.1 for homologs).

sidered to be able to describe correctly the actual situation, since each MII belongs to a subunit which comprises four helices, alternately down and up from the synaptic side to the cytoplasm, so that the dipole moment per subunit encircling the pore is essentially zero. Thus, even though the MIIs are the closest to the pore, the unfavorable effect of their parallel dipoles on the passage of a cation must be considerably reduced by the presence of the other helices of the receptor.

To investigate explicitly this cancelling effect, the profile must be computed in the presence of all helices. Since such a calculation involves a very large number of interactions, we have chosen, in a first step, to demonstrate the effect involved by considering only the influence of five of these helices (one per subunit), i.e. those which appear to be closest to the pore after the MIIs. According to the model defined in [8,10], the conically disposed MII helices should be surrounded by five others, denoted X, one per subunit, disposed just behind (fig.4a,b). In view of the relative shortness of the loops separating MI and MIII from MII, either of them could play the role of X, both necessarily oriented antiparallel to MII. Consistently with the hypotheses of the model, we assume that the five Xs, antiparallel to the MIIs, themselves form a second conical pentagonal bundle surrounding the inner one (fig.5a,b). Having verified that, in an α-helix, the nature of the amino acid side chains has little effect on the global dipole moment compared to that due to the cumulated peptide bonds, we have used, to mimic X, an α-helical polyglycine with the length of MII, but adopting for the interaxial distance between X and MII the optimal distance found [17] between two adjacent α-helices with bulky hydrocarbon side chains at their interface. This allowed us to calculate the positions of the axes of the X helices forming the second cone.

The energy profile of Na^+ was then recomputed in the double cone (fig.6). It indicates very strikingly that: (i) the interaction energy E of Na^+ with the whole 'double' bundle (10 helices) is now negative everywhere, and (ii) the unfavorable evolution of E from top to bottom of the channel present in the previous profiles has disappeared. We have ascertained that in the course of optimizations of the Na^+ positions no artefact minimum occurred near a polyglycine. In fact, the sites reached by Na^+ upon energy minimization are the same as in the cone of five MIIs.

Here again the key feature in the variation of the total interaction energy of Na^+ with the channel resides in the variations of its electrostatic component, however, this component is considerably modified with respect to that obtained in the MII-helix bundle: appreciably less favorable than before in the upper part of the channel, it is appreciably more favorable in the lower part. The inset in fig.6 shows the contributions, inside this electrostatic component, of the part El_{MII}, due to the five MII helices, and of El_X, due to the five X helices: one observes that, owing to the long-range character of the electrostatic interactions, the X helices, although situated further away from the

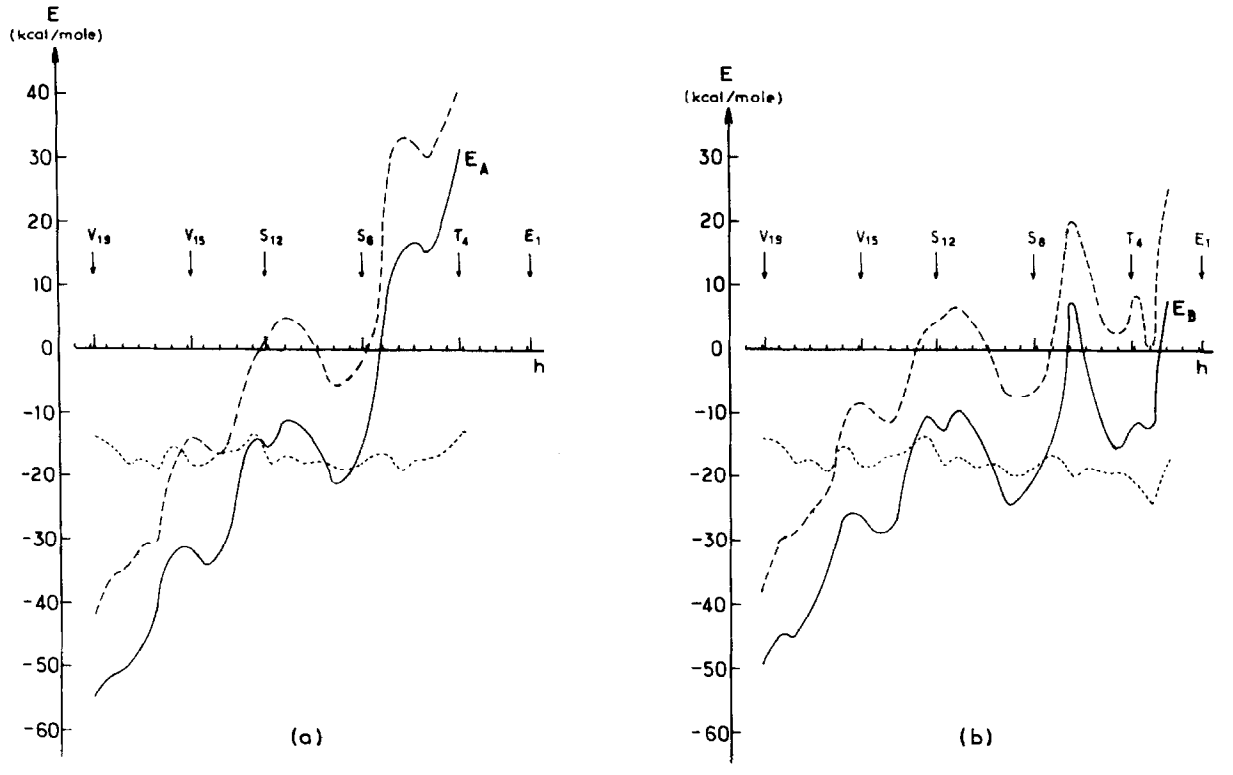

Fig.3. Energy profiles defined as in fig.2: (——) total interaction energy E, (– – –) electrostatic component of E, (· · ·) polarization component of E.

central hole than the five MIIs, nevertheless have a strong influence inside it which counteracts the effect due to the MIIs.

It is easily seen that the computation of the effect of the Xs is sufficient to foresee the effect of all the helices. A third helix Y (MI or MIII if X is MIII or MI) will be at a comparable (or slightly larger) distance from the pore, and hence will add an effect similar to (or slightly smaller than) that of X; the fourth helix, MIV, necessarily further out and of opposite orientation, will add in turn a con-

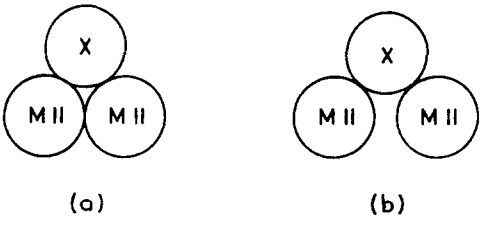

Fig.4. Schematic view of the mutual disposition of two adjacent MII helices and another helix X in (a) lower part and (b) upper part of the conical model of the channel.

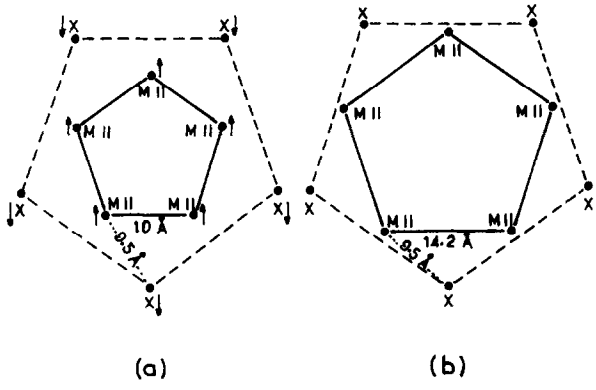

Fig.5. Disposition of the axes of the 5 MII inner helices and the five X helices surrounding them, viewed schematically from the synaptic side; (a,b) as in fig.4; (↑, ↓) orientation of the helices, from their N- to their C-terminal.

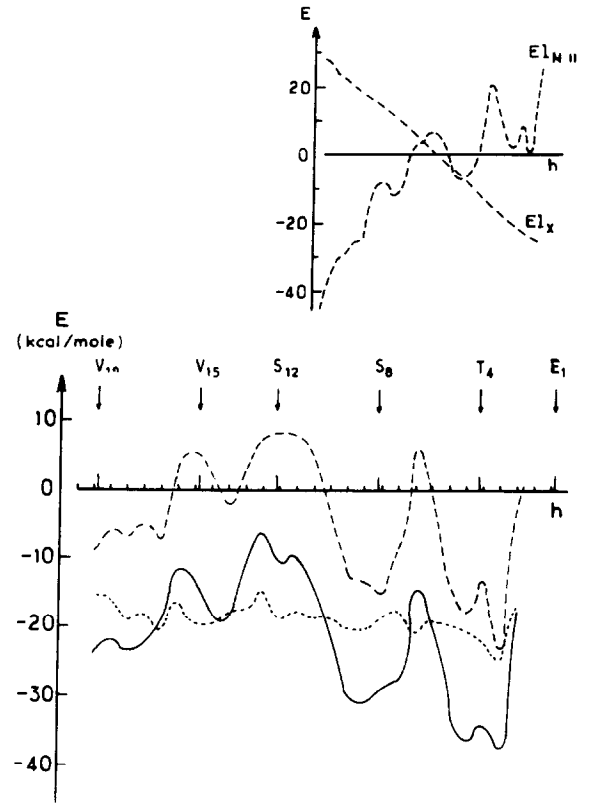

Fig.6. Energy profile of Na^+ in the double cone of fig.5 (charged residues 1 and 2 of MII included in the calculation). Curves as in fig.3. Inset (upper right): El_{MII} and El_X – electrostatic interaction energy of Na^+ with the five MIIs only and the five Xs only, respectively.

tribution of opposite slope but smaller. Finally, the global effect of the three external helices will be essentially that of X somewhat enhanced, leading to a favorable profile as in fig.6.

In conclusion, it can thus be stated that the obtaining of a favorable energy profile requires the proper inclusion in the calculation of the dipole effects of all the helices. The question then arises as to the role played in the favorable profile by the N-terminal charged residues. To assess this role, we have recalculated the profile in the double cone, suppressing residues 1 and 2 of MII (and two corresponding residues in X). The results indicate that, as in fig.2 (E_A), the ion encounters an energy barrier for its exit from the pore: E is 6.2 kcal/mol at the level of Cα of T4, while it is -34.7 kcal/mol in the profile of fig.6: indeed, close inspection of the evolution of E in this figure shows this deepening towards the end of the channel.

A final remark concerns the respective roles, in the profiles, of elements of the channel inner wall other than the charged end residues. It is observed that in the upper part of the channel, where bulky non-polar side chains point inside, favorable energies are nevertheless obtained: these are due to favorable interactions between the cation and the carbonyl oxygens of the peptide backbone of one or another of the MII helices, in spite of the presence of the bulky hydrocarbon side chains lining the wall. This result confirms our previous conclusion [12] (based on a calculation of the energy profile of Na^+ in a bundle composed of five polyalanines), namely that polar side chains are not necessary to create a favorable energy path for a cation and emphasizes again the role in this respect of the peptide carbonyls. In the lower half of the pore, the minima observed are due to both the carbonyl oxygens and the hydroxyl oxygen of the polar residues present which favor the interaction (a result again confirming the result of our previous model calculations [13]). The internal barriers found in the energy profiles can be readily explained by the nature and strength of the interactions existing at the level concerned. As shown in [12,13], they would easily be removed or decreased upon relaxation of the whole system without, however, altering the overall pattern.

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